



Determination of Cyanobacteria Toxins in Ambient and Drinking Water by ELISA

S-001-OWQ-WAP-TGM-21-T-R4

Technical Standard Operating Procedure (TSOP)

Office: Office of Water Quality

Branch: Watershed Assessment and Planning Branch

Section: Targeted Monitoring Section

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Purpose (of the TSOP)

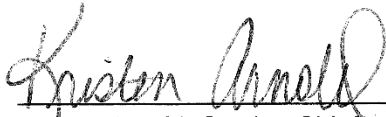
This technical standard operating procedure (TSOP) ensures sample preparation and analysis, using the enzyme-linked immunosorbent assay (ELISA) kits for microcystins, cylindrospermopsin, anatoxin-a, saxitoxin, and β -N-methylamino-L-alanine (BMAA), are conducted in a consistent manner to yield high quality, reproducible, and reportable data.

This TSOP should be used by:

This TSOP applies to agency staff located in the Office of Water Quality (OWQ), Watershed Assessment and Planning Branch (WAPB) responsible for preparing and analyzing cyanobacteria toxins in samples collected from surface and drinking water as part of the agency's ambient and drinking water quality monitoring programs.

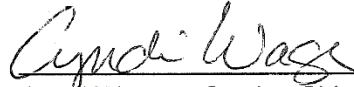
Authorizing Signatures

I approve and authorize this technical standard operating procedure:



Kristen Arnold, Section Chief
OWQ Technical and Logistical Services Section

6/29/2021
Date



Cyndi Wagner, Section Chief
OWQ Targeted Monitoring Section

6/29/2021
Date



Marylou Renshaw, Branch Chief
OWQ Watershed Assessment and Planning Branch

6/29/2021
Date

This technical standard operating procedure is consistent with agency requirements.



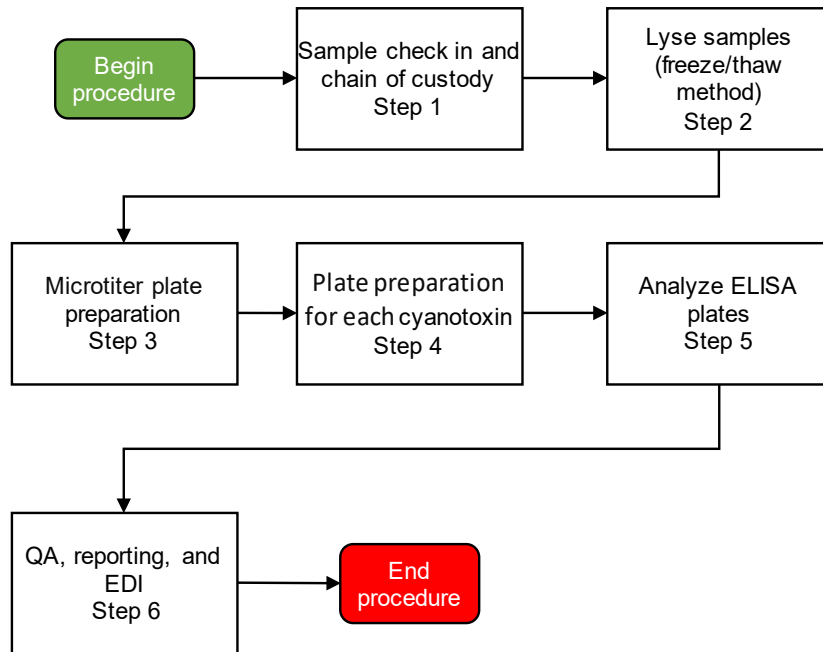
Quality Assurance Staff
Office of Program Support

30 June 2021
Date

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1.0. Overview Flowchart



2.0. Procedures

2.1. Procedural Flowchart:

Since a procedural flowchart for the whole process would be complicated and too large, each step in Section 2.2 contains a procedural flowchart for the step followed by the detailed written steps.

2.2. Procedural Steps:

Step 1. Transferring Samples and Completing the OWQ Chain of Custody (COC) Form (Appendix 9.1.)

1. A laboratory staff (LS) verifies the sample collector (SC) signed the COC form. Thus, certifies the listed samples were collected by or in the presence of the SC. The LS also verifies the date the samples were submitted to the lab and the sample type (e.g., algae). As a good practice, ask the SC to print his name next to the signature for signature verification.
2. LS verifies each sample is recorded on the field COC form. LS ensures the event identification number (ID) and the IDEM sample numbers on the COC form match the event ID and the IDEM sample numbers on the sample vials.

