Microcystin Sampling and Analysis

Presented by Rhonda Morris

Microcystins

Cyanobacteria

- Bacteria that photosynthesize like algae
  - Commonly called blue-green algae
- Multiply rapidly to form blooms in late summer and early fall under optimal conditions
  - Adequate amounts of phosphorus, nitrogen, and organic matter
  - Temperatures between 5 - 30°C
  - pH between 6 - 9

Cyanotoxins

- Some genera of cyanobacteria are able to produce toxins that may be harmful to humans, domestic animals, and wildlife
- Most toxins are stored within cells and are only released into water when cell membranes rupture (cell lysis) or upon cell death
- Once released, toxins can be stable in water for several weeks
- Toxins may be present in water even if cyanobacteria are not visible

Cyanotoxins

- Routes of human exposure
  - Drinking contaminated water
  - Inhalation and dermal contact during bathing, showering, and recreational use
  - Ingesting contaminated food

Cyanotoxins

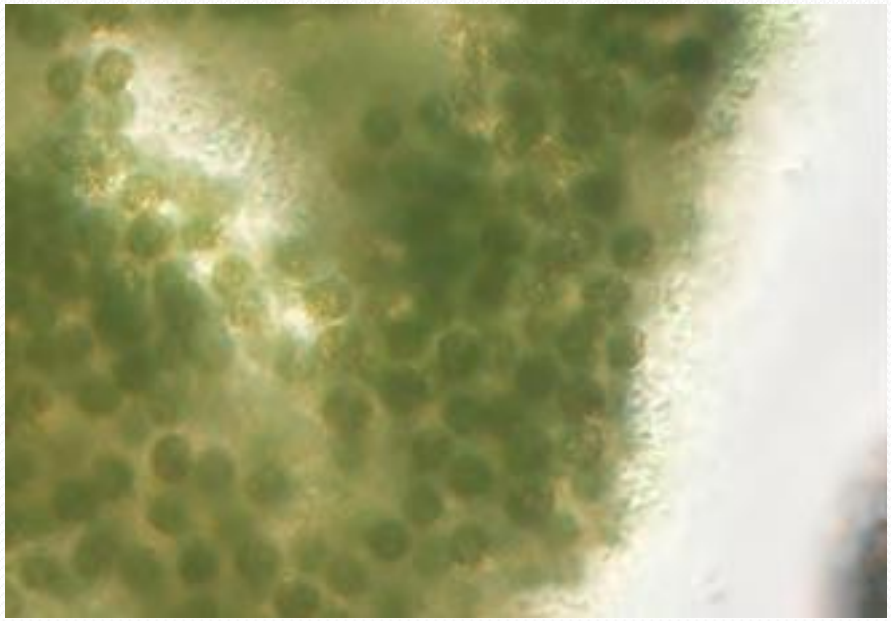
- Different classes of cyanotoxins
  - Dermatotoxins
  - Hepatotoxins
  - Neurotoxins
- Type and toxicity of cyanotoxins produced are dependent on the types of cyanobacteria present and can vary over time or over distances within the same body of water

Cyanotoxin	Type	Symptoms	Cyanobacteria
Lyngbyatoxin	Dermatotoxin	Skin redness Skin burning Skin itching Skin blistering	Lynbya
Cylindrospermopsin	Hepatotoxin	Abdominal pain Vomiting Diarrhea Liver inflammation & hemorrhage Acute pneumonia Acute dermatitis Kidney damage	*Anabaena* *Aphanizomenon* *Cylindrospermopsis* *Lyngbya* *Umezakia*
Microcystin	Hepatotoxin		*Anabaena* *Anabaenopsis* *Microcystis* *Nostoc* *Oscillatoria*

Cyanotoxin	Type	Symptoms	Cyanobacteria
Anatoxin-a	Neurotoxin	Tingling sensation Burning sensation Numbness Drowsiness Incoherent speech Respiratory paralysis leading to death	*Anabaena* *Aphanizomenon* *Oscillatoria*
Anatoxin-a (s)	Neurotoxin		*Anabaena*
Saxitoxin	Neurotoxin		*Anabaena* *Aphanizomenon* *Cylindrospermopsis* *Lyngbya* *Oscillatoria*

