Rapid Simple Analysis of Harmful Algae Bloom Toxins Via Waveguide Enabled ELISA

2023 Environmental Measurement Symposium
Minneapolis, MN

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Hach Company

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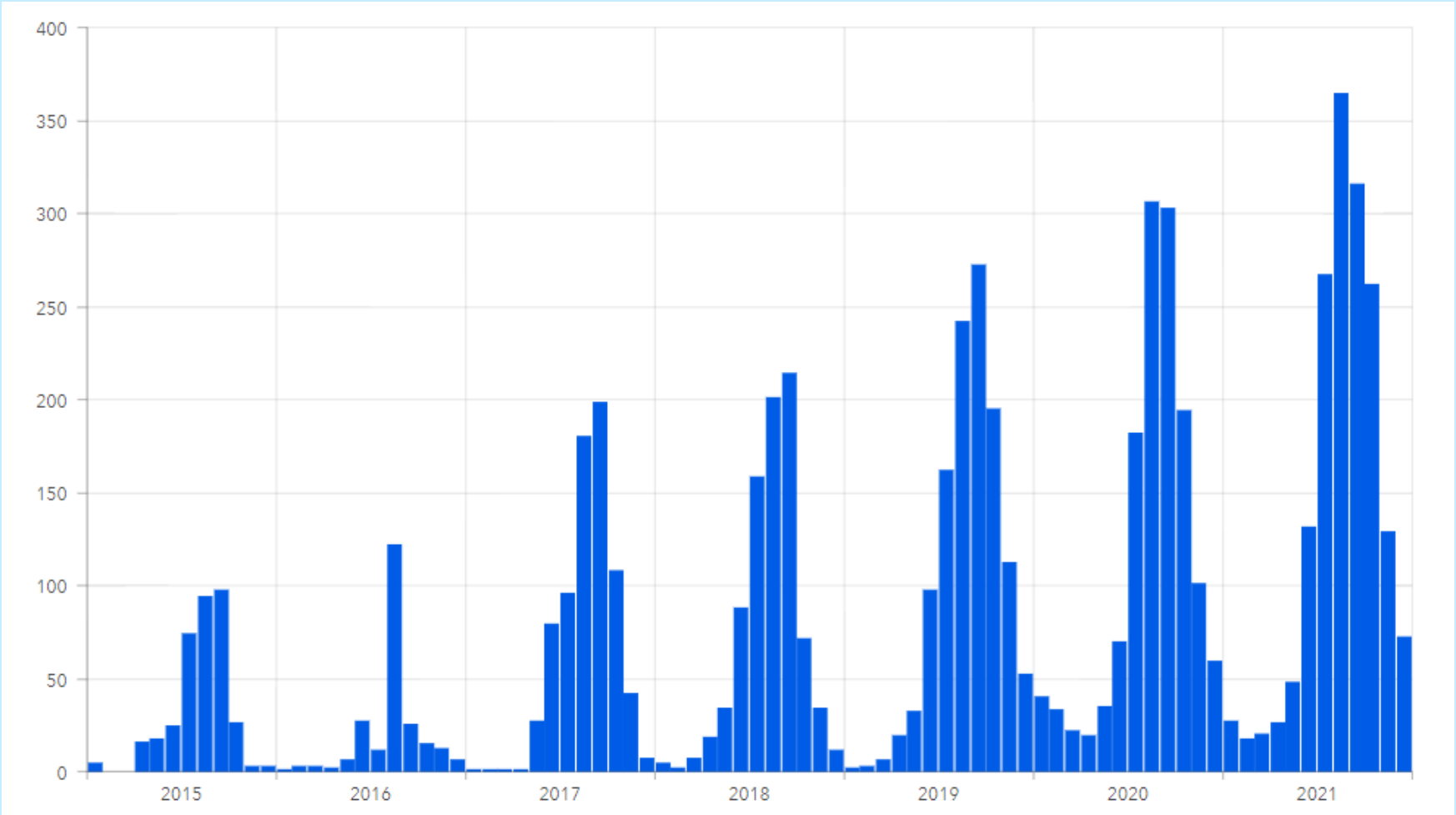
ANNUAL HARMFUL ALGAL BLOOMS, BEACH CLOSURES & ADVISORIES (EPA)



Note: Many states started publicly reporting freshwater HABs advisories since the EPA published its Drinking Water Health Advisories for two cyanotoxins, microcystins and cylindrospermopsin, in 2015.

Source: EPA, https://storymaps.arcgis.com/stories/d4a87e6cdfd44d6ea7b97477969cb1dd

HAB Events increasing (increased reporting + increased prevalence)

HARMFUL ALGAL BLOOMS

Why the rise in HAB event reports?

-Rainwater runoff brings superabundance of nutrient -Rising water temperatures
-Sunlight exposure
-Slow water velocity/mixing

Can float, hang suspended in the water column, or sink to the sediment

Bloom decay creates eutrophic/hypoxic conditions (killing flora and fauna)

Dozens of unique cyanobacteria, not all produce cyanotoxins (mechanism not well understood)

Those that release toxins, do so primarily during/after cell death or lysis (disintegration)

-can leach for months after the living bloom is gone
-cannot tell by looking at, smelling, or tasting contaminated water whether it is toxic or not

CYANOTOXINS

HEPATOTOXINS (symptoms: nausea, vomiting, diarrhea, inflammation, kidney damage)

Microystin (MC)

-Most common cyanobacteria

-Too few studies to formally declare as carcinogenic

-5,000-11,600 µg/kg body weight causes liver damage i.e., 2 mg in 10 kg child[2]

Cylindrospermopsin (CYN)