3. LS ensures the correct number of vials is listed in the container column on the COC form. (Note: Ambient samples are collected in three 40-mL vials. The drinking water program uses both 40mL and 60 mL vials). Then the LS ensures the correct type of vial (i.e., amber, or clear) and preservative (noted on sample vial label) were used (Appendix 9.5). Sample vials should arrive at the laboratory chilled to a temperature less than 10 °C. If the sample temperature is above this requirement, document on the COC and immediately inform the project manager.
4. When samples must be frozen before analysis, the vials should be no more than half full, (e.g., BMAA should be frozen or analyzed immediately). (Note: Before placing the vial into the freezer, adequately mix the sample and then remove enough sample to prevent vial breakage during freezing.) If the volume of sample is insufficient for analysis, document on the COC and immediately inform the project manager.
5. LS checks the collection date (mm/dd/yyyy) and time (24 hr) for each sample. Ambient monitoring analysis sets require field duplicate (FD) and field blank (FB) samples, identified on the COC form with a “D” or “B” respectively. FDs and samples from the same analysis set should have the same collection date and time. If field QC samples are missing, request an explanation from the SC, and document the explanation on the COC and in the Cyanotoxin Run Log.
6. LS fills in the sample delivery location at the bottom of the COC next to Lab: and Address: lines.
7. SC signs next to “Relinquished by:” to relinquish the samples to the LS. If a lab staff is not available, the SC records the date and time in the appropriate spot on the COC and places the samples directly in the laboratory refrigerator. Step 1. 10. provides the location and conditions for storage.
8. LS signs next to the “Received by:” stating all samples listed on the field COC form were physically verified and received. If the SC is present, document the date and time samples were received from SC. If the SC is not present, document the date and time samples were removed from the refrigerator and verified.

9. LS signs and records the date and time at the bottom of the COC form.
10. The samples for algae cell enumeration are stored in the laboratory refrigerator in room 117. The samples for the toxin analysis are stored in the lab refrigerator in room 124 until a 2.5 mL aliquot is removed for lysing by the freeze-thaw process (Step 2.). Store the original toxin samples horizontally in the lab freezer. The ambient monitoring anatoxin-a and saxitoxin ELISA samples are received in separate 40 mL amber vials, with 2 mL of 10X buffer added to each vial before collecting the sample. The 10X buffer is supplied in each kit. The total volume in each bottle is approximately 20 mL or half full.
11. LS scans the field COC form into a portable document format (PDF) file. Save the PDF file to the proper folder on the "S" drive, using the following pathway
 OWQ/WSP/WSP/OWM/Algae/Cyanobacteria\YYYYLab Results\Project Name)\(Trip Number). Name the COC file with COC and the trip number. An example COC file name is COC16LAW001. File the original COC form in the appropriate folder in the room 124 file cabinet.

Step 2. Lyse samples using the freeze-thaw method.
 (Note: LS completes Step 2.)

1. Label a 20 mL borosilicate-glass, screw cap vial, and a corresponding 4 mL glass vial for each sample type.

Sample Type	Type of Vial Required
Microcystins ADDA ELISA	amber or clear glass
Cylindrospermopsin ELISA	amber or clear glass
Anatoxin_a ELISA	amber glass
Saxitoxin ELISA	amber or clear glass
BMAA	clear glass

Use either the Dymo® label writer or labeling tape. (Note: Cut labels with scissors.)

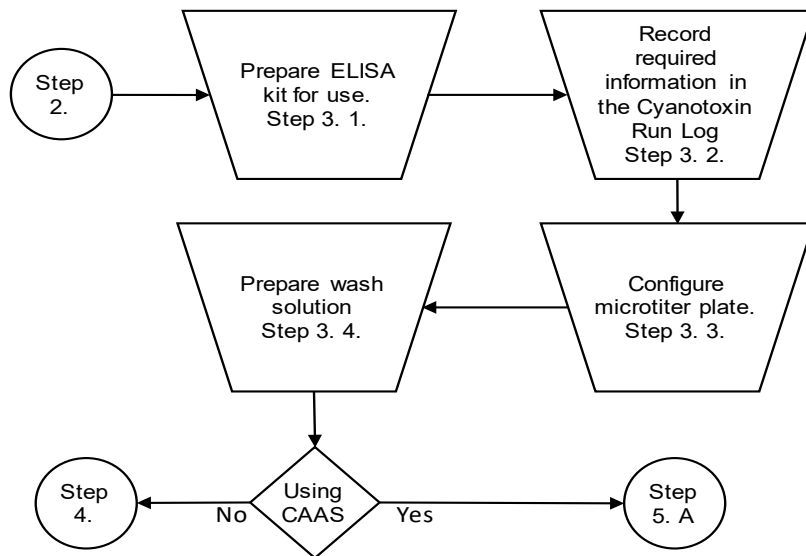
2. Record the AB or DK number of the sample, the sample collection date, and the LS's last name on the label for tracking the sample through the analysis process.
3. Use the 0.5-5.0 mL micropipette and corresponding tip to transfer 5.0 mL of well-mixed raw sample into the corresponding 20 mL borosilicate-glass, screw-cap vial

(analysis samples). Record pipettes used in the Cyanotoxin Run Log (Appendix 9.2).