Microcystins

- Most common cyanotoxin found in surface waters worldwide
- Over 90 different congeners
  - Microcystin-LR one of the most toxic and widely studied



Microcystins

- Harmful algal blooms have been increasing in severity and frequency in recent years raising concerns over drinking water safety
- EPA health advisory levels for total microcystins
  - 0.3 µg/L for children < 6 years old and susceptible groups (pregnant & nursing women, the elderly, and those who are immune-compromised or receiving dialysis treatment)
  - 1.6 µg/L for children > 6 years old to adults

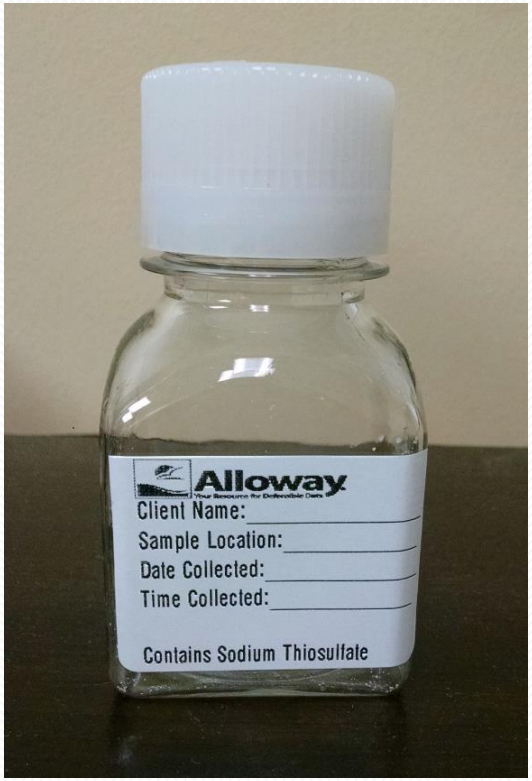
Microcystins

- Increased microcystin monitoring
  - HAB Rule – Senate Bill 1/OAC 3745.50
    - Monitoring requirements for total microcystins
  - UCMR4
    - Monitoring requirements for total microcystins, 6 individual congeners of microcystin, nodularians, anatoxin-a, and cylindrospermopsin

Sample Collection

Sample Collection

- Sample bottles must be glass or PETG
- Samples must be quenched with sodium thiosulfate to remove any residual chlorine
- Samples must be placed on ice immediately after collection and during shipment



Sample Collection

- Avoid direct contact with the sample
  - Wear gloves and safety glasses
  - Wash hands after sample collection or handling
- Collect at least 100 mL of sample
- Clean any sampling equipment with DI water in between sampling locations and when finished

Sample Collection

- The COC must include the following information in order for results to be reported to OEPA on time
  - PWS name and ID #
  - Facility State Code (STUID #)
  - Sample point ID
  - Sample collection date and time
  - Sample type (routine, resample, or repeat)

Ohio Environmental Protection Agency

Division of Drinking and Ground Waters

Central Office
50 W Town St
Columbus Ohio 43215
(614) 644-2752 FAX (614) 644-2909

CHEMICAL SAMPLE SUBMISSION REPORT (SSR)

PUBLIC WATER SYSTEM INFORMATION:

PWS ID: OH _____

PWS Name:_____

Facility Code:_____

Facility Name:_____

Address:_____

City, State, Zip:_____

County:_____

LABORATORY INFORMATION:

Reporting Lab Name:_____

Reporting Lab Certification No.:_____

Lab Receipt Date:_____

Data Quality Results:

Analysis: ☐ --Accepted ☐ -- Rejected

☐ --Invalid Sampling Point ☐ --Broken
☐ --Exceeds Holding Time ☐ --Chlorine Present
☐ --Excessive Head Space ☐ --Frozen Sample
☐ --Lab Accident ☐ --Leaked in Transit
☐ --Insufficient Sample Information
☐ --Invalid Sampling Protocol

SAMPLE INFORMATION:

Lab Sample Number:_____

Sample Monitoring Point_____

Sample Type:

☐ -- Routine (compliance)
☐ -- Special (non-compliance)

Sample Collection Date:_____

Sample Collection Time:_____

Street Address and Tap Location:_____

_____

_____

Lead/Copper Location Type:

☐ -At Source ☐ --Flushed
☐ -First Draw ☐ --Lead Service Line

Comments

Sample Results:

Analyte	Analyte Code	Method Code	Results Sign	Results Value	Results Units	Analytical Lab ID#	Analyst #	Analysis Date	QC Date
Microcystin Total		701.0							

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_____
_____

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Comments

Resample

For compliance samples, either check that it is a routine sample or indicate that it is a repeat or resample

Sample Results:

Analyte	Analyte Code	Method Code	Results Sign	Results Value	Results Units	Analytical Lab ID#	Analyst #	Analysis Date	QC Date
Microcystin Total		701.0							

Sample Receipt

- Upon receipt at the laboratory, the following checks will be performed:
  - Temperature – must be 0-4°C
  - Ice – solid ice must still be present or ice packs must still be frozen
  - pH – must be 5-11
  - Residual chlorine – must be <0.1 mg/L

Sample Receipt

- Sample temperature may be above 4°C only if samples were collected the same day they are received and if ice is present in the cooler

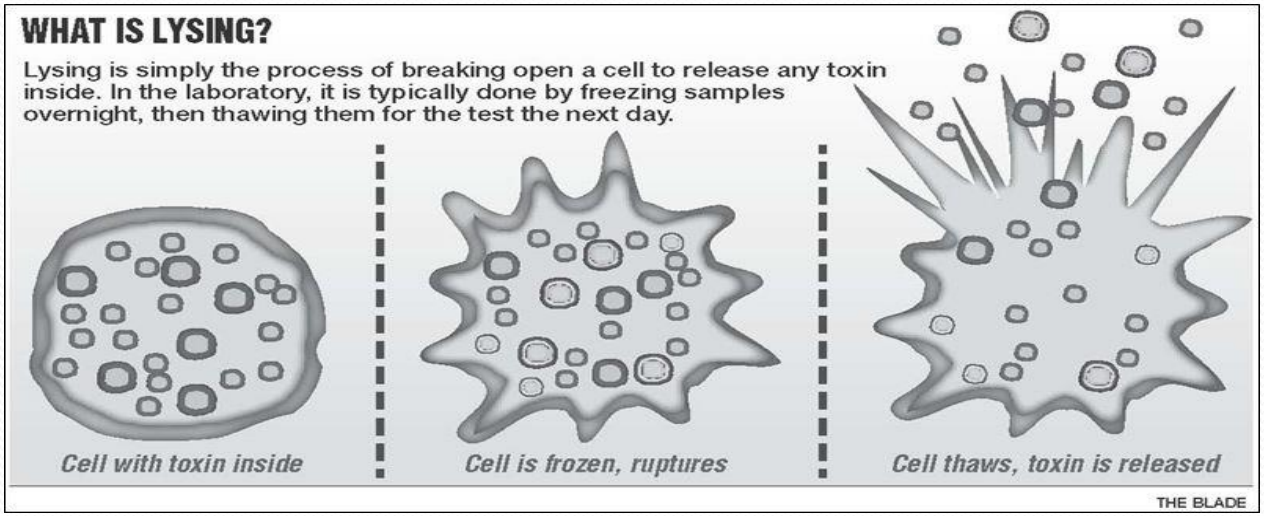
Sample Receipt

- If sample pH is not 5-11, it can be adjusted upon receipt by adding HCl or NaOH
- If sample residual Cl2 is >0.1 mg/L, it can be adjusted upon receipt by adding additional sodium thiosulfate
  - Beginning June 1, 2016, any sample with residual Cl2 >0.1 mg/L will be considered invalid and will require resampling

Sample Analysis

Sample Analysis

- Microcystins can be measured as free or total
  - Free = extracellular
  - Total = intracellular + extracellular
- For total microcystins, cells must be lysed prior to analysis