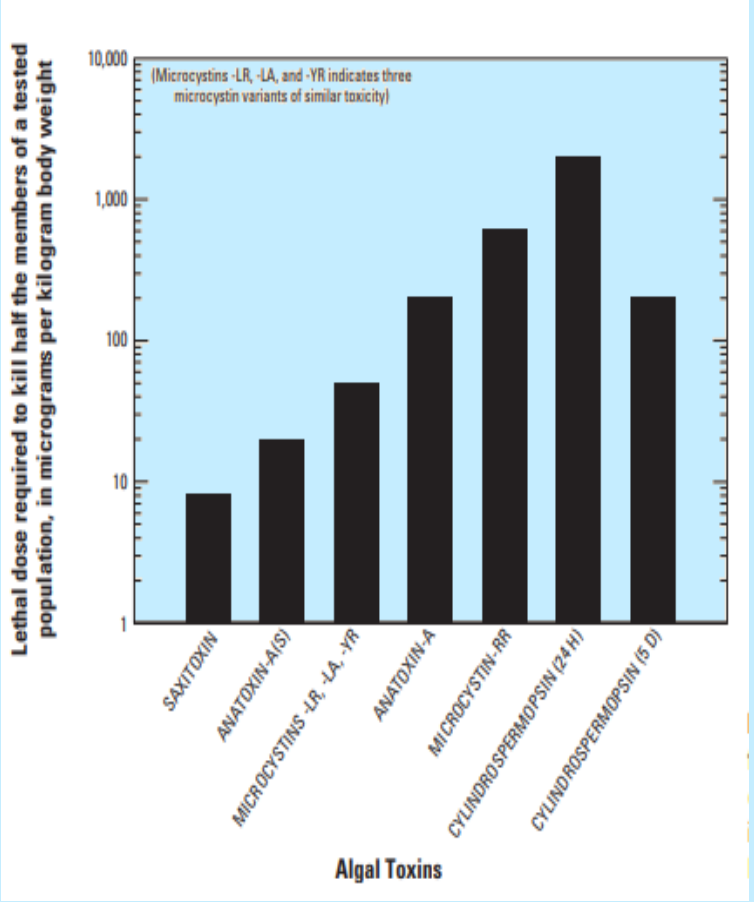
Used to be rare, now observed with increasing frequency

NEUROTOXINS (Symptoms: tingling, numbness, slurring words, paralysis)

Anatoxin-A (AN-A)

Saxitoxin (STX)

-Family of Paralytic Shellfish Toxins (used by CIA as a replacement to the WWII cyanide pill)



HABs in the News

WDBJ7.com Your Hometown Station

Smith Mountain Lake swimming advisory continues; harmful algae bloom still an issue

abc 7 NEWS

Toxic algae bloom shuts down 2 popular East Bay lakes. Here's what this means for visitors

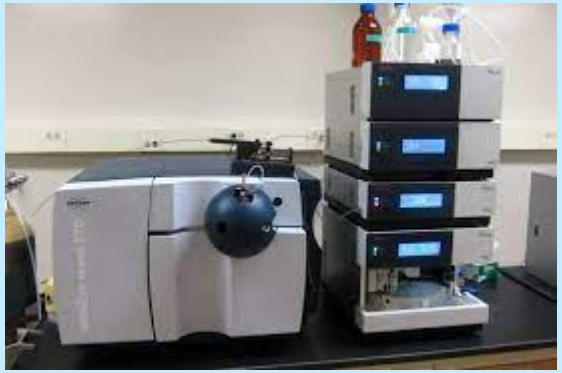
FOX NEWS channel

Toxic algae suspected in deaths of sea lions and dolphins on Southern California coast

Detection Methods for Cyanotoxins

There is a diverse range of rapid screen tests and laboratory methods available to detect and identify cyanobacteria cells and cyanotoxins in water.

- Enzyme–linked immunosorbent assays (ELISA) test strips, laboratory, field
- Protein phosphatase inhibition assay (PPIA)
- Reversed-phase high performance liquid chromatographic methods (HPLC) combined with mass spectrometric (MS, MS/MS) or ultraviolet/photodiode array detectors (UV/PDA).
- Liquid chromatography/mass spectrometry (LC/MS)
- Conventional polymerase chain reaction (PCR), quantitative real–time PCR (qPCR) and microarrays/DNA chips

