Cyanotoxin Round Robin Data Presentation

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Objectives

- Background- Through toxicological analysis a draft number of 0.07 ug/L for total microcystins (MC) was proposed by DSR for finished drinking water. EPA 546, which is a <u>biochemical</u> analysis for total MC has a MRL of 0.3 ug/L

- Many laboratories use Eurofins Abraxis, a vendor which makes ELISA plates
  - Two detection chemistries, both target the "ADDA" region of the molecule, but the reporting chemistry is slightly different
    - ADDA-OH (520011) – Limit of Detection is 0.1 ug/L | Ref range is 0.3 to 5.000
    - SAES (520011SAES) – Limit of Detection is 0.016 ug/L | Ref range is 0.05 to 5.000

Objectives

- There is a 15% "swing" allowed for samples – so objectively detection is possible on either kit on the low end assuming full recovery
- Question is about the confidence in those results – and how reliable on the low end these results actually are:
  - Standards for ADDA-OH – 0, 0.15, 0.40, 1.0, 2.0, 5.0
  - Standards for SAES – 0, 0.05, 0.15, 0.4, 1.5, 5
- Round Robin was split into 2 parts:
  - One part was conducted at DEP to measure the repeatability and reliability
    - 10 paired replicates
      - 0.07, 0.1, 0.3, 2, 1 (in Quenched Tap Water), 0 [all ug/L]
  - Second Part was 5 samples randomized and sent to 4 labs for lab analysis
    - Similar to Abraxis PT testing
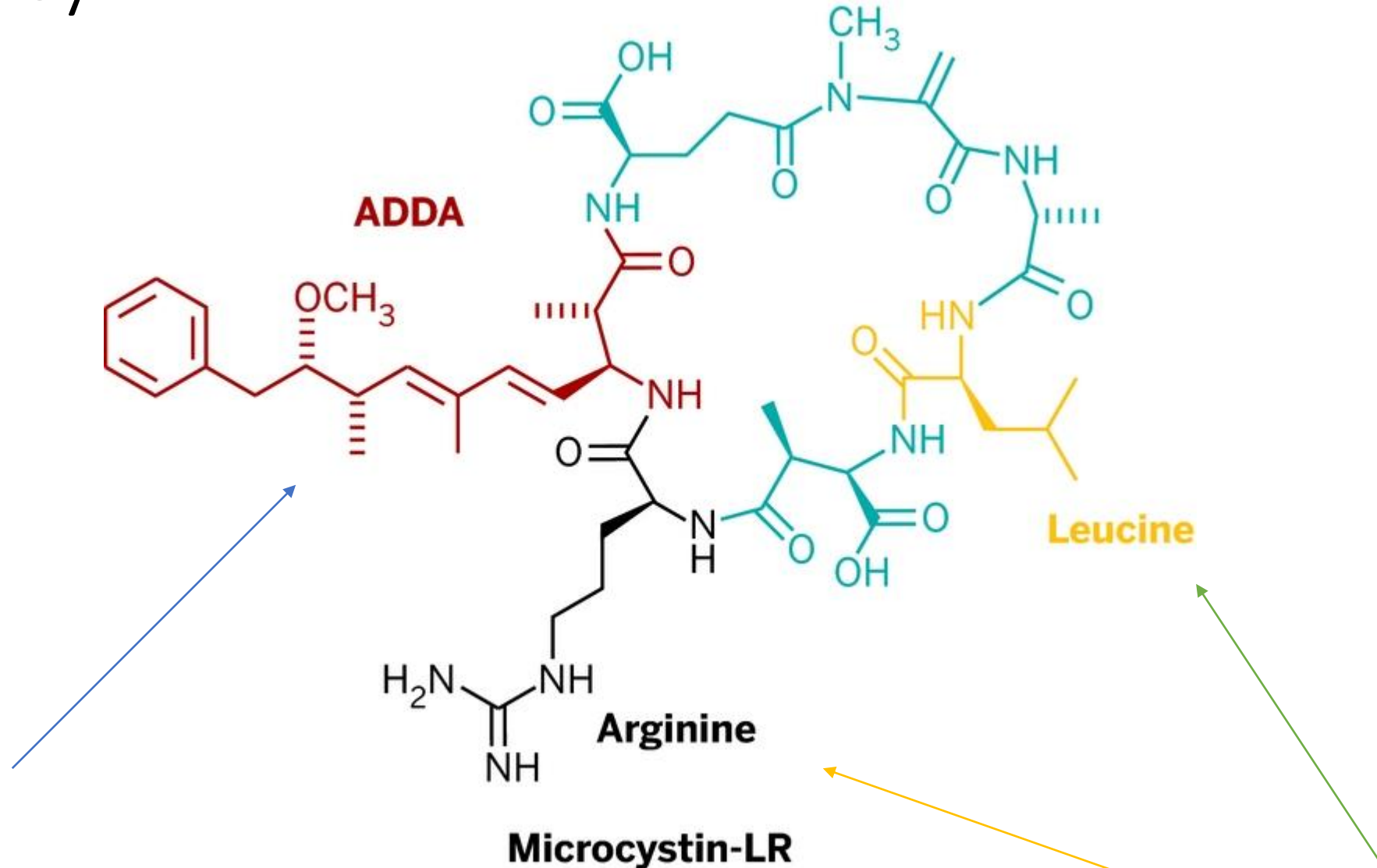   - Concentrations were identical to above

