Cyanotoxin ELISA Testing in Delaware

Process Development by the State of Delaware Environmental Laboratory

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Why Develop Cyanotoxin ELISA Testing Capability?

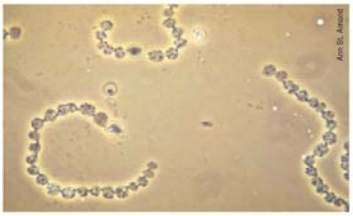
- Quicker turn around time for sample analysis
- Reduced analysis cost
- Facilitate timely management decisions impacting recreational waters

Microcystins Test Elements:

- Measurable Results Range: 0.15 - 5.00 ppb
  Higher concentrations require dilution of the Sample with the ELISA Plate Kit Diluent.

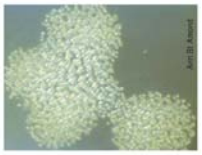
- Method Detection Limit (MDL) of 0.10 ppb
  (*manufacturer's specification*)
- Limit of Quantitation (LOQ) of 0.15 ppb
  (*0.15 ppb is the lowest ELISA Kit Standard*)
- Precision, as determined by 4 replicates within an Analysis Run, is 9.3%
  (*acceptable Test Precision of < 20%*)

Six Major Process Elements

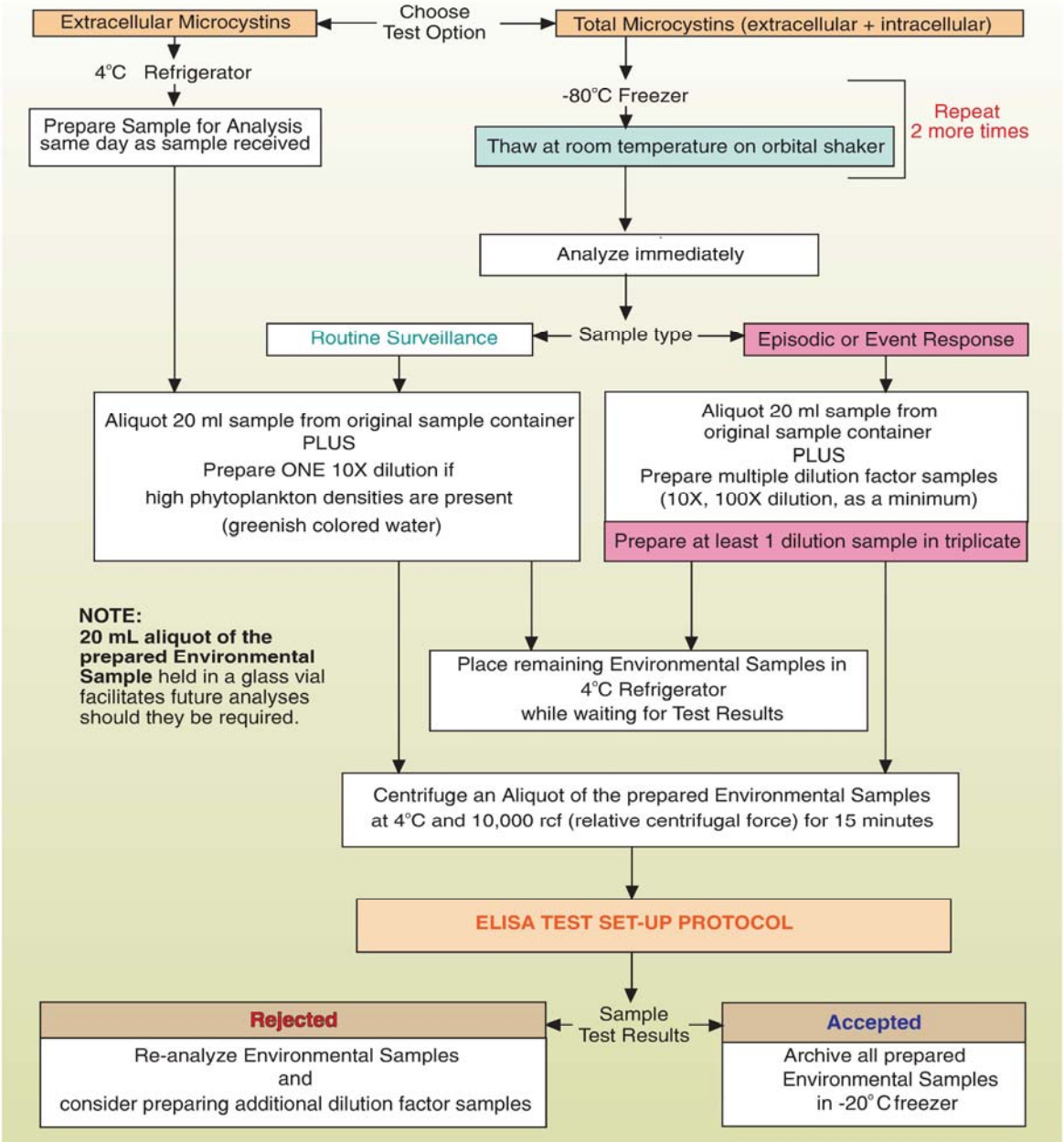


1. Sample Collection/Preservation Requirements:

- 500 mL amber glass bottle
- Transport sample on ICE



2. Sample Preparation for the determination of Total Microcystins requires a method to release the cell-bound toxin. In 2007, Delaware followed a freeze/thaw method followed by centrifugation, which reduced the interference by cellular debris. In 2008, Delaware will be testing a lysing agent (QuikLyse), which should eliminate the freeze/thaw step of the sample preparation.



3. ELISA Test Set-Up Requirements and Special Acceptance Criteria:

- Sample concentrations are determined from absorbance readings compared to the Standard Curve.
- There should be a laboratory duplicate for every 10 Environmental Samples. Increase the number of replicates when there is a presence of blue-green algae scum or elevated microscopic cell counts which may be indicative of a potential human health or environmental risk.

CV% for Concentrations of Sample Replicates ≤ 20% ► NO ► **Accept, but Qualify Sample Result and report Sample CV%**

▼ YES

▼ **Accept**

- There should be 1 Method Blank per Analysis Run.
  (*Method Blank is equivalent to 0 ppb Standard, which is supplied with the Microcystin ADDA Microtiter Plate Kit*)