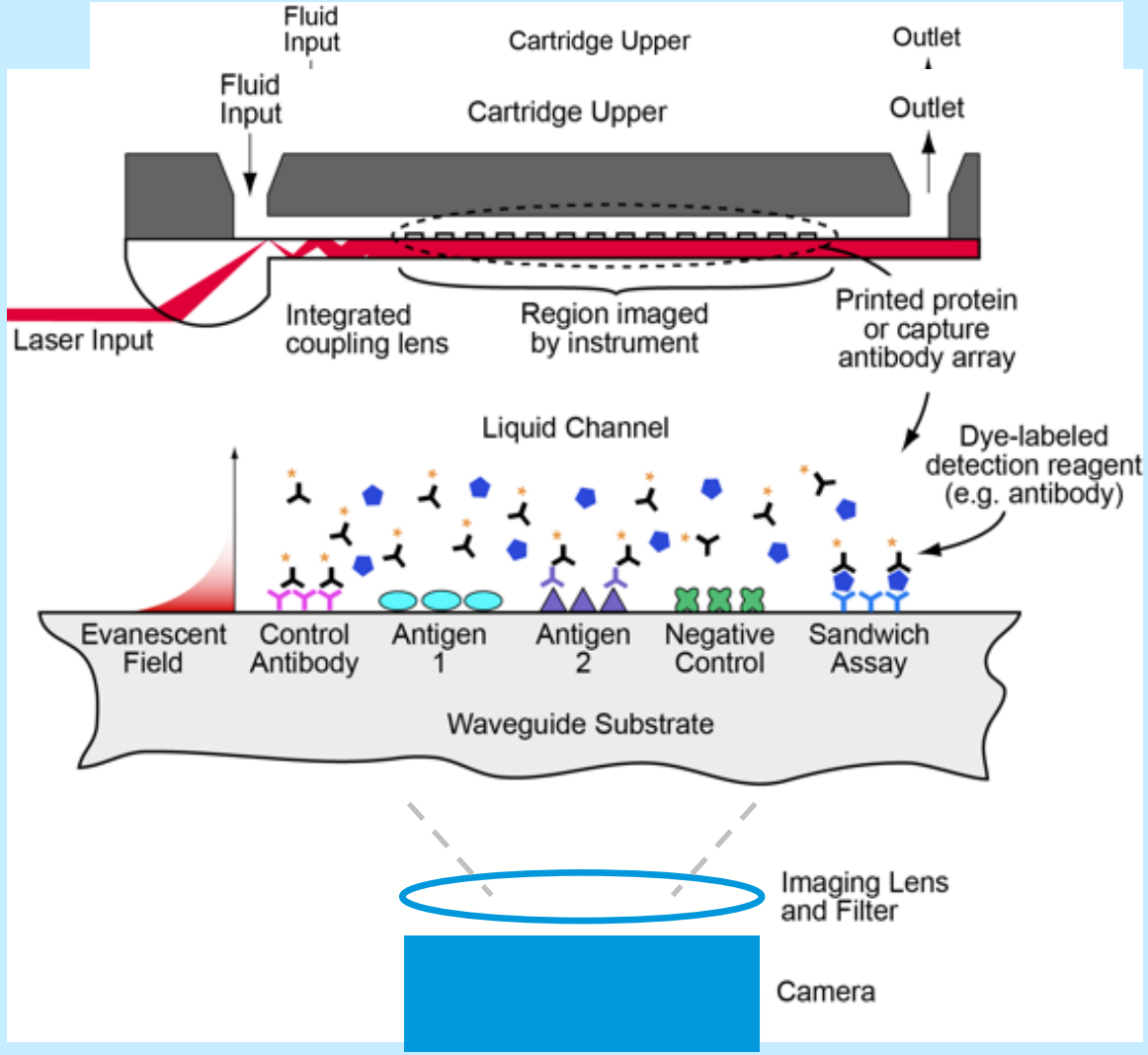


Draw Backs: Expensive equipment, Handraulic and prone to user technique errors, Lack of sensitivity.

LightDeck Platform: Waveguide Enabled Performance

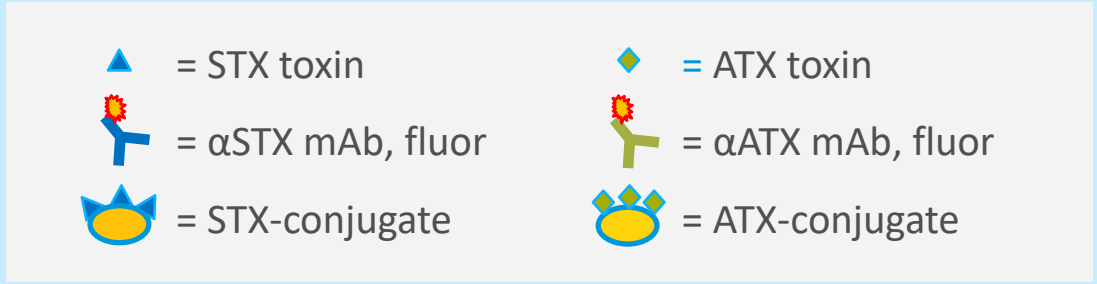
- Cartridge-based fluorescence assay system

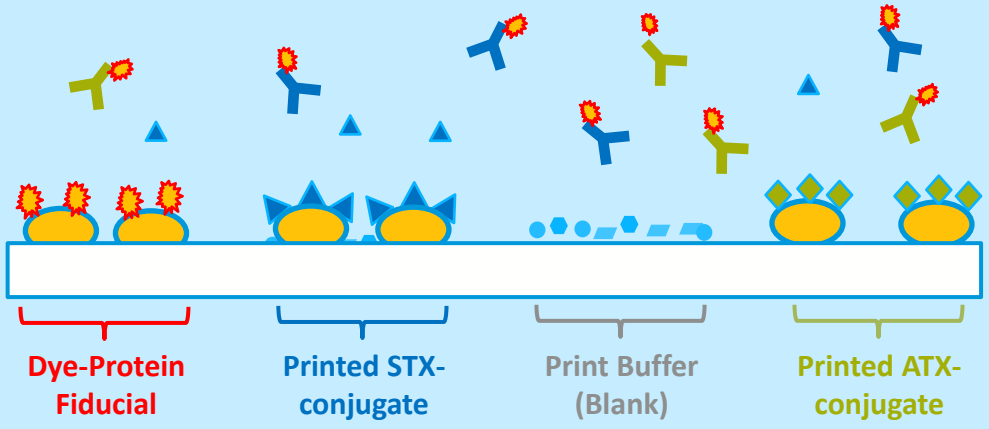
Built on best-in-class planar waveguide approach

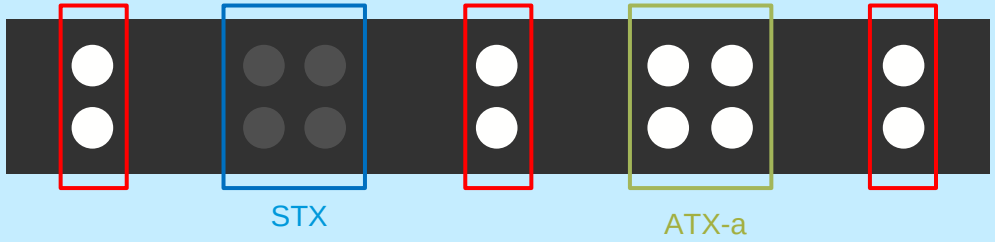


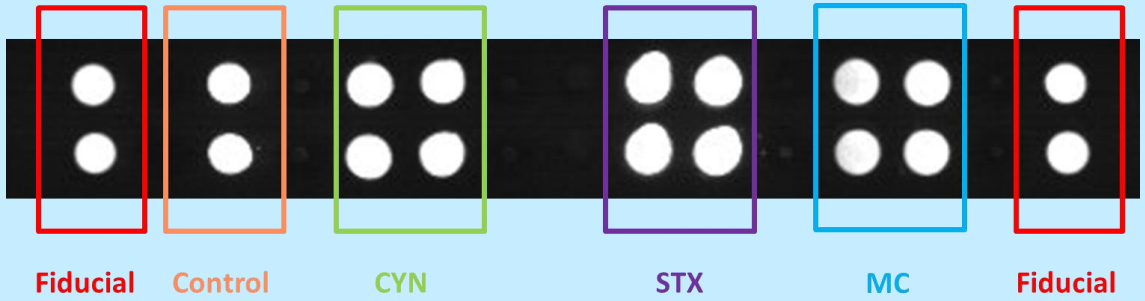
Printed microarray on planar waveguide (injection molded) w/ simple micro-fluidic channel

Multiplexed Competitive Assay Format









Allows for testing of multiple analytes on a single chip.