4. Prepare quality control samples as follows:
 - a. Lab duplicate sample – Transfer an additional 5.0 mL aliquot from one of the field samples into a different 20 mL glass vial. Label the lab duplicate sample with the original sample number with LD added.
 - b. Lab blank sample – Transfer 5.0 mL of deionized (DI) or Millipore water from the laboratory into a 20 mL glass vial. Label the lab blank LRB, per naming protocol.
 - c. Fortified blank sample – Transfer 5.0 mL of Millipore or DI water into a 20 mL glass vial and add the appropriate amount and type of spiking solution. Label the lab fortified blank LFB per naming protocol.
 - d. Matrix spike or matrix spike duplicate (MS or MSD) samples – Transfer 5.0 mL of the sample to be spiked into two separate 20 mL glass vials. Then, add the appropriate amount and type of spiking solution into each vial. Label the MS or MSD samples with the original sample number, with MS or MSD added.
5. Place the original raw sample vials into the appropriate lab refrigerator or freezer for storage.
6. Vortex each analysis sample vial for 5-10 seconds for proper mixing. Then, place the 20 mL analysis vials horizontally in the freezer until completely frozen. Record the freeze start time in the Cyanotoxin Run Log.
7. Remove the analysis vials from freezer and allow to thaw completely. Ensure no visible ice crystals remain in analysis samples. A warm water bath (up to 35°C) is typically used to decrease the thaw time. Record the thaw time start in the Cyanotoxin Run Log.
8. Repeat the freeze and thaw cycle two more times mixing each sample after each thaw. Record each freeze and thaw time in the Cyanotoxin Run Log. (Note: Each freeze step should take between 15 and 30 minutes. Each thaw step should take between 5 and 30 minutes depending on whether a warm water bath is used).

9. Filter 1 to 2 mL of each lysed sample into a 4 mL glass vial using a disposable 5 mL plastic syringe and a 25mm, 1.2 μm pore sized glass-fiber syringe filter.
10. If needed, make sample dilutions at this time. Dilute the sample with reagent water which matches the sample matrix. (e.g., Dilute samples preserved with sodium thiosulfate with Millipore water containing sodium thiosulfate.)
11. Store capped samples in the refrigerator until ready for analysis. After the sample aliquot is removed for ELISA analysis, store remaining sample in the refrigerator for possible repeat analysis.

Step 3. Microtiter Plate Preparation
(Note: LS completes Step 3.)



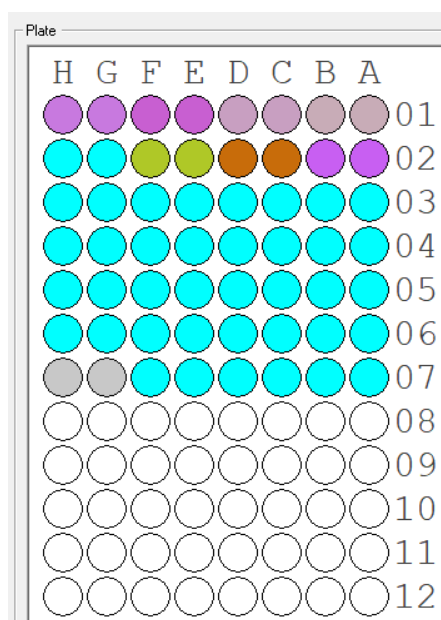
1. Open the cyanobacteria toxin kit. Before use, check each kits' expiration date. Discard expired kits. With a marker or pen, write date opened and LS initials on the kit. Remove metal seals from the standards and controls. (Note: Laboratory forceps are a good instrument for quickly peeling off the seals.) If one kit or combining more than one kit for an analysis, record the lot numbers of each ELISA kit in the Cyanotoxin Run Log worksheet.
2. For the toxin samples analyzed on the Cyanotoxin Automated Assay System (CAAS) or Chromate Reader (plate reader only, not automated), document the following

on the Cyanotoxin Run Log worksheet: analyses date, analysts' names, analyses set numbers, assay names, and any additional notes in the comments section. Ensure freeze and thaw dates and times are recorded in the run log from Step 2. 8.

3. Configure Microtiter Plate

(Note: Each microtiter plate, by definition, is an analysis batch, regardless of the number of wells used.)

- a. For analysis, determine the number of strips needed in the microtiter plate. Each microtiter plate contains 12 strips (01-12), and each strip contains 8 wells (A-H).



- b. Number of strips = $(\text{Total Samples} \times 2) / 8$
(Note: Round any fractions up to the next integer.)

Where:

Total samples = The sum of analysis samples, QC samples, and calibration samples.

- c. If using a full plate, remove strips from the plate to make the number of strips in the plate equal to the number calculated.

If reusing an empty plate, place strips into the plate to equal the number calculated.