- There should be a minimum of 1 Matrix Spike per waterbody in each Analysis Run. A Spike concentration of 1 ppb is preferred as this concentration level is in the most stable portion of the 4 parameter Standard Curve.

Matrix Spike Recovery ± 20% of Theoretical Value
(*Spike Concentration + Unspiked Sample Concentration*) ► NO ▼ **If within ± 50 %, report Sample Result as biased low or high.**
▲ **If outside ± 50 %, reject Sample Result**

▼ YES

▼ **Accept**

4. Microcystin ELISA Plate Kit Analysis

The Kit is supplied with the necessary Microcystin Standards and Control solutions, process reagents, and prepared Microtiter Plate. All test components, including the Environmental Sample, must be at room temperature. Good pipetting techniques are vital to ensure accurate results for this test. A simple sequence of steps, depicted in the flowchart, makes this test easy to complete.

Verify that the sample type and sequence record in the software program of the Plate Reader coincides with the sample type dispensed into the wells of the Microtiter Plate: duplicate each
Plate sequence: 6 Standards (0 through 5 ppb), Control (0.75 ppb) followed by Environmental Samples

▼

Add 50 uL of Standards, Control, and Samples to each respective well
(Vortex Environmental Samples for 15 seconds prior to removing aliquot)

▼

Immediately add 50 uL of **Antibody Solution** to each well

▼

Cover top of Microtiter Plate with Parafilm©
Rapidly circulate plate for 30 seconds on bench top
Cover top of Microtiter Plate with foil, and incubate for **90 minutes at room temperature**

▼

Remove foil and Parafilm©
Shake out solution in wells into sink - Invert Plate on paper towels, gently pat to remove excess solution
Wash wells 3 times with **1 X Washing Buffer Solution** (minimum of 250 uL per well)
Following each Wash - remove excess buffer in wells by patting wells up side down on paper towels

▼

Add 100 uL of **Enzyme Conjugate Solution** to each well

▼

Cover top of Microtiter Plate with new Parafilm©
Rapidly circulate plate for 30 seconds on bench top
Cover top of Microtiter Plate with foil, and incubate for **30 minutes at room temperature**

▼

Remove foil and Parafilm© and follow the wash procedure above.

