qPCR and Total Microcystin Analysis : How it Works and What Your Results Mean

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Analyst II

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Toxins Detected by qPCR Analysis

- 16S – Total cyanobacteria
- Microcystins
- Cylindrospermopsins
- Saxitoxins

16S – Total Cyanobacteria

- Commonly called Blue-Green Algae
- It is a Bacteria that can photosynthesize like a plant
- Cyanobacteria grows like any other plant or organism
- The toxins are typically contained within the cells
  - Once released, toxins can remain stable in the water for weeks
- Toxins can be present even when a visible bloom is not
- Not all Toxins are TOXIC
- Blue-green algae, typically lives on the surface of water
- The scum can cause decreased levels of oxygen and prevent sunlight from penetrating the water column
- Toxic blue-green algae can cause lower reproduction and growth rates in the aquatic wildlife, as well as fatality

Microcystin

- Most commonly occurring and toxic cyanobacteria
- Microcystins are a Hepatoxin
- Exposure can come from dermal, ingestion, or inhalation
- Symptoms include:
  - Skin Rashes
  - Abdominal Pain, nausea, vomiting and diarrhea
  - Headaches
  - Sore throat and dry cough
  - Blistering around mouth
  - Pneumonia
  - Liver Disease – interhepatic hemorrhage or hemorragic shock
  - Kidney Failure
  - Heart Failure
  - Neurological effects
- Can be fatal to humans and animals

Cylindrospermopsin

- Hepatoxin
  - Liver and Kidney damage
- Exposure is most commonly oral
- Relatively Stable in the dark
- Survives for up to 8 weeks at:
  - 4 - 50°C
  - pH 4-10
- Toxins remain potent after 15 minutes of boiling

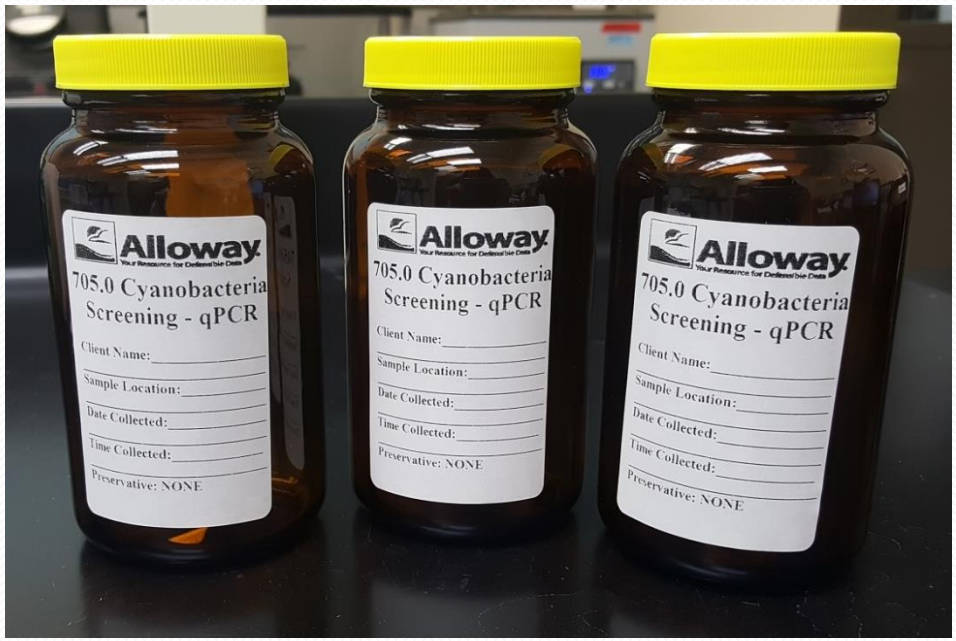
Saxitoxin

- Neurotoxin
- Are a large family of toxins that are known as the Paralytic Shellfish Poisoning (PSP)
- Most common exposure is from consuming contaminated shellfish
- Symptoms include:
  - Numbness
  - Headache
  - Dizziness
  - Nausea
  - Loss of Coordination
  - Floating Sensation
  - Muscle Paralysis or Respiratory Failure