(Note: Reused plates are located next to the CAAS.)

(Note: Often the last strip is not complete (i.e., Not all eight

wells are needed.), use dummy wells located next to the CAAS to complete a partial strip.)

- d. Reseal any remaining strips in the zip-lock foil bag and place into the analysis kit for later use.
- e. If using the CAAS, place the completed microtiter plate in the unit.
If using the Chromate reader, place the plate on the bench top.
- f. Place the reagents for use in the analysis, and the lysed samples on the bench top. Allow the reagents and samples to warm to room temperature.

4. Prepare Wash Solution

- a. Each ELISA kit contains a 100 mL wash buffer (5X concentrate) which is the same for microcystins, cylindrospermopsin, anatoxin-a, saxitoxin, and BMAA analyses. Manual well washes or in the CAAS wash reservoir require wash solution made from the wash buffer (5x concentrate).
- b. Prepare the wash solution by adding one volume of 5X concentrate wash buffer with four equal volumes of DI water in a 1 L container. (Note: Use the solution until the first occurs of either depletion or expiration.)
- c. Record the lot numbers and volume of the wash solution made in the Cyanotoxin Run Log, Reagents Made section.

Step 4. Cyanotoxins' Plates Preparation

(Notes: Step 4. describes backup methods for ELISA.

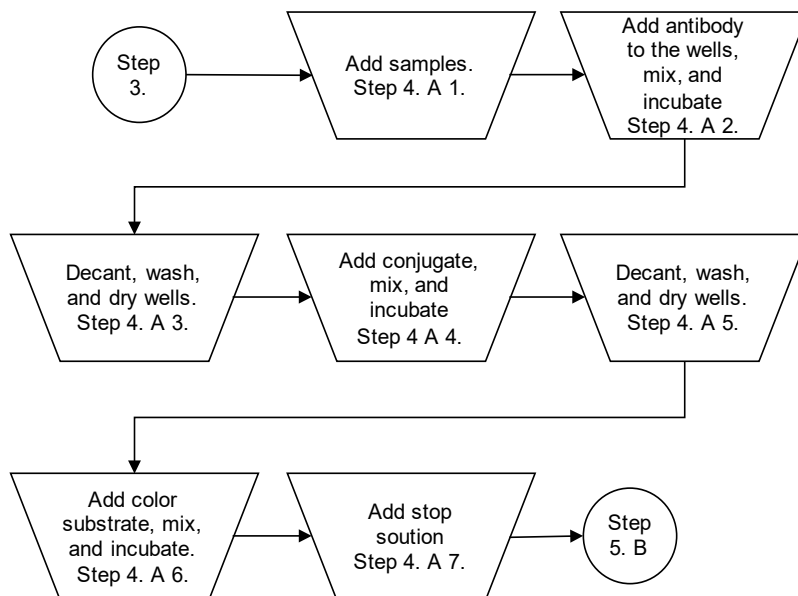
The primary method uses CAAS in Step 5. A.

The subsets below give the manual assay procedures to determine each toxin. However, each Eurofins Abraxis cyanotoxin kit contains working instructions for the ELISA kit. Before use, please review the working instructions provided in the kits for any possible procedural changes.)

A Microcystins ELISA Plate Preparation

(Notes: Takes approximately 3.5 hours.

Ensure all solutions are equilibrated to room temperature before starting the procedure.)