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Analytical Methods

- Quantitative Polymerase Chain Reaction (qPCR)
- Enzyme-Linked Immunosorbent Assays (ELISA)
- Liquid Chromatography with Tandem Mass Spectrophotometry (LC-MS/MS)

qPCR

- Currently no EPA certification program in place
  - All compliance samples must be analyzed by OEPA Division of Environmental Services
- Identifies and quantifies the genes responsible for microcystin production
  - Results reported in number of gene copies per volume of sample (copies/mL)
- Assumes a correlation between number of gene copies and concentration of microcystins

qPCR – Sample Prep

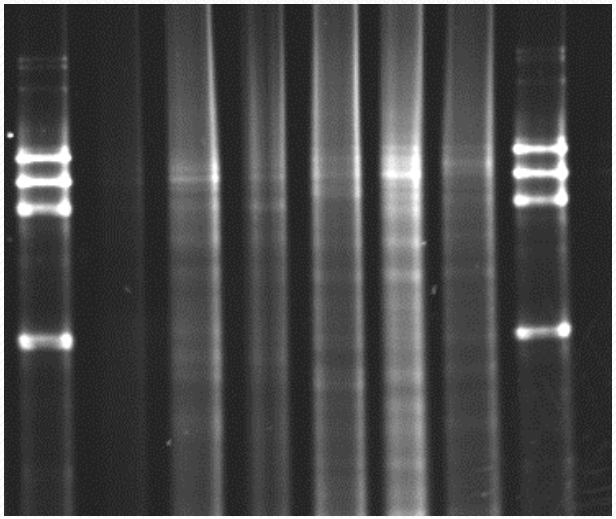
- Cells lysed by adding microbeads to the sample and placing it in a vortex mixer
- Sample centrifuged to remove any particulates
- DNA extracted from the sample
- Reference cultures used to generate standard curves and act as controls

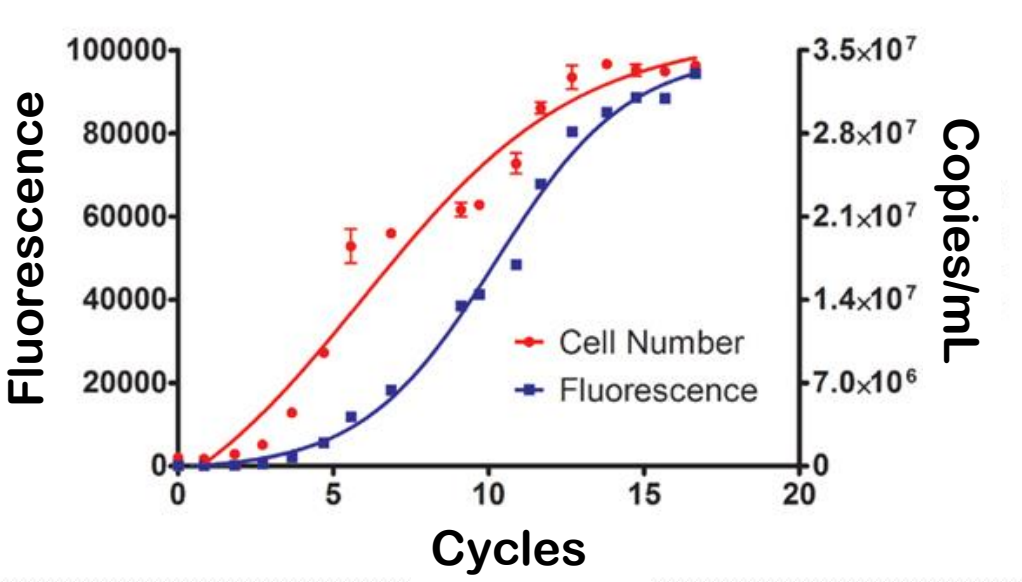
qPCR - Analysis

- Certain genetic sequences are specific to different toxic cyanobacteria
- Those sequences are amplified to be better identified and quantitated
  - Heat is applied separating DNA into 2 separate strands
  - Enzyme is added which builds a copy of the strands using the original strand as a template
- Amplification process is repeated multiple times

qPCR - Analysis

- Copies are measured after each round of amplification using a fluorescent signal
  - Fluorescence is positively correlated to the number of gene copies present





qPCR

Pros

- More sensitive and accurate than identifying and counting cells by microscope
- Able to detect and quantify multiple toxic genotypes
- Able to detect the presence of toxic cyanobacteria even if toxins are absent