qPCR Analysis

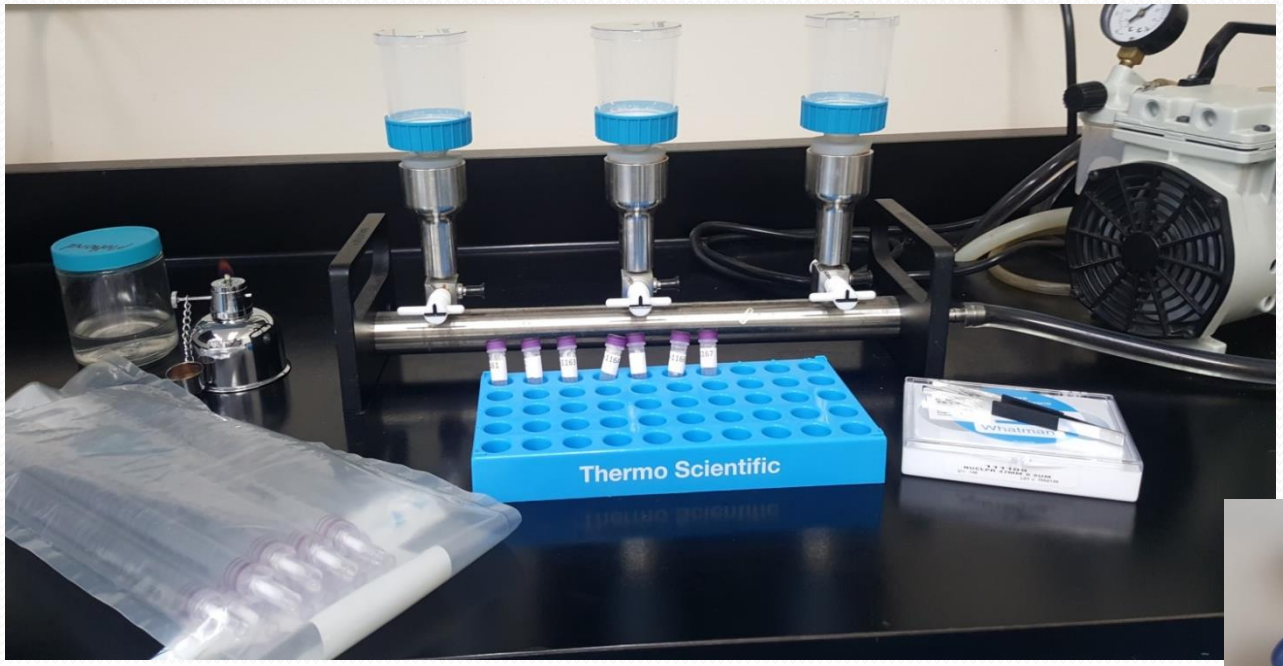
- Sample Collection
- Extraction
- Analysis

Sample Collection



- Samples must be collected in an Amber Glass Bottle
- Stored at 0-4°C immediately after collection
- Samples then have to be extracted within 48 hours of collection
- Once extracted and frozen the hold time is extended