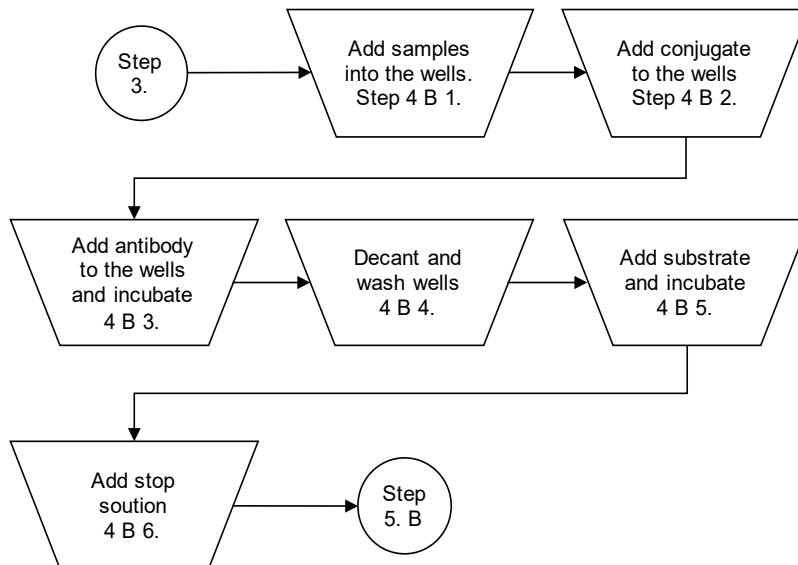


1. Add 50 μL of the standard solutions, control samples, and analysis samples into the wells of the test strips, in accordance with the working scheme provided in the kit instructions. Duplicates of all samples and solutions are added for the analysis.
2. Add 50 μL of the antibody solution to the individual wells, using a multichannel pipette. Cover the wells with parafilm or tape (cover). Carefully move the strip holder in a circular motion on the benchtop for 30 seconds, preventing spillage of the contents (mix). Incubate the strips for 90 minutes at room temperature.
3. Remove the covering. Decant the contents of the wells into a sink (decant). Blot the inverted plate on a stack of paper towels (blot). Wash the strips three times using at least 250 μL diluted wash buffer for each well and each washing step (triple wash). Blot after each wash. After the last wash and blot, check the wells for any remaining buffer, if necessary, remove with additional blotting.

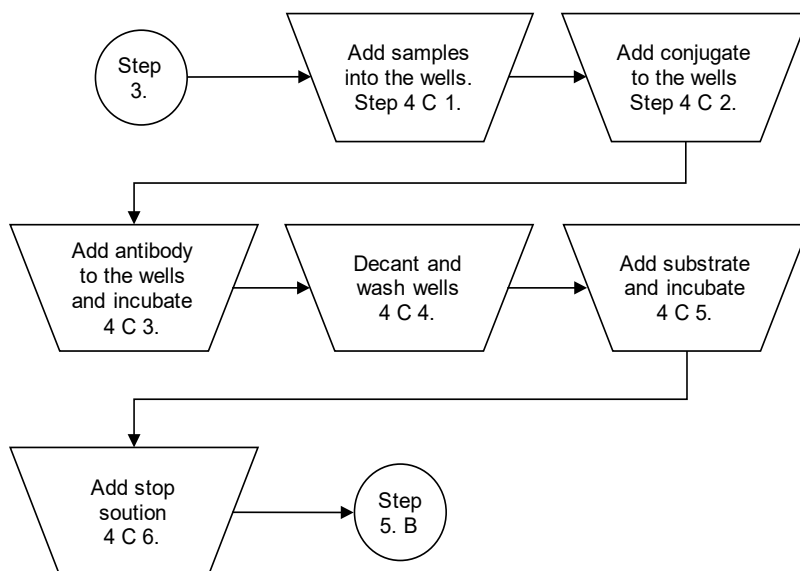
4. Add 100 μL of enzyme conjugate solution to the individual wells, using a multichannel pipette. Cover. Mix. Incubate the strips for 30 minutes at room temperature.
5. Remove the covering. Decant. Blot. Triple wash. Blot after each wash. After the last wash and blot, check the wells for any remaining buffer in the wells. If necessary, remove with additional blotting.
6. Add 100 μL of color substrate solution to the individual wells, using a multichannel pipette. Cover. Mix for 30 seconds. Incubate the strips for 20-30 minutes at room temperature under a light-safe box or cover.
7. Add 50 μL of stop solution into each well, using a multichannel pipette. The color of the liquid in the wells instantly turns from blue to yellow.
8. Continue to Step 5. B (Note: Insert the tray into the chromate reader and read within 15 minutes of adding stop solution.)

B Procedure for the Cylindrospermopsin ELISA

(Note: Takes approximately 2 hours. Ensure all solutions are equilibrated to room temperature before starting this procedure.)