▼

Add 100 uL of **Color (Substrate) Solution** to each well

▼

Volume accuracy critical

Cover top of Microtiter Plate with **new** Parafilm©
Cover top of Microtiter Plate with foil and incubate for **30 minutes at room temperature**

▼

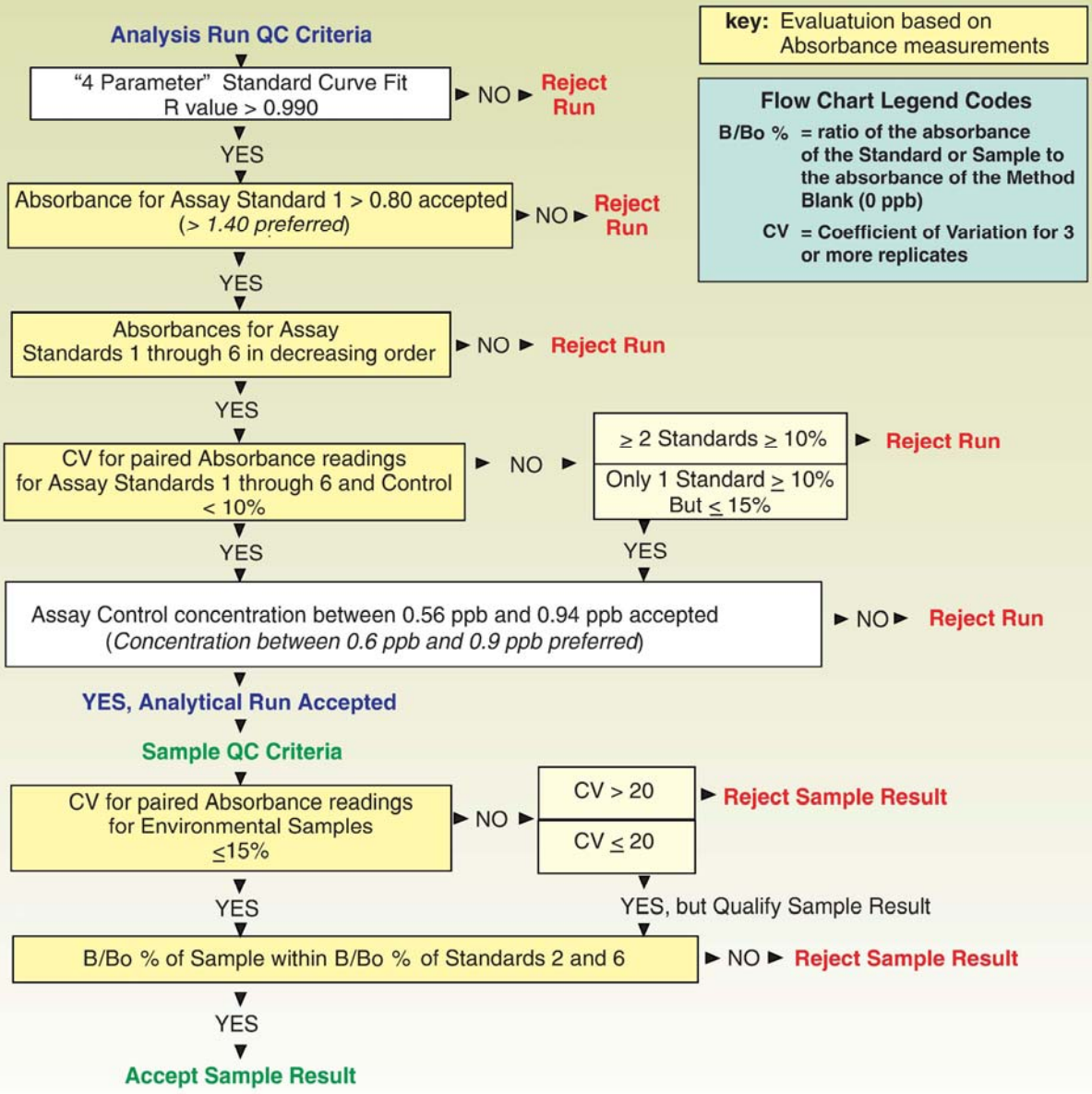
Add 50 uL of **Stop Solution** to each well → *Record Time*