Cons

- Cannot determine if toxins are present or quantitate the amount of toxins present

ELISA

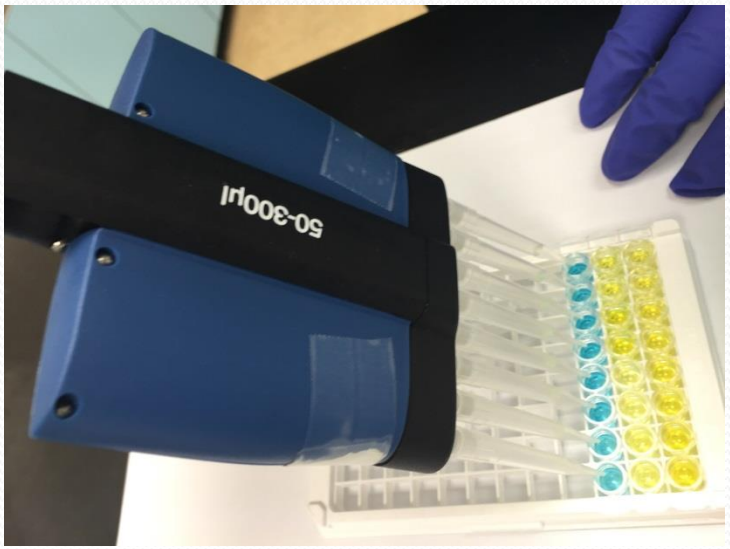
- Ohio EPA Total (Extracellular and Intracellular) Microcystins – ADDA by ELISA Analytical Methodology
- Laboratory approval possible with certification beginning in 2017
- Determines the total concentration of microcystins present
  - Reported as µg/L

ELISA – Sample Prep

- Samples must be stored at 0-4°C and must be analyzed within 5 days of collection
- Cells lysed with 3 separate freeze/thaw cycles
- Lysed sample is filtered to remove particulates

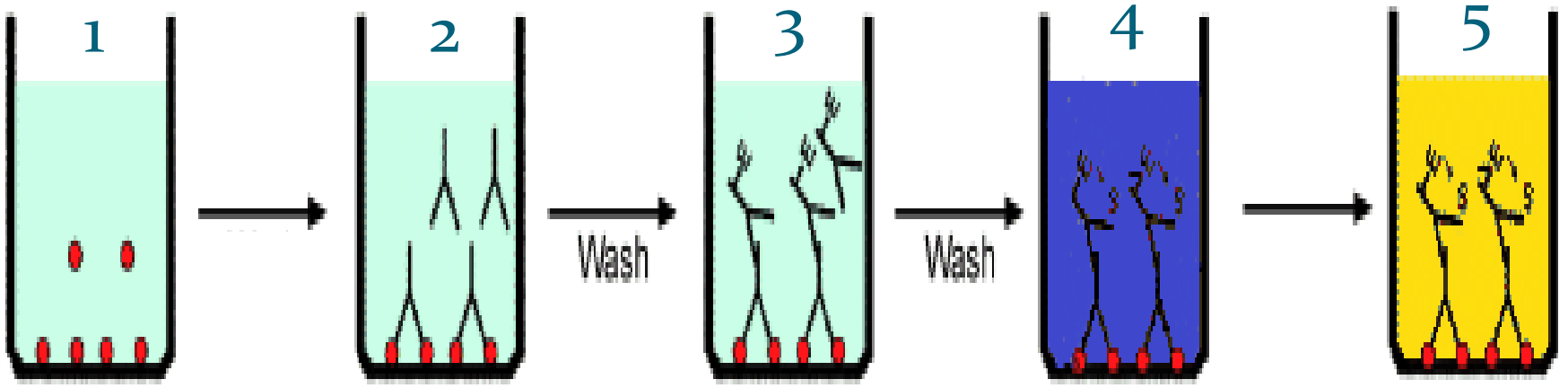
ELISA – Sample Analysis

- Microtiter plate wells coated with antigens that only recognize and bind to the specific cyanotoxins being analyzed
  - Abraxis Microcystin–ADDA kit required for analyzing total microcystins
- Detection level of 0.15 µg/L