Current Findings

- DEP Findings
  - 0.07 Detection is possible on both kits, but it's not 100% consistent
    - Plotting below the DL on ADDA-OH requires you to manually export the data as well; If using the CAAS-Cube, values will show <DL
  - 0.1 faired better on both kits with lower CV values
    - Still a concern if running ADDA-OH only and outside the 15% swing that software might say <DL, though actually at or above the limit
    - LRB is higher on ADDA-OH kit generally, when plotting raw data.
      - DEP avg LRB 546 ~ 0.036 ug/L (which is < 0.15 ug/L | ppb)
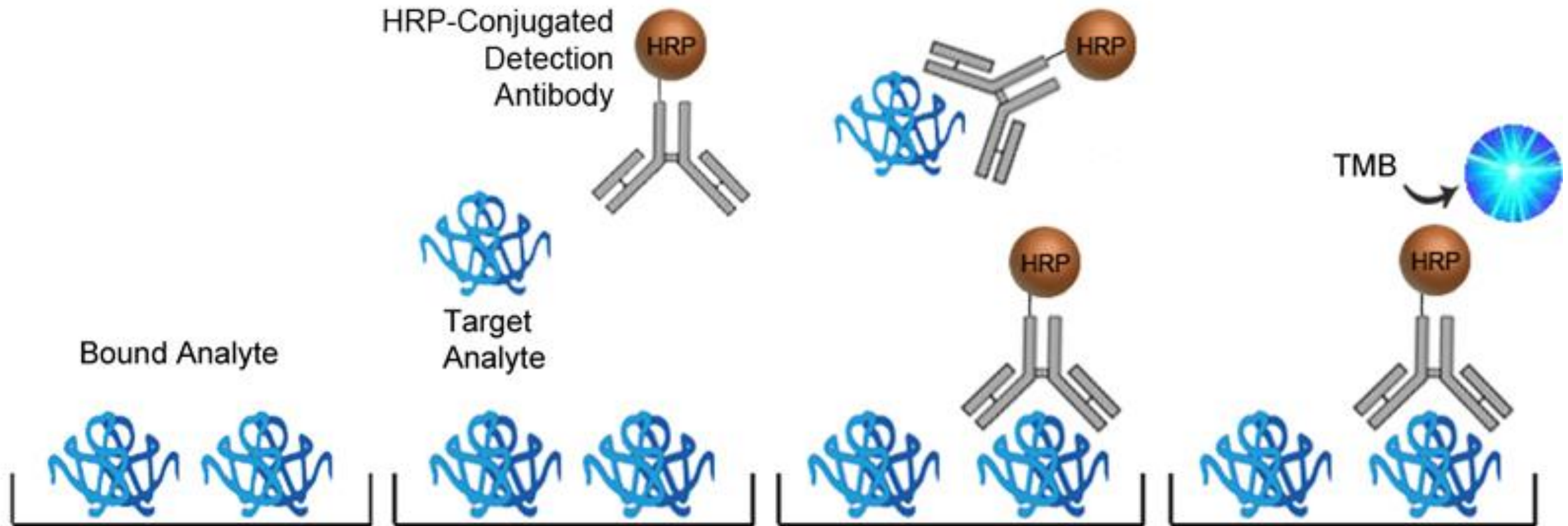   - Avg LRB 546 ~ 0.005 ug/L for SAES

Test Background

- At its core, this ELISA works by measuring fluorescence against a black background. Known concentrations of a target analyte are used and the intensity of the color is used as a proportion to indicate the quantitative amount present in the sample.
  - This means you can, in theory, measure the analyte in a sample, because so long as the analyte is in the sample, *some* reaction will occur that will be greater than the background of 0.
  - The software will generally report this as below detection limit but if you look at the raw data you can see where the data plots between 0 (your first true standard), and the lowest calibrator.
  - CAAS CUBE does not show raw data plots, CAAS does; In both software managers you can export the raw data.