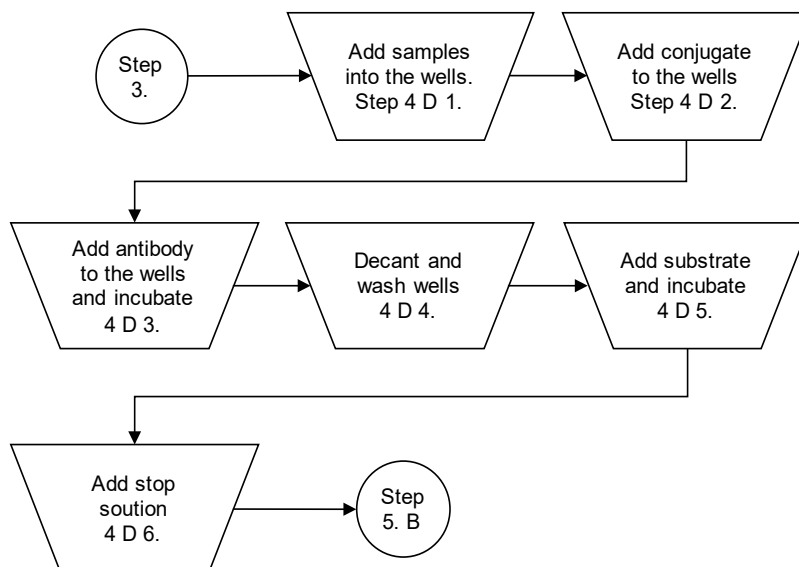


1. Add 50 μL of the standard solutions, control samples, and analysis samples into the wells of the test strips, according to the working scheme provided. Duplicates of all samples and solutions are added for the analysis.
 2. Add 50 μL of enzyme conjugate solution into each well, using a multichannel pipette. Vortex the enzyme conjugate solution.
 3. Add 50 μL of antibody solution into each well, using a multichannel pipette. Cover the titer tray with Titer-Tops or other brand of clear adhesive film. Mix for 30 seconds. Incubate for 45 minutes at room temperature.
 4. Remove the covering. Decant. Blot. Quadruple wash. Blot after each wash step. After the last wash and blot, check the wells for any remaining buffer in the wells. If necessary, remove with additional blotting.
 5. Add 100 μL of color substrate solution into each well, using a multichannel pipette. Cover. Incubate for 30-45 minutes at room temperature under a light-safe box or cover.
 6. Add 100 μL of stop solution into each well, using a multichannel pipette. The color of the liquid in the wells instantly turns yellow.
 7. Continue to Step 5. B (Note: Insert the tray into the chromate reader and read within 15 minutes of adding stop solution.)
- C Procedure for the Anatoxin-a ELISA
(Note: Takes approximately 2.5 hours)



1. Add 50 μL of the standard solutions, control samples, and analysis samples into the wells of the test strips, according to the working scheme provided. Duplicates of all samples and solutions are added for the analysis.
2. Add 50 μL of the reconstituted enzyme conjugate solution to the individual wells, using a multichannel pipette.
3. Add 50 μL of the reconstituted antibody solution to the individual wells, using a multichannel pipette. Cover. Mix for 60 seconds. Incubate the strips for 60 minutes at room temperature.
4. Remove the covering. Decant. Quadruple wash. Blot.
5. Add 100 μL of the substrate color solution to the individual wells, using a multichannel pipette. Protect the strips from sunlight. Cover. Mix for 30 seconds. Incubate the strips for 20-30 minutes at room temperature.
6. Add 100 μL of the stop solution to the wells in the same sequence as the substrate color solution sequence, using a multichannel pipette or a stepping pipette.
7. Continue to Step 5. B (Note: Read the absorbance at 450 nm using a microtiter plate ELISA photometer within 15 minutes after the addition of the stop solution.)

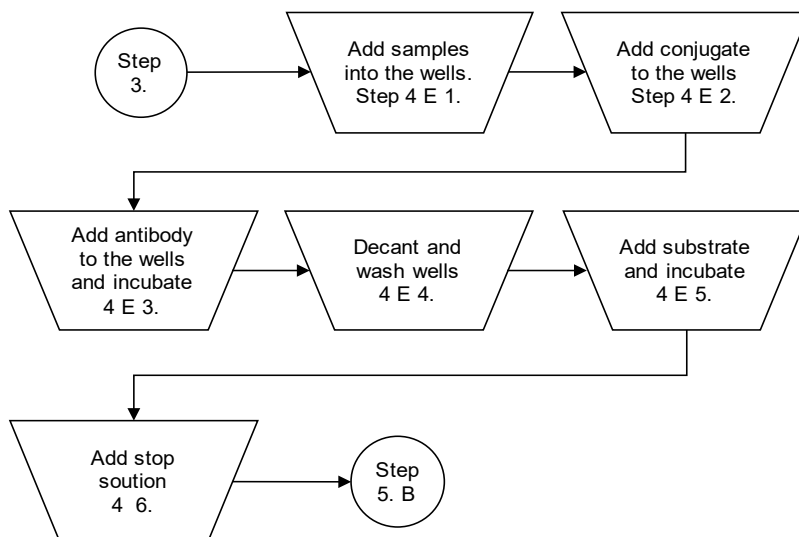
D Procedure for the Saxitoxin ELISA (Note: Takes approximately 1.5 hours.)



1. Add 50 μL of the standard solutions, control samples, or analysis samples into the wells of the test strips, according to the working scheme provided. Duplicates of all samples and solutions are added for the analysis.
2. Add 50 μL of the enzyme conjugate solution to the individual wells, using a multichannel pipette.
3. Add 50 μL of the antibody solution to the individual wells, using a multichannel pipette. Cover. Mix for 60 seconds. Incubate the strips for 30 minutes at room temperature.
4. Remove the covering. Decant. Quadruple wash. Blot. After the last wash and blot, check the wells for any remaining buffer in the wells. If necessary, remove with additional blotting.
5. Add 100 μL of the substrate color solution to the individual wells, using a multichannel pipette. Protect the strips from sunlight. Cover. Mix for 30 seconds. Incubate the strips for 30 minutes at room temperature.
6. Add 100 μL of the stop solution to the wells in the same sequence as the substrate color solution sequence, using a multichannel pipette.