LightDeck® Technology:
Speed, Performance and Portability

- **Multiplexing** – 6 or more targets
- **Quantitative** – readout
- **Simple** – workflow
- **Fast** – results in 10 minutes or less
- **Compact** – small format packaging
- **Robust** – fluorescence reader

HAB Toxin System: Workflow

10-minute assay with sample added to a tube then the cartridge

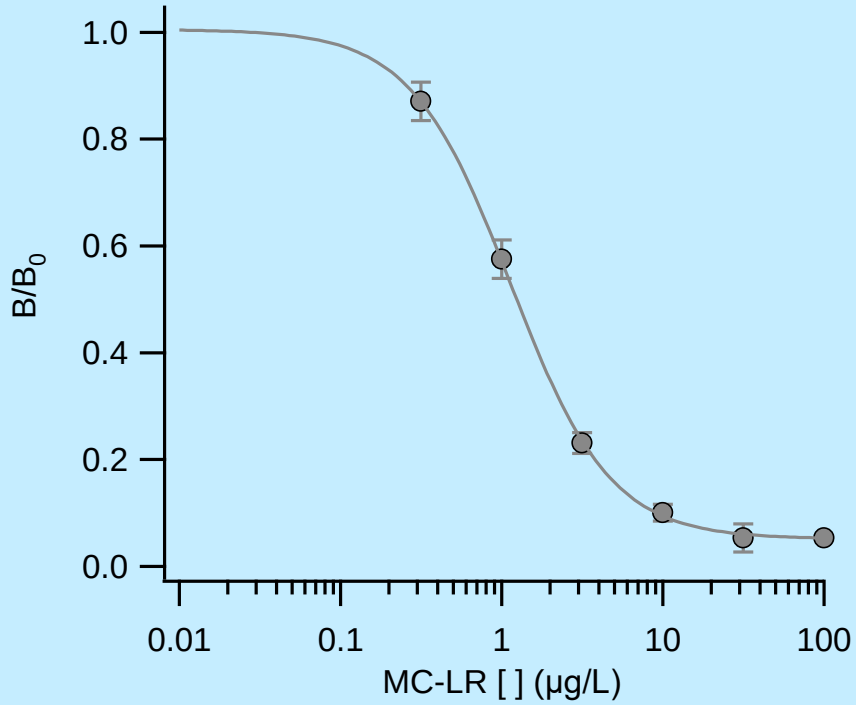
m·bio® DIAGNOSTICS

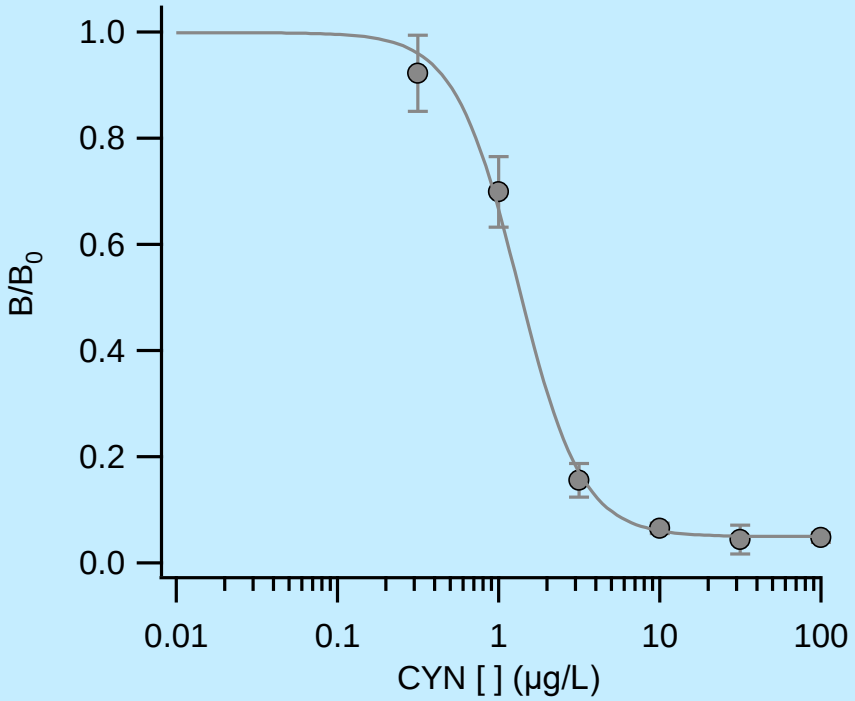
MBio HAB System
MC/CYN Gen 2

Sample Information	
Sample ID:	Lake4_drag2
Sampler ID:	MJL
Test Type:	MC/CYN
Collection Date:	03/25/2018
Collection Time:	01:45 PM
Tester ID:	SRB
Test Type:	MC/CYN
Test Date:	04/13/2018
Test Time:	03:00 PM
Time:	03:00 PM
Cartridge Lot ID:	001056
Cartridge ID:	ZZ-02005-136
Expiration Date:	12/31/2018
Reader ID:	98708740872
Software Version:	SnapEsi-LS 3.2.0.3