Microcystin

ADDA

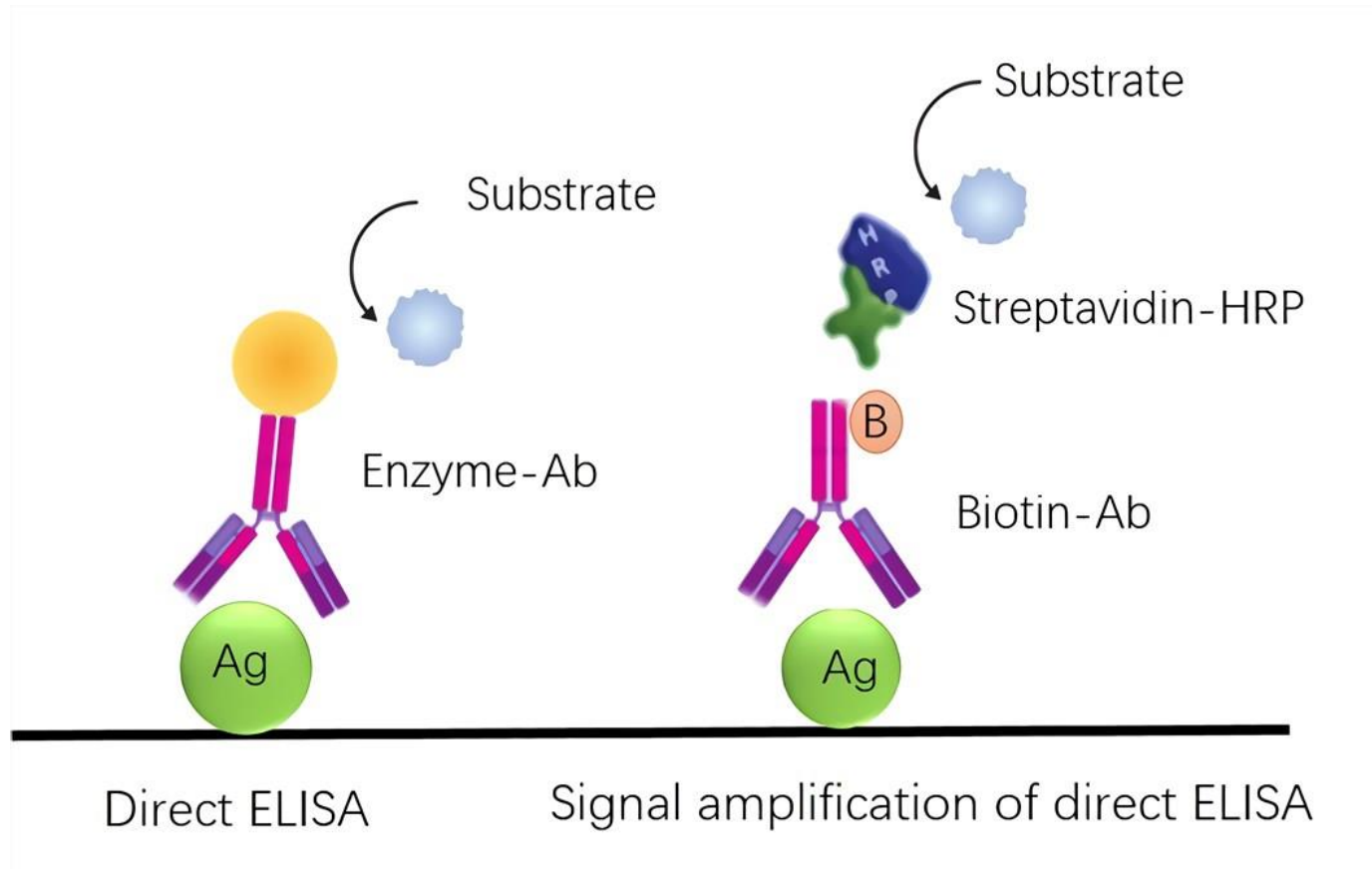Leucine

Arginine

Microcystin-LR
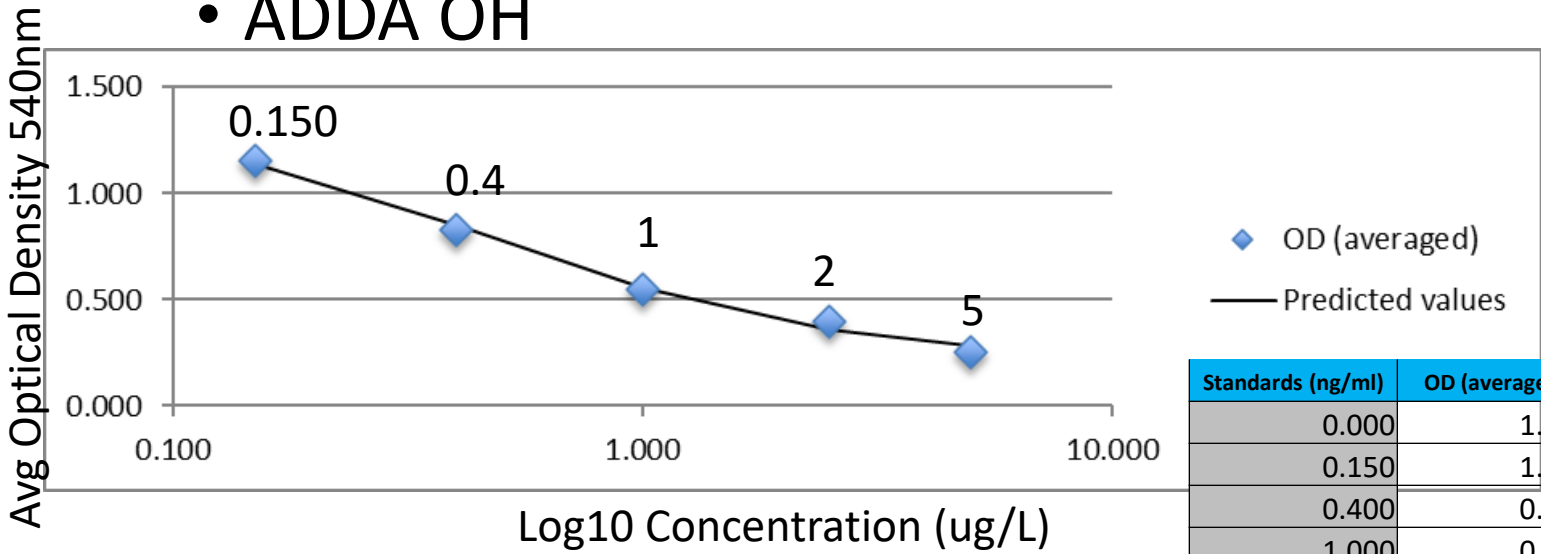
Test Background

HRP-Conjugated Detection Antibody

Target Analyte

Bound Analyte

TMB

https://www.lsbio.com/resources/devkitfaq

Test Background - SAES

Direct ELISA — Signal amplification of direct ELISA

DEP Data

- ADDA OH

Standards (ng/ml)	OD (averaged)	Predicted values	Residual Squares	B/Bo	Std Dev	%CV
0.000	1.408	1.413	2.21E-05		0.006	0.402
0.150	1.156	1.140	2.55E-04	0.821	0.021	1.835
0.400	0.830	0.848	3.07E-04	0.589	0.011	1.363
1.000	0.549	0.554	2.29E-05	0.390	0.000	0.000
2.000	0.396	0.359	1.35E-03	0.281	0.019	4.827
5.000	0.256	0.282	6.62E-04	0.182	0.004	1.657
Sum Of Squares			2.62E-03			

Parameter $(Y=(A-D)/(1+(X/C)^B)+D)$

R-Squared: 0.99741

|		
A	1.413	(Max.)
B	1.127	(Slope)
C	0.447	(IC_{50})
D	0.207	(Min.)