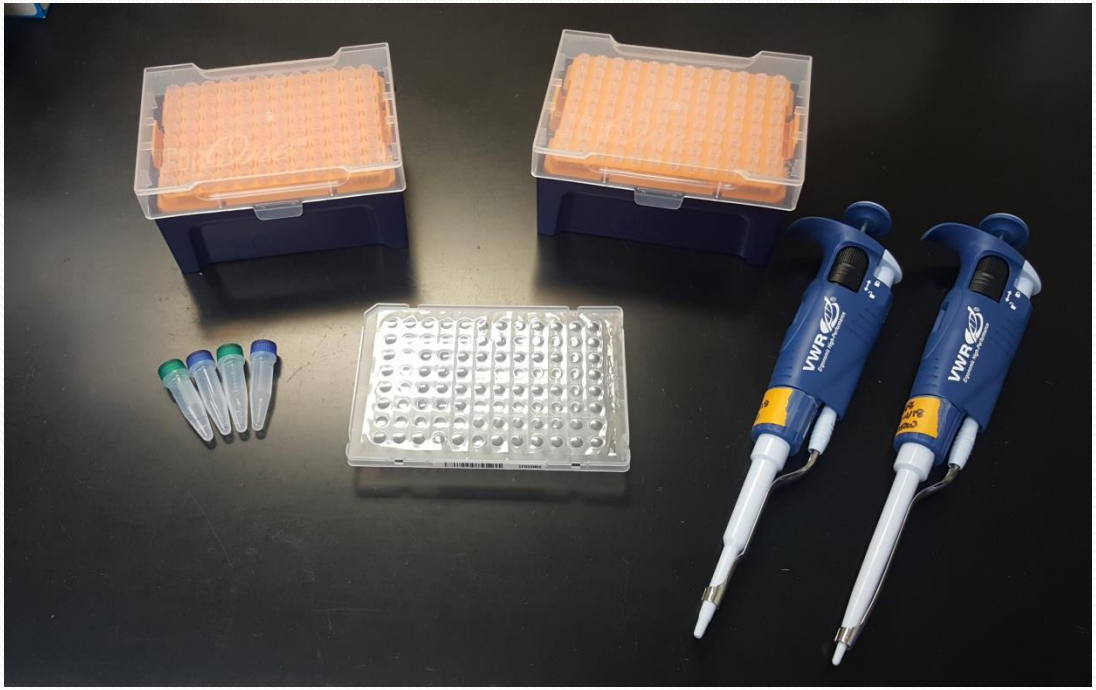
Sample Extraction



- DNA Extraction begins with a filtration step using sterile equipment

Sample Extraction

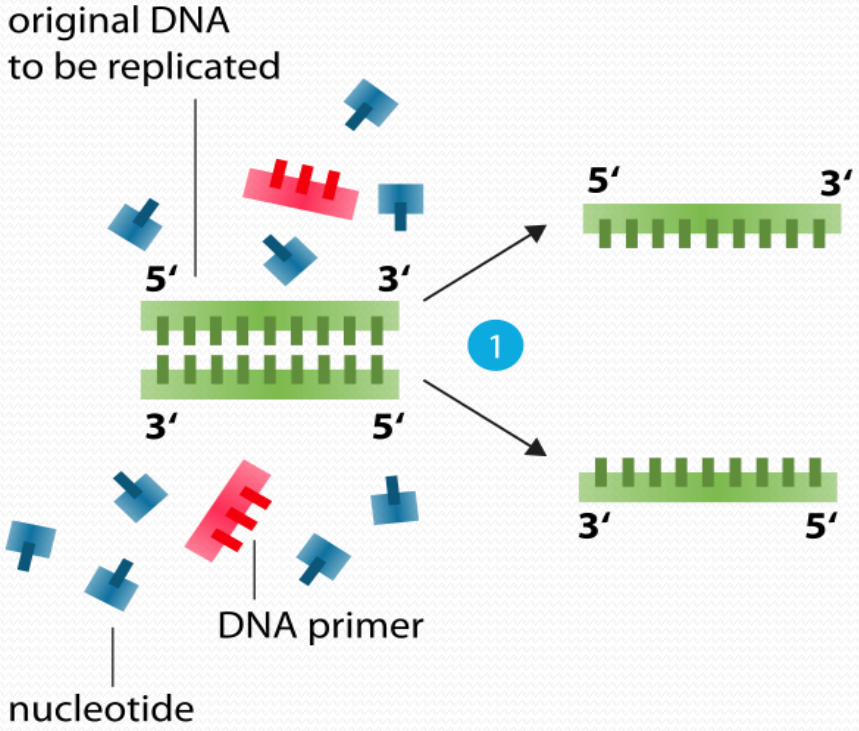
Sample Analysis



- Samples are combined with the Master Mix and pipetted into the plate wells
- Plate is sealed and loaded into the instrument