Control	VALID
Microcystin	2 µg/L
Cylindrosperm.	Less than 0.3 µg/L

Representative Standard Curves





- Reference standards from National Research Council Canada
- 3 replicates

Workflow similar to strip tests, but results demonstrate sensitive detection comparable to ELISA

Performance Benchmarking Against Reference ELISA with Standards

- Calibrators were verified on ADDA ELISA
- 12 cartridge experiment
- Performed 8/10/2018
- All concentrations reported in µg/L
- MC results are consistent with previous testing
- CYN results are slightly higher than expected

Calibrator #	MC Calibrator	CYN Calibrator	Run #	MBio MC	MBio CYN
1	0.0	1.0			
			2	<0.4	1.4
			3	<0.4	1.5
			4	0.5	1.5
2	0.5	1.0	1	<0.4	1.4
			2	0.6	1.5
			3	0.6	1.6
3	1.0	0.5	1	0.9	0.9
			2	0.8	1.0
			3	1.2	0.9
4	2.5	0.0	1	2.8	<0.8
			2	2.9	<0.8
			3	2.9	<0.8

River Water

- All river samples were non-detect for MC and CYN with ELISA
- All river samples were below detection limit on the MBio CYN assay
- Most MBio MC samples were below detection limit, with 3 cartridges out of 24 showing MC detection near threshold
- Measured 11/2018

River water sample 1			River water sample 2			River water sample 3			River water sample 4		
#	MC	CYN	#	MC	CYN	#	MC	CYN	#	MC	CYN
1	< 0.5	< 0.8	1	< 0.6	< 0.9	1	< 0.5	< 0.8	1	< 0.6	< 0.9
2	0.7	< 0.8	2	< 0.6	< 0.9	2	< 0.6	< 0.8	2	< 0.6	< 0.9
3	< 0.6	< 0.8	3	< 0.6	< 0.9	3	0.8	< 0.9	3	< 0.6	< 0.9
4	< 0.6	< 0.9	4	< 0.6	< 0.9	4	< 0.6	< 0.9			
5	< 0.5	< 0.8	5	< 0.6	< 0.9	5	< 0.6	< 0.9			
6	< 0.5	< 0.8	6	0.7	< 0.9	6	< 0.6	< 0.9			
7	< 0.6	< 0.8				7	< 0.6	< 0.9			
						8	< 0.6	< 0.9			

Method Detection Evaluation

- Measured a spike of 0.4 µg/L 7 times
- Data taken over two days in July and August
- All MDL samples detected. MBio reports slightly high relative to spike.
- Possible causes could be user preparation, sample aging, or calibrator error
- Operator-to-operator variation could be isolated by measuring cartridges with no toxin sample for validation

Sample ID	MBio Result for MC (ppb or µg/L)
MDL 1	1.1
MDL 2	1.0
MDL 3	0.5
MDL 4	1.4
MDL 5	0.8
MDL 6	0.8
MDL 7	1.4
Spike Concentration	0.4
Average	1.0
STD Deviation	0.3

Spikes into River Water

- Three samples of river water was spiked with MC and CYN to determine matrix effects
- Measured 11/2018-12/2018
- 36 cartridge experiment

Sample #1: River Water Samples Spike and Recovery

- Results are within expected range

Spike #	MC Spike	CYN Spike	Run #	MBio MC	MBio CYN
1	5.0	0.0	1	>4	<0.9
			2	>4.1	<0.9
			3	>4.1	<0.9
2	1.0	3.0	1	1.2	>2.4
			2	1.9	>2.4
			3	2.1	>2.4
3	3.0	1.0	1	4.2	1.2
			2	>4.3	1.2
			3	2.7	1.5
4	0.0	5.0	1	<0.7	>2.5
			2	<0.7	>2.5
			3	<0.7	>2.5

Sample #2: River Water Samples Spike and Recovery

- Results are within expected range

Spike #	MC Spike	CYN Spike	Run #	MBio MC	MBio CYN
1	5.0	0.0	1	>4.3	<0.9
			2	>4.3	<0.9
			3	>4.3	<0.9
2	1.0	3.0	1	1.3	>2.5
			2	2.5	>2.5
			3	1.8	>2.5
3	3.0	1.0	1	4.1	1.4
			2	2.8	1.2
			3	3.6	1.2
4	0.0	5.0	1	<0.7	>2.5
			2	<0.7	>2.5
			3	<0.7	>2.5

Sample #3: River Water Samples Spike and Recovery

- Results are within expected range

Spike #	MC Spike	CYN Spike	Run #	MBio MC	MBio CYN
1	5.0	0.0	1	2.2	<0.8
			2	>3.5	<0.9
			3	>3.6	<0.9
2	1.2	2.9	1	1.4	>2.4
			2	1.4	>2.4
			3	1.6	>2.4
3	1.3	0.8	1	<0.6	1.1
			2	1.5	1.2
			3	1.4	1.6
4	0.0	5.0	1	<0.3	>2.5
			2	<0.3	>2.5
			3	<0.3	>2.5

MBio Interpretation of GCWW Results

- Spiked calibrators and river water samples gave expected results within error
- River water matrix study showed expected negatives in CYN and a small number of border cases on MC
- MDLs show slight overreporting of MC concentration
- MBio will continue to look at tuning MC assay compared to ADDA ELISA

Western Lake Erie, OH Trip #1

- 4 samples measured in duplicate for dissolved toxin and total toxin
- All concentrations reported in µg/L
- Results show reasonable agreement between MBio field data and reference ELISA run in the laboratory