DEP Data

Sample/Control	Absorbances	Std Dev	%CV	Sample Concentration (ng/ml)	Sample Concentration Avg (ng/ml)	Comments
LRB	1.309			0.055		LESS than Standard 1, outside of quantifiable range
LRB	1.269	0.028	2.194	0.076	0.065	
QCS	0.691			0.637		
QCS	0.694	0.002	0.306	0.631	0.634	
LCRC	0.855			0.391		
LCRC	0.830	0.018	2.098	0.421	0.406	
0.07	1.267			0.077		LESS than Standard 1, outside of quantifiable range
0.07	1.263	0.003	0.224	0.079	0.078	
0.1	1.185			0.123		LESS than Standard 1, outside of quantifiable range
0.1	1.180	0.004	0.299	0.126	0.124	
0.3	0.918			0.324		
0.3	0.912	0.004	0.464	0.330	0.327	
2	0.395			2.003		
2	0.395	0.000	0.000	2.003	2.003	
1 in Q	0.555			0.996		
1 in Q	0.542	0.009	1.676	1.044	1.020	

DEP Data

- SAES
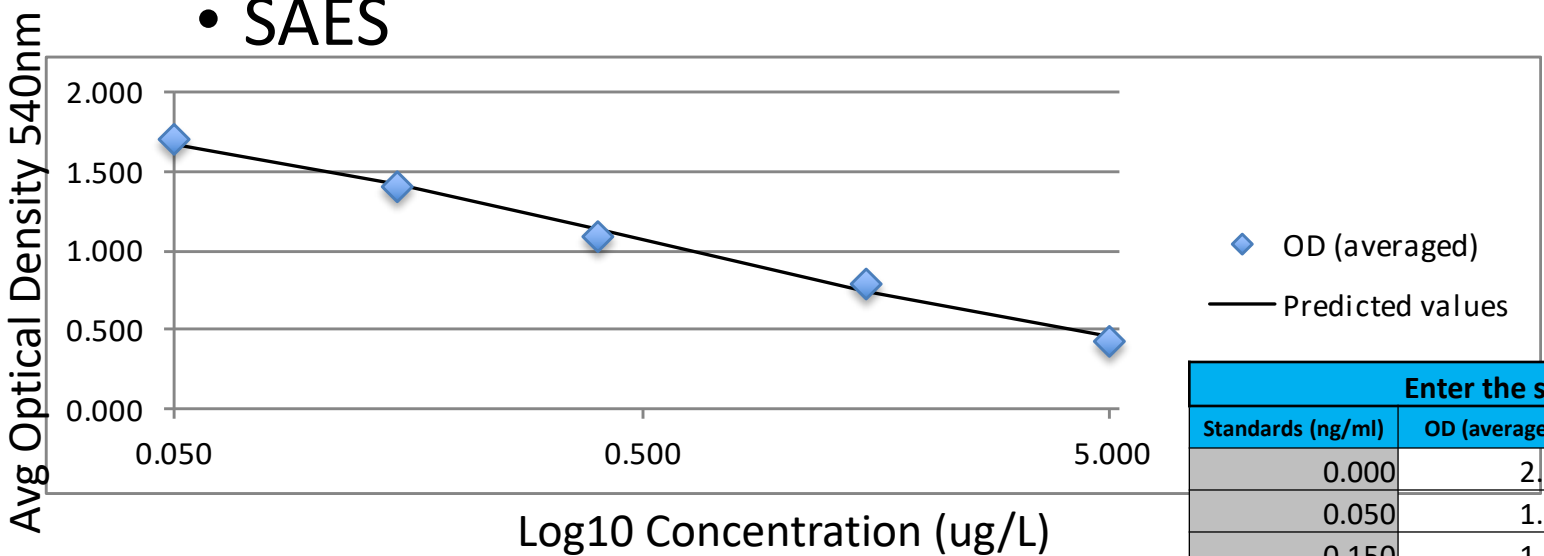
Enter the standard concentrations of each standard into the grey boxes						
Standards (ng/ml)	OD (averaged)	Predicted values	Residual Squares	B/Bo	Std Dev	%CV
0.000	2.074	2.080	4.88E-05		0.012	0.580
0.050	1.713	1.680	1.09E-03	0.826	0.052	3.014
0.150	1.400	1.420	4.04E-04	0.675	0.006	0.404
0.400	1.096	1.132	1.26E-03	0.529	0.035	3.226
1.500	0.788	0.736	2.63E-03	0.380	0.009	1.167
5.000	0.432	0.453	4.66E-04	0.208	0.004	0.819
Sum Of Squares			5.89E-03			

Parameter $(Y=(A-D)/(1+(X/C)^B)+D)$

			R-Squared
A	2.080	(Max.)	0.99677
B	0.614	(Slope)	
C	0.484	(IC_{50})	
D	0.065	(Min.)	