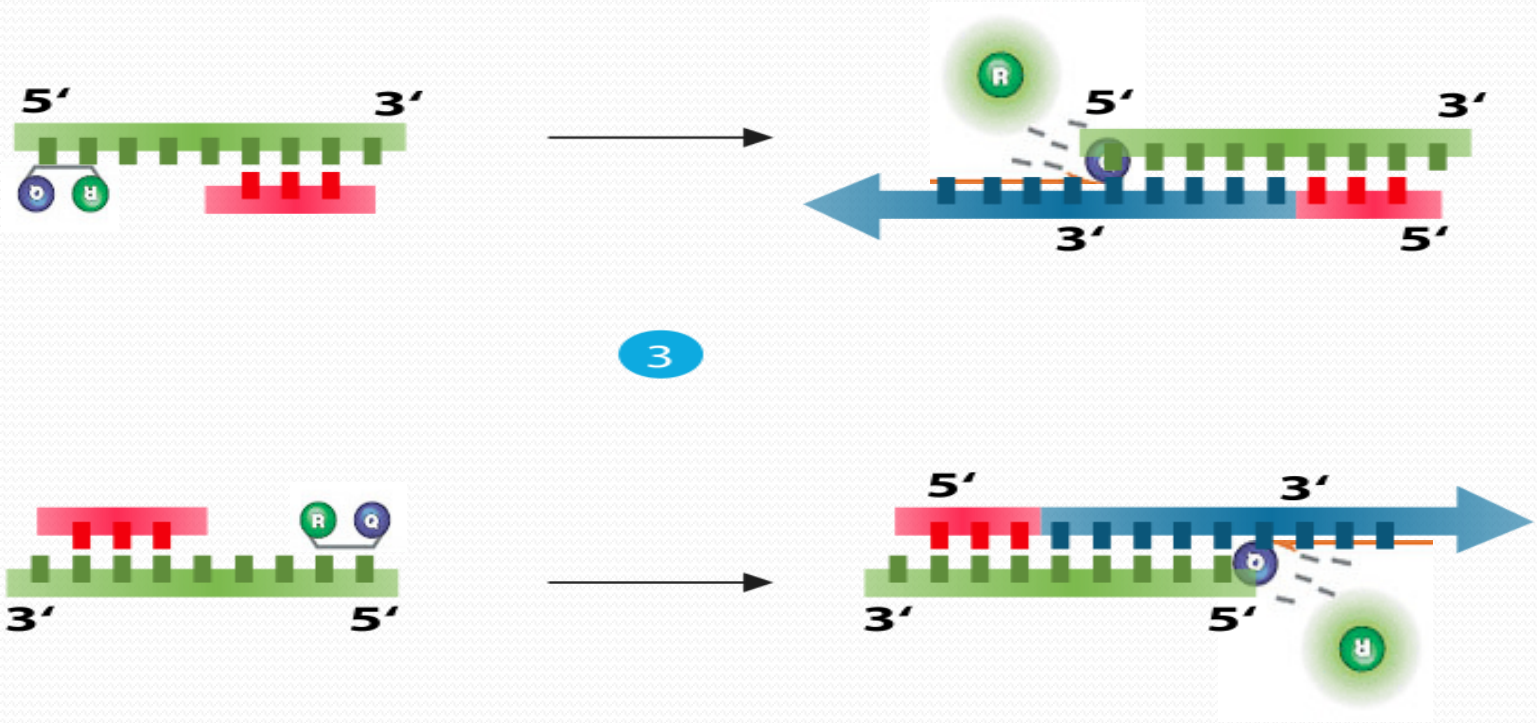
Sample Analysis

- **Step 1- Denaturation**: heat plate to 95°C for 15 seconds



Sample Analysis

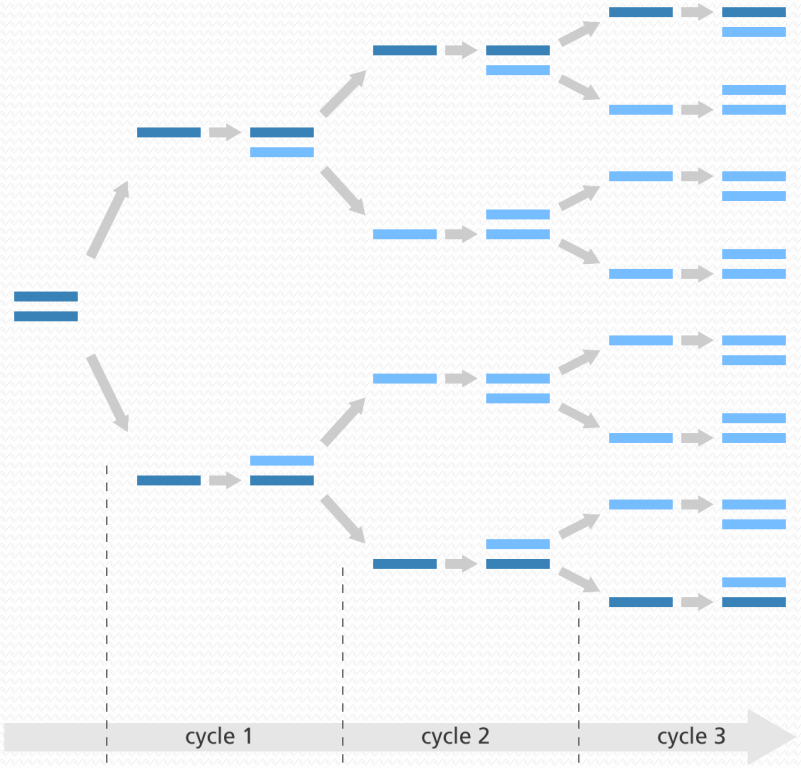
- **Step 2 - Annealing**: cool plate to 65°C



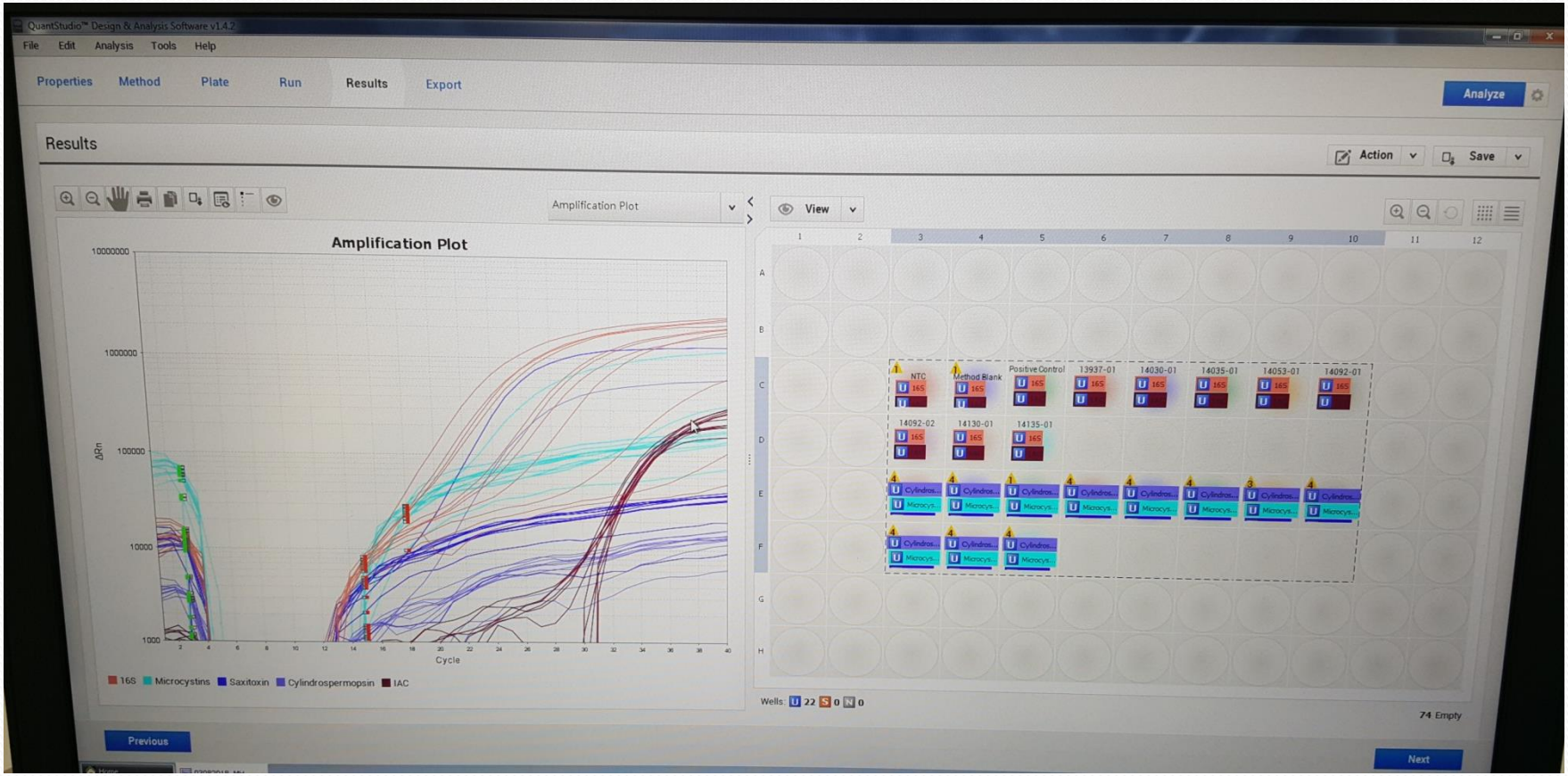
Sample Analysis

- **Step 3: the plate repeats step 1 and 2 40 times**

- **The 40 cycles create an exponential growth of DNA strands**



Sample Analysis



Sample Analysis

https://www.youtube.com/watch?v=fkUDu042xic

Results-16S

- 16S does not distinguish between toxic and non-toxic
- Can be a helpful guide for anticipating a bloom and assessing source water