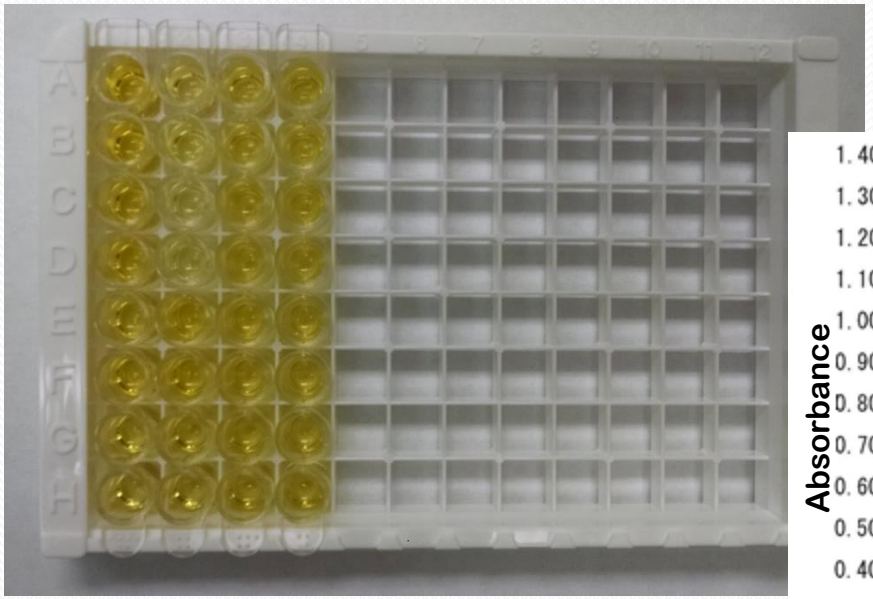
ELISA – Sample Analysis

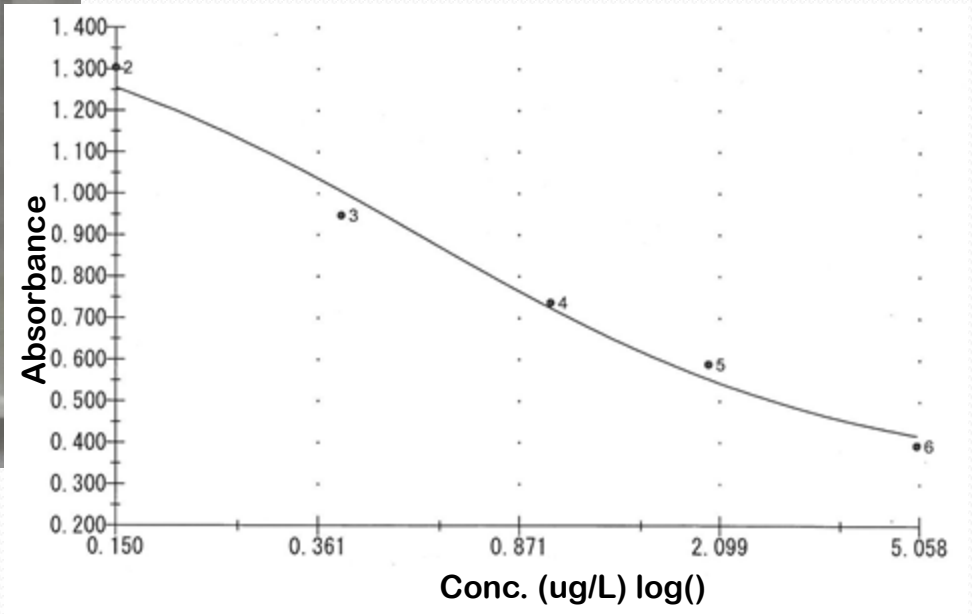
1. Known standards/samples added with antibody solution to each antigen-coated well
2. Toxin and antibodies compete to bind with the antigens
3. Enzyme conjugate added which only binds to the antibodies
4. Substrate added which reacts with the enzyme and turns blue
5. Stop solution added to stop color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