7. Continue to Step 5. B (Note: Insert the tray into the chromate reader and read within 15 minutes of adding stop solution.)

E Procedure for the BMAA ELISA
(Note: Takes approximately 1.5 hours.)



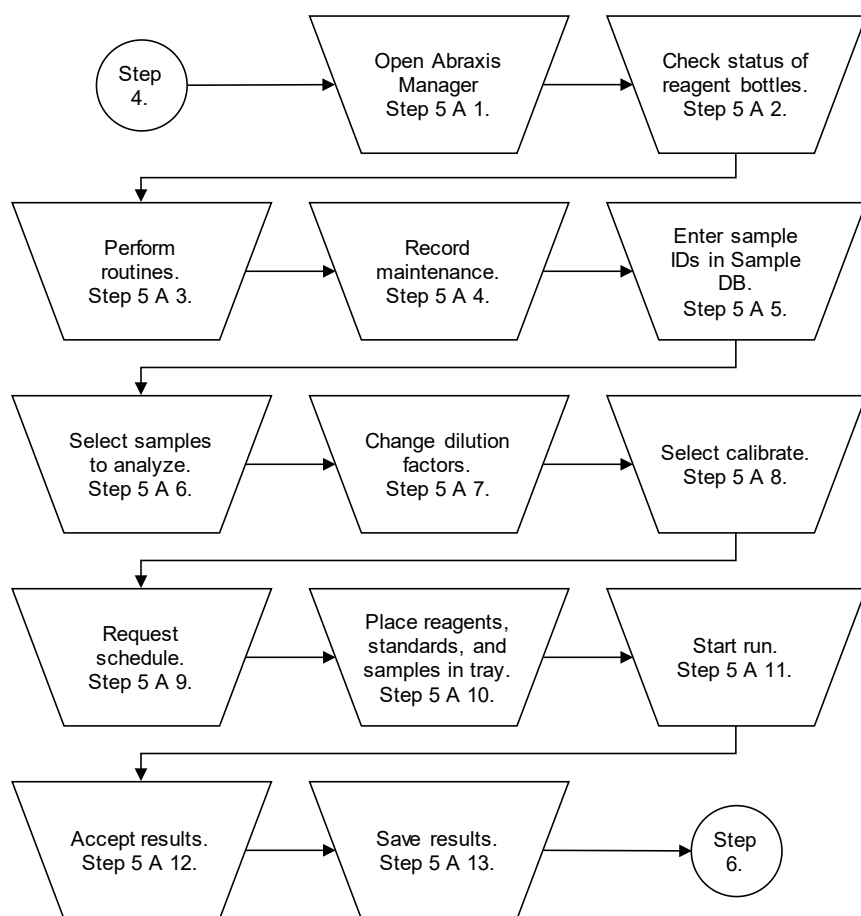
1. Add 100 μL of the reconstituted standard solutions and analysis samples into the wells of the test strips, according to the working scheme provided. Duplicates of all samples and solutions are added for the analysis.
2. Add 50 μL of the enzyme conjugate solution to the individual wells, using a multichannel pipette.
3. Add 50 μL of the antibody solution to the individual wells, using a multichannel pipette. Cover. Mix for 30 seconds. Incubate the strips for 90 minutes at room temperature.
4. Remove the covering. Decant. Quadruple wash. Blot. After the last wash and blot, check the wells for any remaining buffer in the wells. If necessary, remove with additional blotting.
5. Add 150 μL of the substrate color solution to the individual wells, using a multichannel pipette. Protect the strips from sunlight. Cover. Mix for 30 seconds. Incubate the strips for 30 minutes at room temperature.

6. Add 100 μL of the stop solution to the wells in the same sequence as for the substrate color solution using a multichannel pipette or a stepping pipette.
7. Continue to Step 5. B (Note: Read the absorbance at 450 nm using a microtiter plate ELISA photometer within 15 minutes after the addition of the stopping solution.)

Step 5. Analyzing ELISA Plates

A CAAS Abraxis Manager

(Note: For further information on the CAAS and Abraxis Manager software including pictures and screenshots please review (Abraxis 2015) [Abraxis CAAS Operators Manual.](#))



1. Ensure the CAAS is on and the USB cord is connected to the computer. The toggle switch is in the back. Open Abraxis Manager icon on the computer. Software connects with CAAS and begins initialization.