Results - Microcystins

- Generally you will see Gene Detections prior to microcystin Detections
- During the Ohio EPA paired sampling study they found:
  - 100% of Microcystin detections >1.6 µg/L had mcyE detections
  - 90% of these had detections >5.0 gc/µL
  - 100% of Microcystin detections >5.0 µg/L had mcyE detections >5.0 gc/µL
  - <2% of samples had Micrcystin Detections without mcyE Detections

Results – Saxitoxin and Cylindrospermopsin

- In the Ohio EPA paired study <1% of Saxitoxin detections did not have a paired gene detection
- There were no detections for cylindrospermopsin during the study

Microcystin Analysis

Sample Collection

Cell Lysing

Analysis

Sample Collection

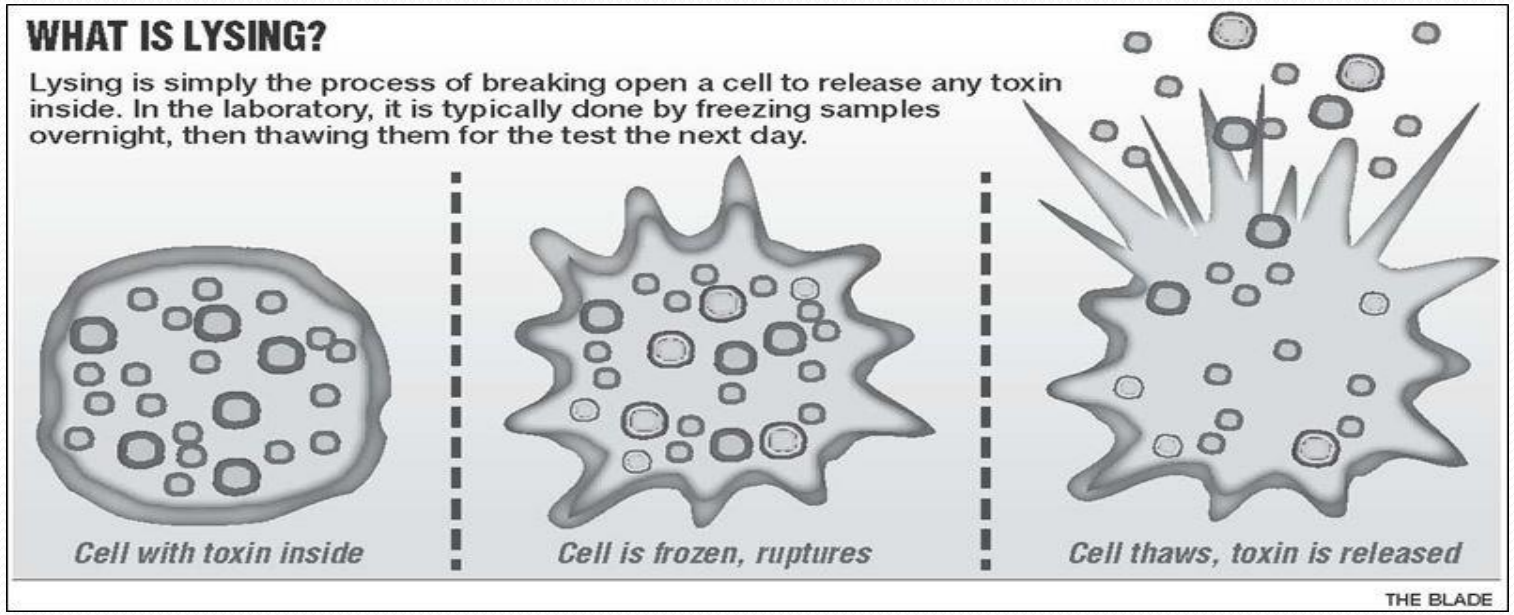
- Samples are collected in 125 mL Polyethylene Terephthalate Glycol (PETG) or glass bottles
- Preserved with Sodium Thiosulfate
- Stored at 0-4°C immediately after collection
- Samples must be analyzed within 5 days

Cell Lysis

- Samples are prepared for analysis
  - Samples are frozen in a dry ice and ethanol mixture
  - Samples are thawed in approximately a 35°C water bath
  - This process is repeated 3 times