Sample #	MC ADDA ELISA	Dissolved vs Total	Run #	MBio MC	MBio CYN
GL 01	0.05 ± 0.01	Dissolved	1	< 0.5	< 3.0
			2	< 0.5	< 3.0
	0.98 ± 0.10	Total	1	2.6	< 2.7
			2	0.8	< 2.8
GL 02	0.10 ± 0.01	Dissolved	1	< 0.5	< 3.0
			2	< 0.5	< 3.1
	2.17 ± 0.10	Total	1	2.9	< 2.9
			2	2.7	< 2.9
GL 03	0.07 ± 0.04	Dissolved	1	< 0.5	< 3.1
			2	< 0.5	< 3.2
	2.15 ± 0.43	Total	1	2.6	< 3.0
			2	2.7	< 3.0
GL 04	0.15 ± 0.03	Dissolved	1	< 0.6	< 3.2
			2	< 0.6	< 3.2
	0.49 ± 0.00	Total	1	1.2	< 3.0
			2	< 0.5	< 3.0

Western Lake Erie, OH Trip #2

- 4 samples measured in duplicate for dissolved toxin and total toxin
- All concentrations reported in µg/L
- Results show reasonable agreement between MBio field data and reference ELISA run in the laboratory

Sample #	MC ADDA ELISA	Dissolved vs Total	Run #	MBio MC	MBio CYN
GL 13	0.08 ± 0.03	Dissolved	1	0.4	< 0.7
			2	< 0.3	< 0.7
	2.29 ± 0.04	Total	1	1.7	0.7
			2	1.3	< 0.6
GL 14	0.18 ± 0.02	Dissolved	1	0.4	< 0.7
			2	0.3	< 0.7
	2.77 ± 0.30	Total	1	2.8	0.9
			2	2.1	0.6
GL 15	0.10 ± 0.03	Dissolved	1	0.5	0.7
			2	0.3	< 0.6
	2.20 ± 0.32	Total	1	3.6	1.1
			2	1.4	0.6
GL 16	0.68 ± 0.03	Dissolved	1	0.8	0.7
			2	0.9	< 0.7
	3.20 ± 0.25	Total	1	1.8	0.8
			2	1.4	< 0.5

Sandusky Bay, OH Trip #1

- 4 samples measured in duplicate for dissolved toxin and total toxin
- All concentrations reported in µg/L
- Sandusky Bay is known to have a diverse mix of algae, producing a range of microcystin congeners
- ACT verbally said that preliminary LC-MS/MS results do not agree with ADDA ELISA on these samples, which they say is typical for these samples
- Data is consistent with Sandusky Bay samples measured on MBio System previously[1]

[1]Bickman et. al., Env. Sci. Tech 2018

Sample #	MC ADDA ELISA	Dissolved vs Total	Run #	MBio MC	MBio CYN
GL 05	1.90 ± 0.47	Dissolved	1	0.7	< 0.5
			2	< 0.5	< 0.4
	9.52 ± 1.64	Total	1	2.8	0.2
			2	2.8	< 0.1
GL 06	0.77 ± 0.08	Dissolved	1	0.7	< 0.4
			2	< 0.6	< 0.4
	6.51 ± 0.58	Total	1	2.5	0.3
			2	2.3	0
GL 07	0.82 ± 0.02	Dissolved	1	< 0.5	< 0.5
			2	< 0.5	< 0.5
	6.00 ± 0.76	Total	1	2.7	< 0.0
			2	2.9	< 0.0
GL 08	0.74 ± 0.07	Dissolved	1	< 0.6	< 0.3
			2	< 0.6	< 0.3
	4.73 ± 0.57	Total	1	2.5	< 0.3
			2	2.7	0.3

Sandusky Bay, OH Trip #2

- 4 samples measured in duplicate for dissolved toxin and total toxin
- All concentrations reported in µg/L
- Sandusky Bay is known to have a diverse mix of algae, producing a range of microcystin congeners
- ACT verbally said that preliminary LC-MS/MS results do not agree with ADDA ELISA on these samples, which they say is typical for these samples
- Data is consistent with Sandusky Bay samples measured on MBio System previously[1]