DEP Data

Sample/Control	Absorbances	Std Dev	%CV	Sample Concentration (ng/ml)	Sample Concentration Avg (ng/ml)	Comments
LRB	1.986			0.004		LESS than Standard 1, outside of quantifiable range
LRB	1.922	0.045	2.316	0.009	0.006	
QCS	0.978			0.658		
QCS	0.995	0.012	1.219	0.622	0.640	
0.07	1.560			0.087		
0.07	1.554	0.004	0.272	0.089	0.088	
0.1	1.580			0.080		
0.1	1.594	0.010	0.624	0.075	0.077	
0.3	1.306			0.224		
0.3	1.313	0.005	0.378	0.219	0.222	
2	0.662			1.982		
2	0.646	0.011	1.730	2.110	2.046	
1 in Q	0.819			1.119		
1 in Q	0.756	0.045	5.657	1.397	1.258	
Blank	2.151			Invalid		LESS than Standard 1, outside of quantifiable range
Blank	2.139	0.008	0.396	Invalid	Invalid	

Round Robin

- Three labs routinely running for cyanotoxins participated in NJDEP Round Robin
  - NJDEP supplied 6 tubes containing lot certified concentrations of MC-LR to each lab.
    - 0.07, 1, 0.3, 2, 1 in Quenched Tap, and 0 ug/L
      - Each point was selected to give the best chance of reporting on both the high and low end of the curve under the most ideal conditions.
      - No processing was required from the lab, all labs had to do was thaw the samples and run with other samples, ideally within the same plate, but it wasn't a requirement.

Round Robin Results

Lab	Kit	0.07	A
NJDEP	ADDA		0.068*
NJDEP	SAES		0.088*
1	ADDA		0.101
2	SAES		0.065
3a	ADDA		<DL
3b	SAES		0.049

Lab	Kit	0.1	B
NJDEP	ADDA		0.082*
NJDEP	SAES		0.104*
1	ADDA		0.134
2	SAES		0.108
3a	ADDA		<DL
3b	SAES		0.053

*Average of 10 replicates across different curves

Round Robin Results

Lab	Kit	0.3	C		Lab	Kit	2	D
NJDEP	ADDA		0.328 *		NJDEP	ADDA		1.875*
NJDEP	SAES		0.304*		NJDEP	SAES		2.049*
1	ADDA		0.339		1	ADDA		1.954
2	SAES		0.262		2	SAES		2.098
3a	ADDA		<DL		3a	ADDA		1.595
3b	SAES		0.215		3b	SAES		1.431

*Average of 10 replicates across different curves

Round Robin Results

Lab	Kit	1 Quench E
NJDEP	ADDA	1.004 *
NJDEP	SAES	0.955*
1	ADDA	1.029
2	SAES	0.940
3a	ADDA	0.503
3b	SAES	0.842

Lab	Kit	0 Con	F
NJDEP	ADDA		0.033*
NJDEP	SAES		0.007*
1	ADDA		<0.15
2	SAES		<0.05
3a	ADDA		<0.3
3b	SAES		<0.3

*Average of 10 replicates across different curves

Next Steps

- NJDEP DSR and NJDEP Pesticides Lab with NJDEP OQA are currently in the progress of working to validate 544 and have limited testing capacity
  - Will test comparison of recovery between quenched tap/spiked samples between 546 (ADDA-OH/SAES) and 544 to see performance and recovery.
    - At this point we will test spiking at 0.1 as well as the midpoint of the curve (2 ug/L).

Final Thoughts and Questions

- Controls are crucial to our understanding to how these biological assays perform; particularly since the LFB should be close to background yet has a detectable level of *something* on the ADDA-OH kit.
  - This is greatly reduced on the SAES kit, so it's most likely "noise".

- 0.07 ug/L for total MC can be detected, but accuracy and reliability will very likely be difficulty targets for labs
  - we often had numbers that were below 0.07, and above. It is unlikely a single sample is going to be run 10 times for average, most are run in duplicate on a single ELISA strip.

- 544 analysis would be able to analyze down to ng/L (congener specific), but has a substantially longer lead time and currently lacks any certified labs in NJ.