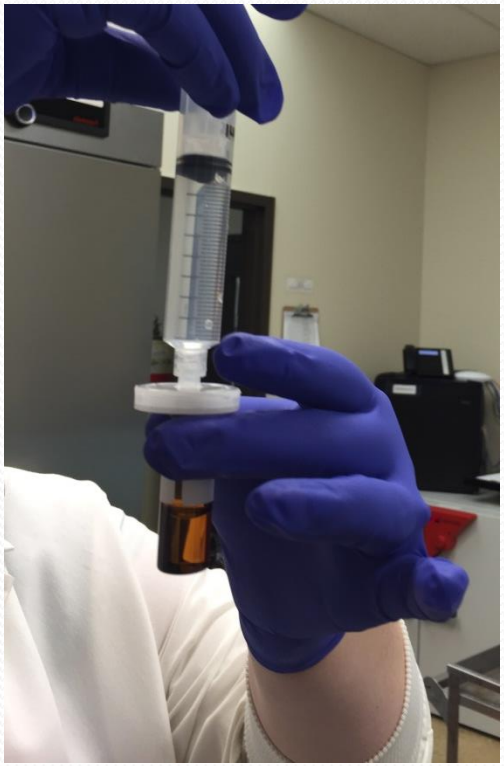
Cell Lysing

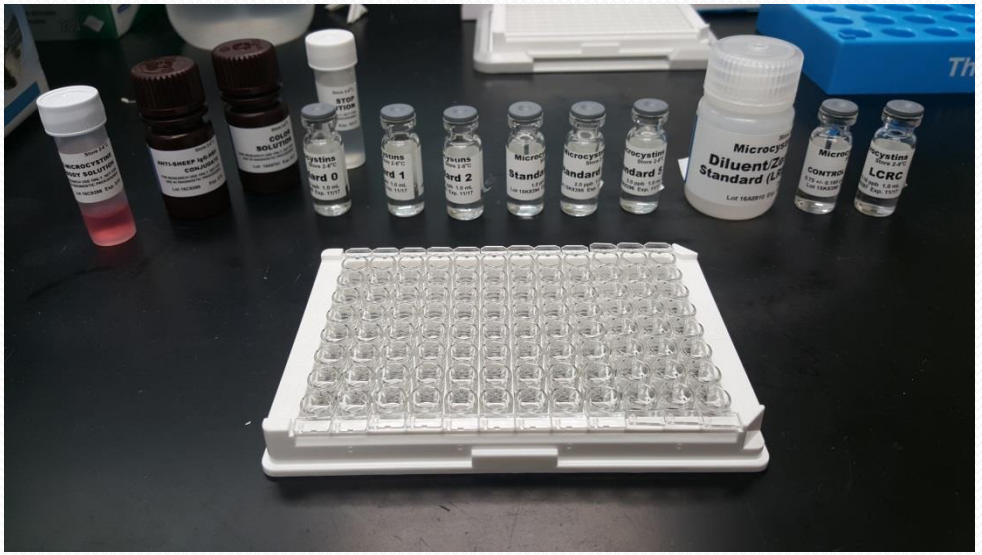
- The freezing and thawing causes cells to lyse
- This is done 3 times to ensure all cells are lysed