[1]Bickman et. al., Env. Sci. Tech 2018

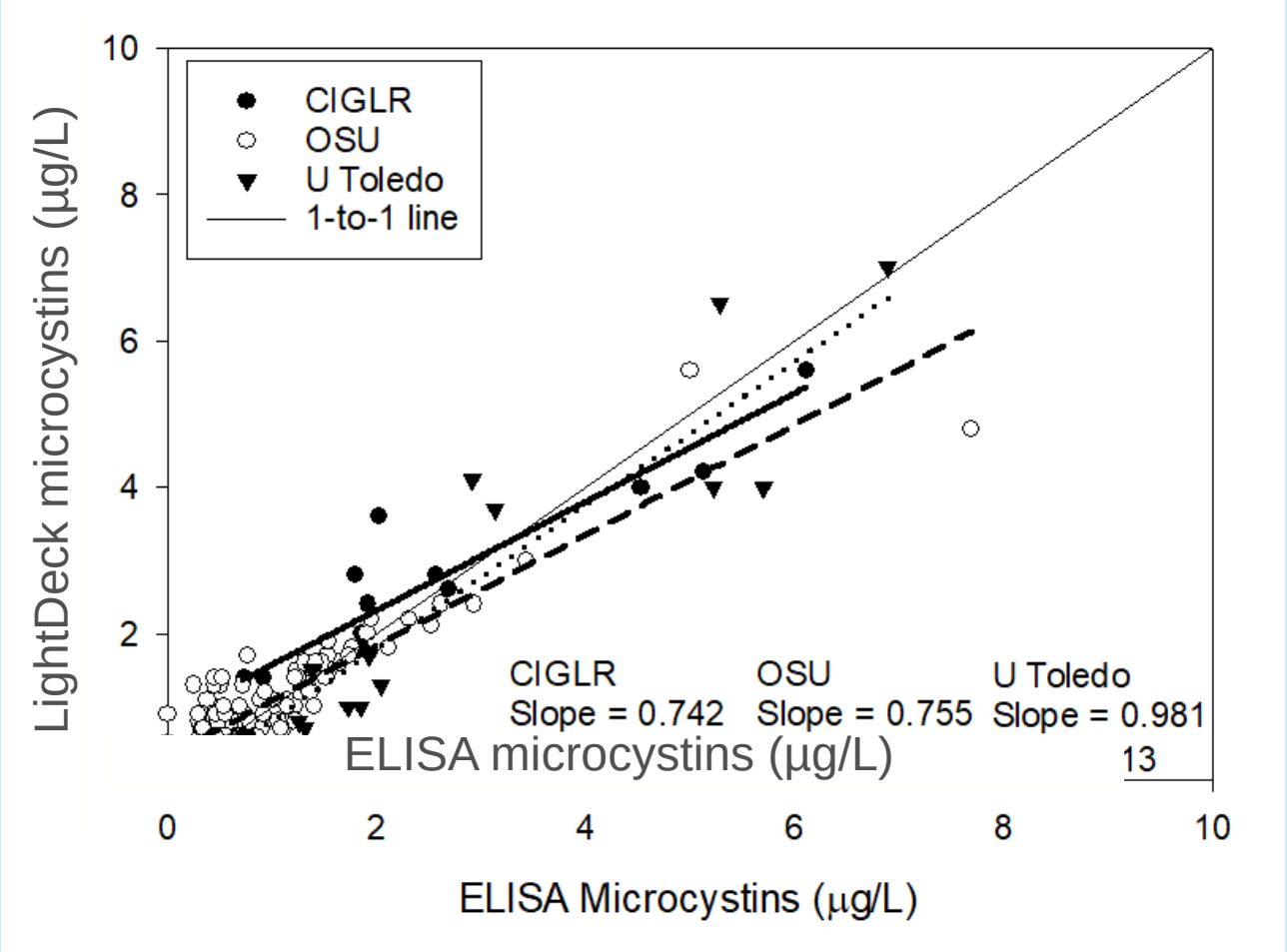
Sample #	MC ADDA ELISA	Dissolved vs Total	Run #	MBio MC	MBio CYN
GL 09	0.7 ± 0.0	Dissolved	1	< 0.2	< 0.7
			2	< 0.3	< 0.7
	6.2 ± 1.5	Total	1	1.8	0.8
			2	2.3	0.8
GL 10	0.7 ± 0.1	Dissolved	1	0.3	< 0.7
			2	0.4	< 0.7
	4.0 ± 0.3	Total	1	1.5	< 0.7
			2	1.2	< 0.7
GL 11	1.9 ± 0.4	Dissolved	1	0.6	< 0.7
			2	0.8	< 0.7
	5.7 ± 1.0	Total	1	2.8	0.8
			2	3	0.8
GL 12	0.6 ± 0.1	Dissolved	1	0.4	< 0.7
			2	< 0.3	< 0.7
	4.1 ± 0.2	Total	1	2.2	< 0.7
			2	1.8	< 0.7

MBio interpretation of ACT Results

- MBio HAB Toxin System was successfully run by independent operators in field settings
- Data showed generally good agreement between MBio and ADDA ELISA for Western Lake Erie
- Quantitative differences between MBio and ADDA ELISA for the Sandusky Bay samples were consistent with previously published results

Comparison of LightDeck and ELISA for Microcystin

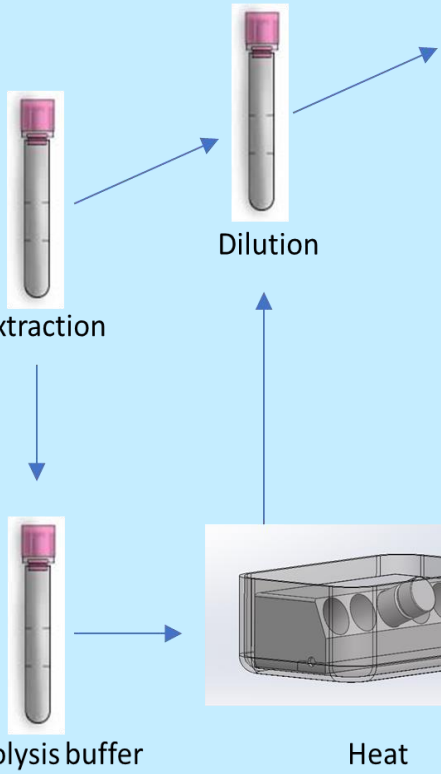
- Samples collected in 2021 and analyzed at Ohio State University Stone Lab, University of Toledo Lake Erie Center, and Cooperative Institute for Great Lakes Research

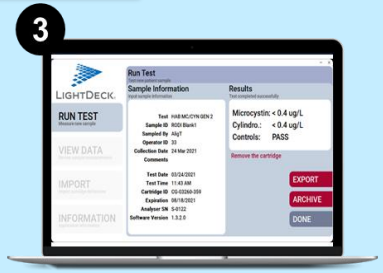


Shellfish and Saltwater

Shellfish

- Homogenized mussel samples are extracted and then diluted with sample buffer before testing
- Extraction process takes 5-10 minutes
- Prepared sample added directly to cartridge
- Duplex assay against ASP and PSP Toxins