▼

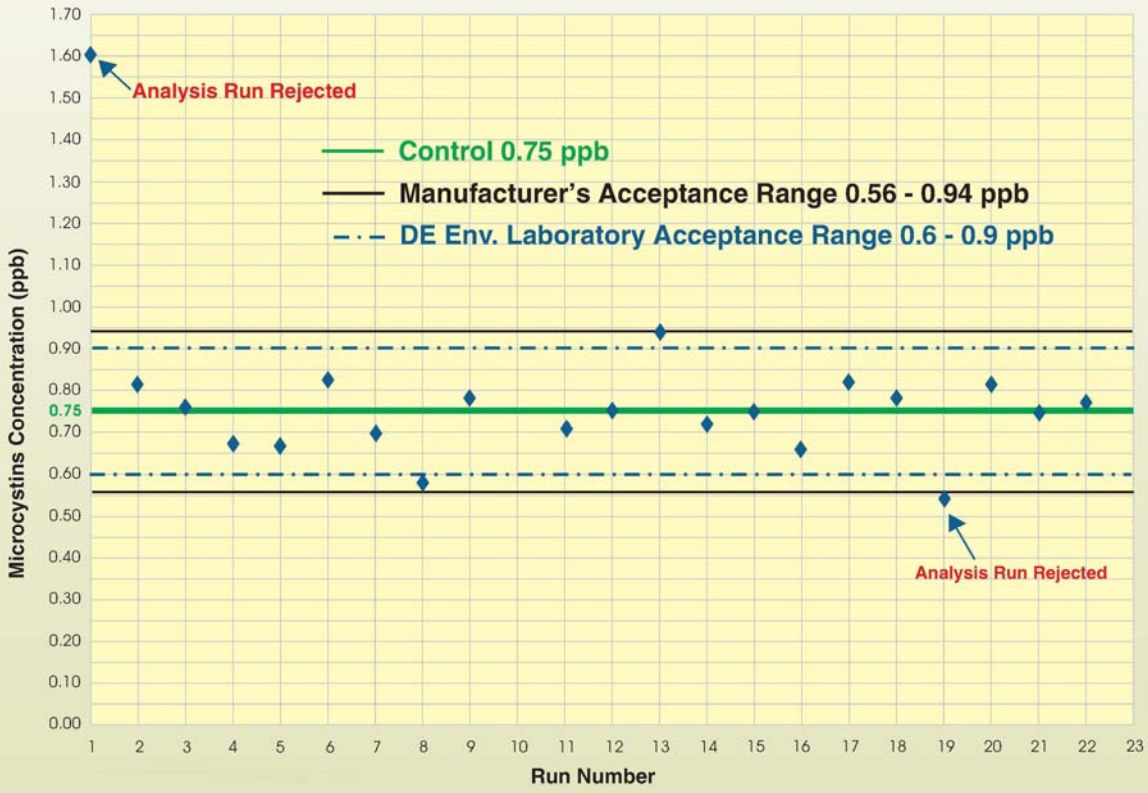
Place Microtiter Plate in Plate Reader
Read Absorbance (450 nm) within 30 minutes (*preferably 15*) from Stop Solution addition

5. Microcystins Test QC Criteria

Evaluation of discrete test parameters is critical for the determination of whether the Analysis Run is acceptable and the Sample results are accurate and repeatable. Review and evaluation of Quality Control measures are depicted in the flowchart.



6. Run Chart for Microcystins Kit Control The Performance Measures of Precision and Accuracy are monitored repeatedly as part of the State Laboratory Quality Assurance Program. Based on 9 Plate Kits representing 4 lot numbers, the Coefficient of Variation amongst the 19 measured Control concentrations was 10.6%.



Acknowledgements

Cyanotoxin Kits (Microcystin ADDA and Cylindrospermopsin) and QuikLyse from Abraxis LLC, Warminster, PA
Microcystin ADDA Kit measures Microcystin congeners, including LR, and Nodularins.

Centrifugation step, personal communication: Keith Loftin, USGS, Kansas Water Science Center

Christy Shaffer, Delaware DNREC

Photographs

Ann St. Amond, Phycotech Inc. Michigan; Ben Pressly, Delaware DNREC; Robin Tyler, Delaware DNREC;
J. Scott Figurski, Figurski Photography, Delaware

* Laboratory protocols and quality assurance/quality control measures beyond those recommended by the kit manufacturer were established in order to obtain maximum confidence in the Enzyme-Linked Immunosorbent Assay (ELISA) test results. The mention of trade names or commercial products is required for this test as the test is based on the use of only the commercial products mentioned. Testing for Cylindrospermopsin follows a similar process.

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