Sample Preparation

- Samples are filtered using a 0.45 µm glass fiber filter





- All samples and standards are warmed to room temperature

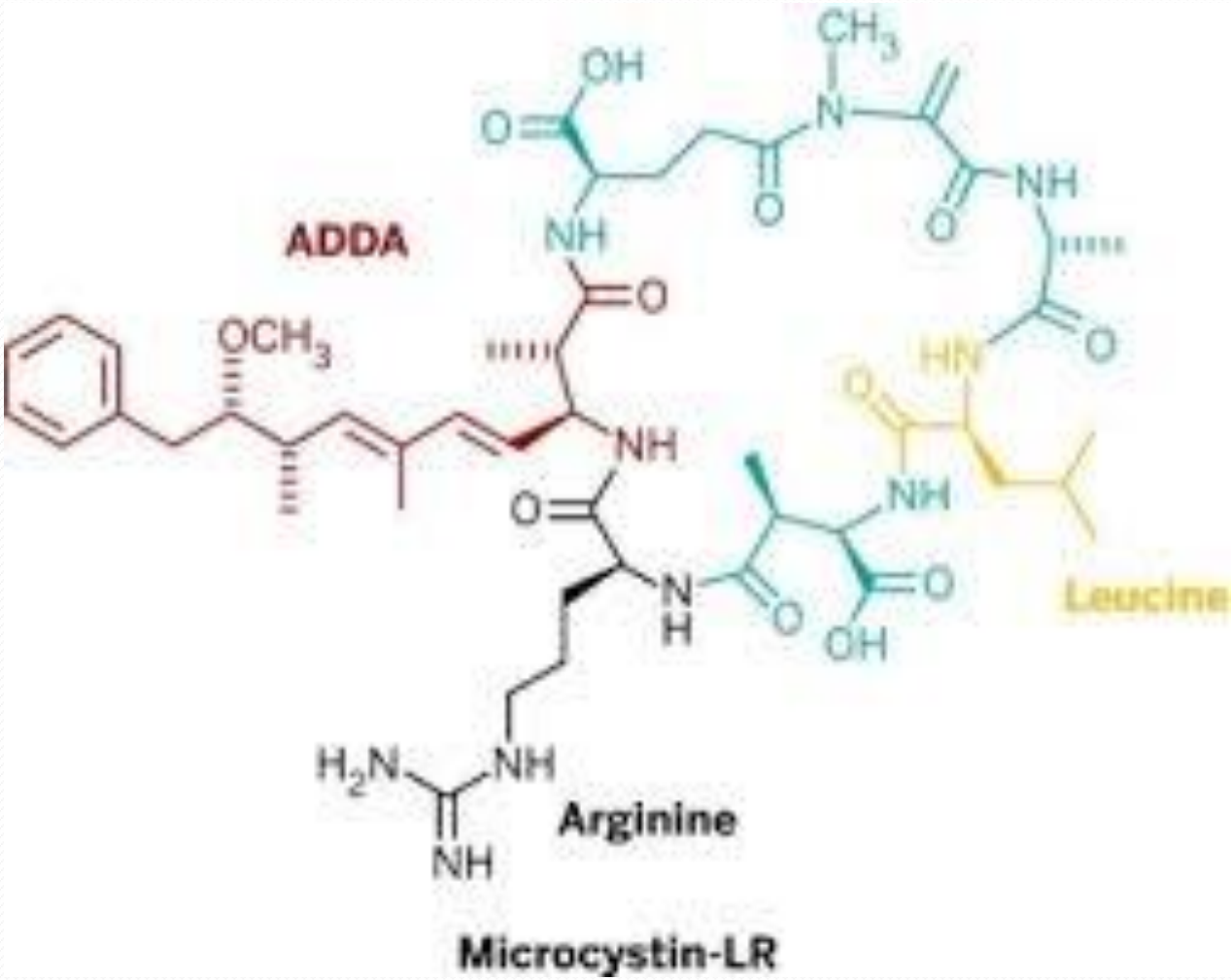
Sample Analysis

Samples and Standards are loaded onto the Cyanotoxin Automated Assay System (CAAS)

or

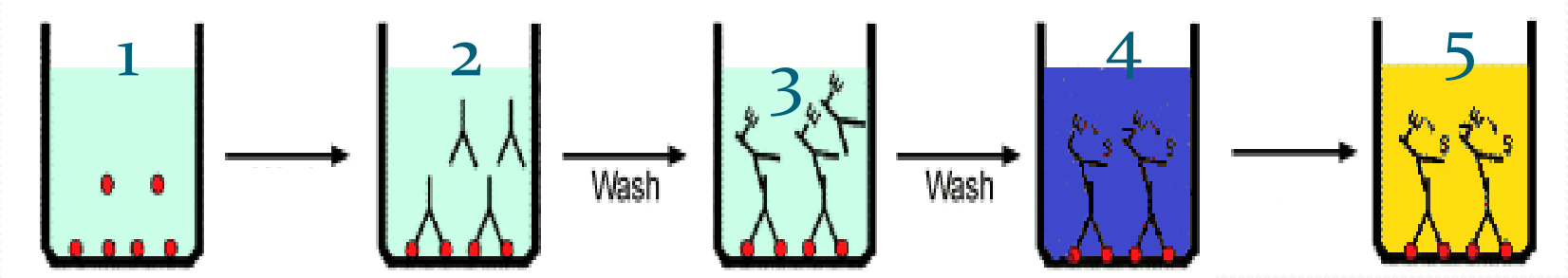
Loaded into a microtiter plate and analyzed with the manual process and reader

Sample Analysis



Sample Analysis

1. Known standards/samples added with antibody solution to each antigen-coated well
2. Toxin and antibodies compete to bind with the antigens
3. Enzyme conjugate added which only binds to the antibodies
4. Substrate added which reacts with the enzyme and turns blue
5. Stop solution added to stop color development and turns blue color to yellow



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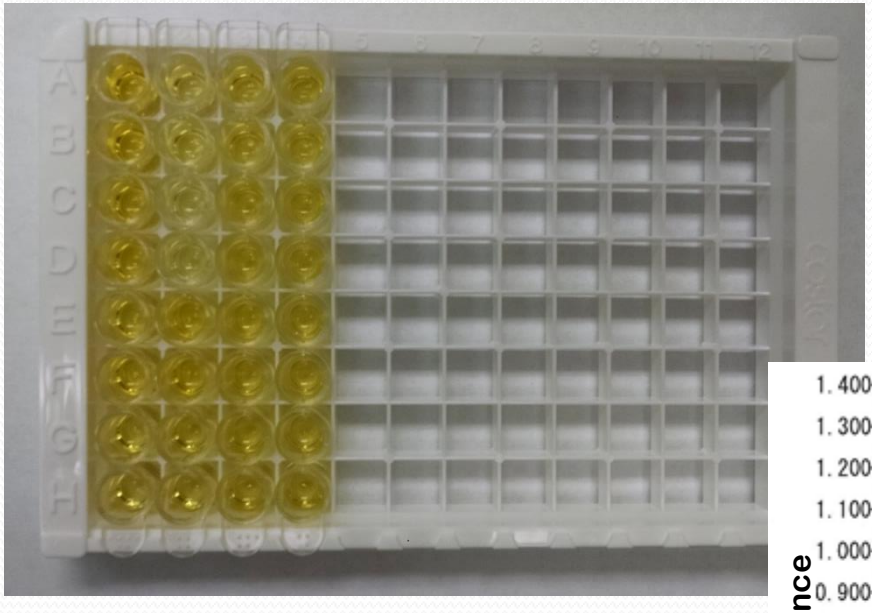