ELISA – Sample Analysis

- Final color intensity is inversely related to the concentration of total microcystins





ELISA

Pros

- Able to quantify all microcystin congeners
- Relatively inexpensive and easy to use
- Rapid analysis

Cons

- Kits are specific to the type of toxin being analyzed
- Highly sensitive to human error
- Cannot distinguish between different congeners

ELISA

- Cyanotoxin Automated Assay System (CASS)

LC-MS/MS

- EPA Method 544
- Not currently an accepted method for analyzing compliance samples for total microcystins
  - Commonly used to confirm detections from ELISA method
  - Required method for UCMR4
- Detection levels down to 0.0012 to 0.0046 µg/L

LC-MS/MS – Sample Collection

- 500 mL amber glass bottles with teflon-lined caps
- Preservation
  - Trizma – buffering reagent agent
  - 2-Chloroacetamide – antimicrobial
  - Ascorbic acid – dechlorinating agent
  - Ethylenediaminetetraacetic acid trisodium salt – inhibits binding of targets to metal
- Samples must be chilled during shipment and must be ≤ 10°C at time of receipt

LC-MS/MS – Sample Prep

- Samples must be stored at ≤ 6°C until extraction and at ≤ -4°C once extracted
- Samples must be extracted within 28 days of collection and analyzed within 28 days after extraction

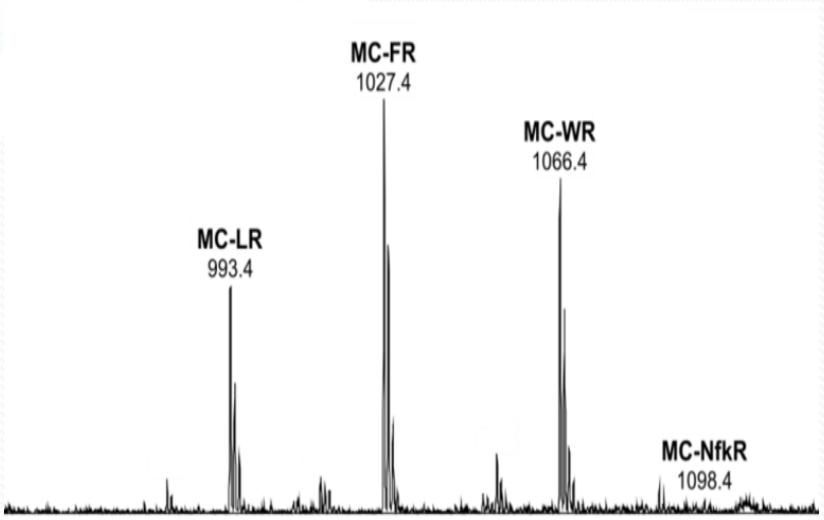
LC-MS/MS – Sample Prep

- Sample filtered
  - Filtrate contains extracellular microcystins
  - Intracellular microcystins extracted off the filter and added back to the filtrate
- Sample extracted using solid phase extraction

LC-MS/MS – Sample Analysis

- Liquid Chromatography
  - Different toxins are separated out along a column based on how well they “stick” to the column
- Mass Spectrophotometry
  - Ions move off the column into the mass detector which separates all ions that have different masses
  - Ions are then fragmented and separated out with the second mass detector

LC-MS/MS – Sample Analysis



LC-MS/MS

Pros

- Low detection levels
- Able to quantify multiple congeners of different cyanotoxins simultaneously
- Minimal matrix interference

Cons

- Requires advanced instrumentation
- Standards only available for a limited number of known congeners

Method Summary

Method	Measures	Units	Approval	Lab Approval
qPCR	Toxin-producing genes	copies/mL	HAB Rule	No
ELISA	Toxins	µg/L	HAB Rule UCMR4	Yes
LC-MS/MS	Toxins	µg/L	UCMR4	Yes

Questions?