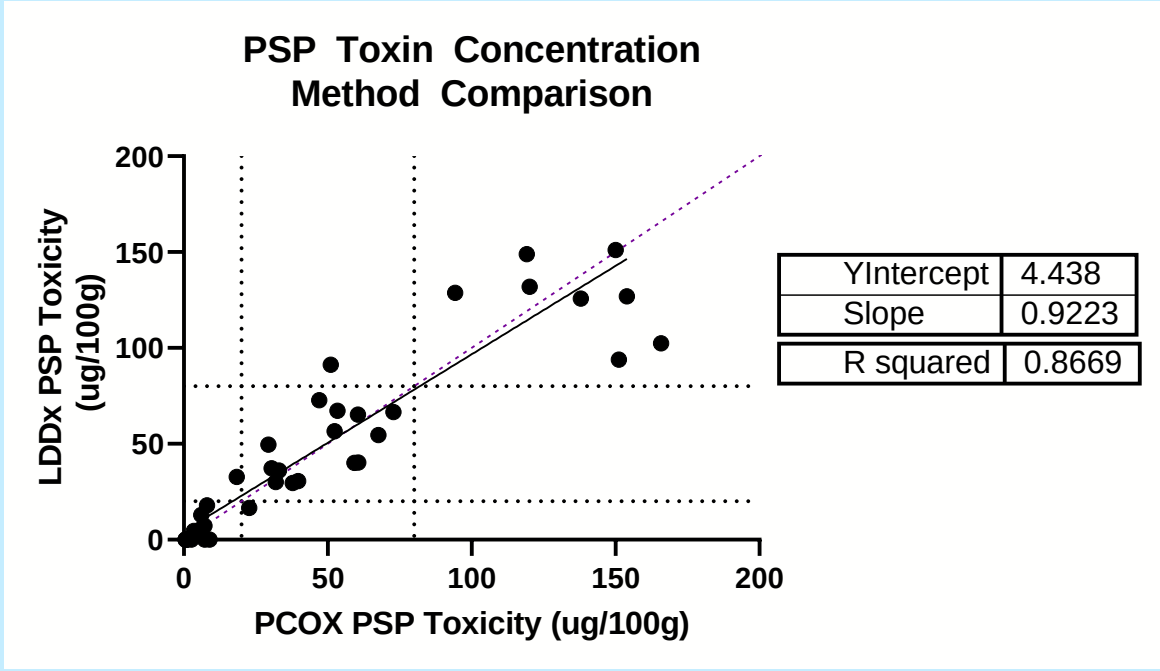


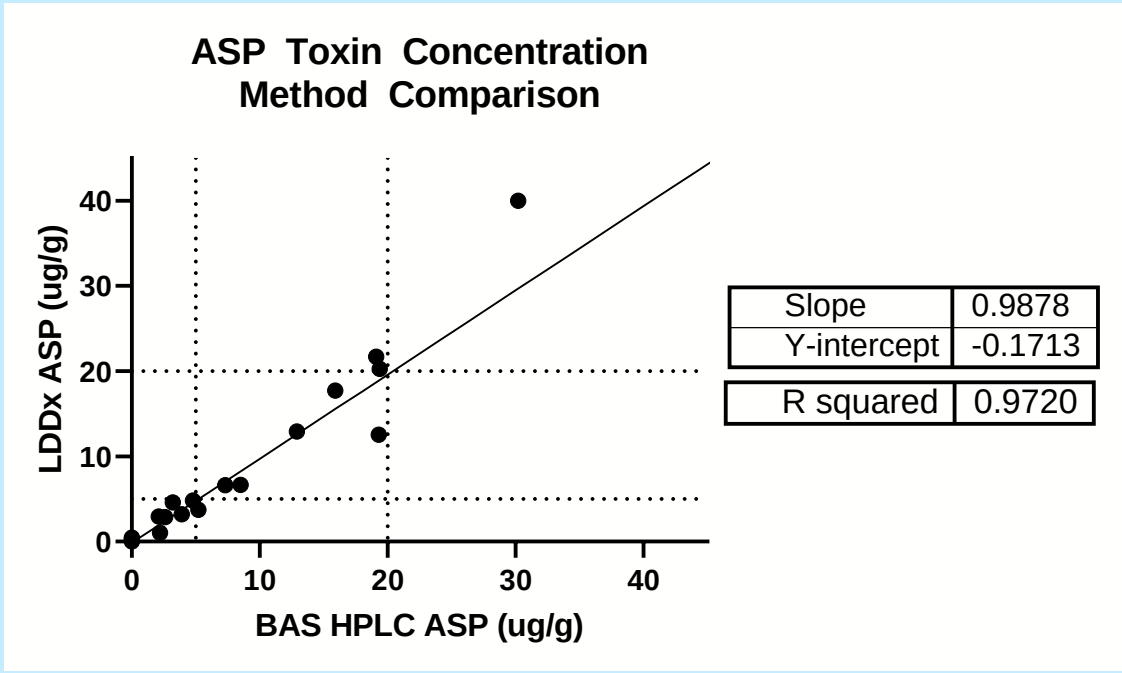


Shellfish

- 46 Naturally contaminated shellfish samples (mussel tissue) analyzed at Bigelow Laboratory for Ocean Sciences
  - Samples collected from Alaska, California, Maine, and Washington
- Tested by LDDx PSP + ASP cartridges against PCOX and HPLC
- 200+ natural samples will be tested during ISSC single-lab validation

Assay Ranges
ASP Toxin: 5-20 ug/g
PSP Toxin: 20-80 ug/100g





Saxitoxins/ Paralytic Shellfish Poisoning (Shellfish)

- Correlation between toxicity and cross reactivity

Green=Within 30% of toxicity

Red=Outside 30% of toxicity

Congener	Toxicity	LightDeck Shellfish STX (relative sensitivity)	Abraxis Shipboard ELISA Shellfish	Scotia/Jellett Rapid Test Shellfish	Beacon Analytical Saxitoxin ELISA	Beacon Analytical NeoSaxitoxin ELISA
STX	100%	100%	100%	100%	100%	11%
NEO	92%	94%	1.3%		0.8%	100%
GTX1&4	99%, 73%	20%	<0.2%	1.8%	<0.1%	<0.1%
GTX2&3	36%, 64%	58%	23%	100%	12%	<1%
dcSTX	51%	17%	29%	100%	18%	<1%
GTX5	6%	29%	23%	62%	25.6%	
C1&2	1%, 10%	1%		<1%	1.4%	
Lyngbia Wollei Toxin		19%	13%			
dcNEO		3%	0.6%	26%	0.7%	0.7%
GTX5&6		16%				

Conclusions

- LightDeck offers a rapid, low-cost multiplexed cyanotoxin detection system
- Currently available test is duplex microcystin and cylindrospermopsin detection
- After lysis, can detect toxins in 10 minutes
- Adding saxitoxin and anatoxin-a detection
- Shellfish test for PSP and ASP preparing for ISSC validation

Questions?????????

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