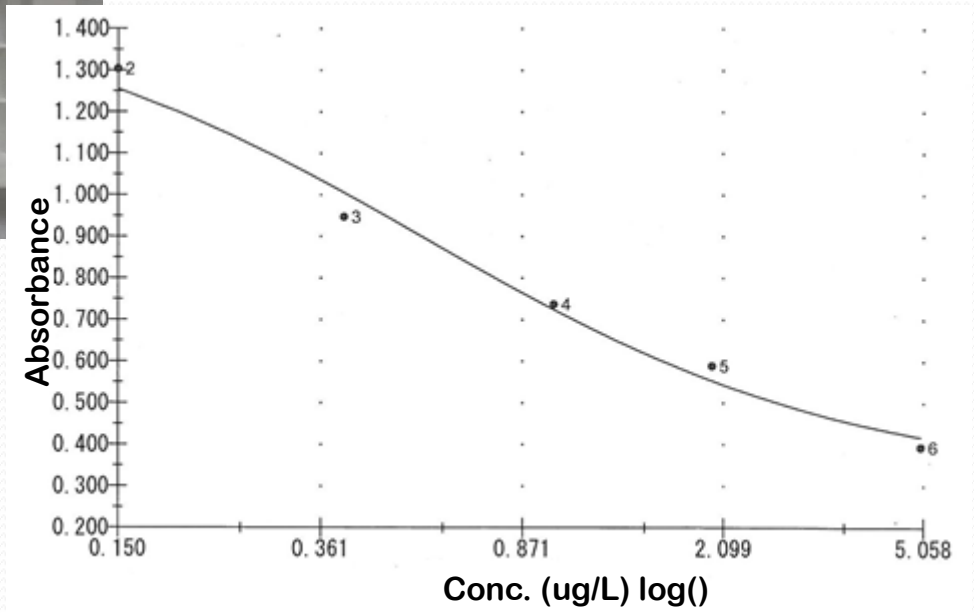

Sample Analysis

Report from the plate reader for total microcystin

We look at the average concentration of the wells and the %CV of the absorbance

Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference
Std1	Microcystins ADDA OH	1.550 Abs	0.002 ug/L		0.000
Std1	Microcystins ADDA OH	1.556 Abs	< 0.000 ug/L		0.000
Std2	Microcystins ADDA OH	1.274 Abs	0.136 ug/L		0.150
Std2	Microcystins ADDA OH	1.256 Abs	0.147 ug/L		0.150
Std3	Microcystins ADDA OH	0.971 Abs	0.406 ug/L		0.400
Std3	Microcystins ADDA OH	0.935 Abs	0.454 ug/L		0.400
Std4	Microcystins ADDA OH	0.699 Abs	0.957 ug/L		1.000
Std4	Microcystins ADDA OH	0.707 Abs	0.932 ug/L		1.000
Std5	Microcystins ADDA OH	0.510 Abs	1.992 ug/L		2.000
Std5	Microcystins ADDA OH	0.505 Abs	2.039 ug/L		2.000
Std6	Microcystins ADDA OH	0.358 Abs	> 5.000 ug/L		5.000
Std6	Microcystins ADDA OH	0.357 Abs	> 5.000 ug/L		5.000
LRB (0.000 - 0.300)	Microcystins ADDA OH	1.664 Abs	< 0.000 ug/L	Out(LR)	
LRB (0.000 - 0.300)	Microcystins ADDA OH	1.634 Abs	< 0.000 ug/L	Out(LR)	
QCS (0.5625 - 0.9375)	Microcystins ADDA OH	0.826 Abs	0.636 ug/L		
QCS (0.5625 - 0.9375)	Microcystins ADDA OH	0.808 Abs	0.673 ug/L		
LCRC (0.240 - 0.560)	Microcystins ADDA OH	1.008 Abs	0.361 ug/L		
LCRC (0.240 - 0.560)	Microcystins ADDA OH	1.078 Abs	0.288 ug/L		
R-17123-01	Microcystins ADDA OH	1.723 Abs	< 0.000 ug/L	Out(LR)	0.300 - 5.000
R-17123-01	Microcystins ADDA OH	1.738 Abs [1.7305] {0.6 CV}	< 0.000 ug/L [< 0.000]	Out(LR) [Out(LR)]	0.300 - 5.000
R-17123-02	Microcystins ADDA OH	1.645 Abs	< 0.000 ug/L	Out(LR)	0.300 - 5.000
R-17123-02	Microcystins ADDA OH	1.704 Abs [1.6745] {2.5 CV}	< 0.000 ug/L [< 0.000]	Out(LR) [Out(LR)]	0.300 - 5.000
R-17123-03	Microcystins ADDA OH	1.650 Abs	< 0.000 ug/L	Out(LR)	0.300 - 5.000
R-17123-03	Microcystins ADDA OH	1.682 Abs [1.6660] {1.4 CV}	< 0.000 ug/L [< 0.000]	Out(LR) [Out(LR)]	0.300 - 5.000
R-17185-01	Microcystins ADDA OH	1.621 Abs	< 0.000 ug/L	Out(LR)	0.300 - 5.000
R-17185-01	Microcystins ADDA OH	1.658 Abs [1.6395] {1.6 CV}	< 0.000 ug/L [< 0.000]	Out(LR) [Out(LR)]	0.300 - 5.000
R-17185-02	Microcystins ADDA OH	1.661 Abs	< 0.000 ug/L	Out(LR)	0.300 - 5.000
R-17185-02	Microcystins ADDA OH	1.647 Abs [1.6540] {0.6 CV}	< 0.000 ug/L [< 0.000]	Out(LR) [Out(LR)]	0.300 - 5.000
R-17185-02 Dup	Microcystins ADDA OH	1.653 Abs	< 0.000 ug/L	Out(LR)	0.300 - 5.000
R-17185-02 Dup	Microcystins ADDA OH	1.654 Abs [1.6535] {0.0 CV}	< 0.000 ug/L [< 0.000]	Out(LR) [Out(LR)]	0.300 - 5.000

Questions?

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