2. Check the status of the wash, rinse, and waste bottles. Also check the status of the water container for priming the syringes. Fill wash and rinse bottles, and empty the waste bottle, if necessary. See Step 3. 4. for preparing wash solution. Check the status of the 1 N HCl bottle in slot 1 of the tray. Fill the container before proceeding.
3. After concluding the initialization, click Routines on the task bar. Then click Start of Day. Click Routines again. Then click Prime Syringes. Click repeat. Use 70% isopropyl alcohol, if necessary, to remove air bubbles. If 70% isopropyl alcohol is used to prime the syringes, immediately remove all alcohol by priming the syringes with two cycles of Millipore water. Click Routines again. Select Prime Washer – Wash Bottle. Click repeat.
4. Record all maintenance performed in Cyanotoxin Run Log in the maintenance log worksheet.
5. Click the “sample” tab located on the lower part of the window. Click Sample DB. Click Edit button. Enter Sample IDs in Sample DB. Family Name refers to the site from which samples were collected. Given Name refers to analysis set number. Middle Name is the QC abbreviations such as LD – Lab Duplicate and FB – Field Blank.
6. Select Samples in Sample DB to run. Click Choose. Samples appear in the left-hand column. Move samples up or down in the order by clicking to highlight the sample. Then use the up and down arrow buttons. Select appropriate assays. Click Add Test below the Choose Tests list. The samples appear on the right side of the window, along with the selected assays, the number of replicates, and the dilution factors.
7. Change the dilution factor, if necessary (e.g., 1:1 when analyzing anatoxin or saxitoxin samples containing added buffer).
8. Click Calibrate below the Work List pane on the lower right-hand corner of the window. When the dialog box appears, click OK.
9. Click Request next to calibrate. When the next window appears, ensure Reset Rack 1, Reset Rack 2, and Reset Plate are checked. Click OK. A schedule for each test

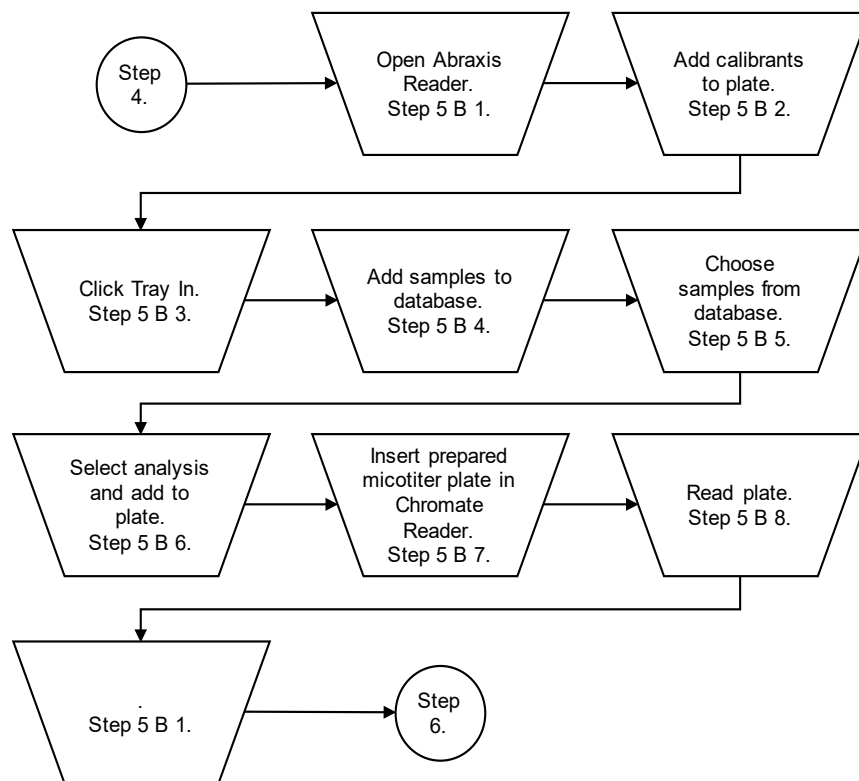
appears in the next window. Select different schedules via the drop-down menu on the window to preferentially change the order, when running multiple tests. Click OK, when satisfied with the schedule.

10. The program automatically switches to the Layout tab. Determine each reagent, standard, or sample's placement, by hovering over the positions on the racks with the cursor. If needed, left click and drag standards and reagents between different positions on a rack, but not between racks.
11. When setup is complete, (Note: The plate for analysis should have previously added in Step 3.) click Start Run in the lower right-hand corner of the program. The CAAS checks volumes of reagents and calibrants. If any volume is insufficient, replace the bottle with a fresh bottle. Click Check Volumes. Once all reagent and calibration standard volumes are sufficient, the CAAS runs the assays.
12. After analysis is complete, click Select All test results. Click Accept test results.
13. Save assay calibration and test reports to the computer desktop. (Note: Both Chromate, Abraxis Reader, and the CAAS Abraxis Manager work in the same way.)
 - a. To observe results for standards and control, click Assay Calibration button on the bottom of the screen.
 - b. Click Print Preview to view the curve, CV%, and R2. Then click print (pull down) to create the PDF file for standards. The following is an example of the naming:
st2012M0614
st denotes a standard curve file
2012 denotes the year
Type of ELISA test is denoted by either:
M for microcystins ELISA
C for cylindrospermopsin ELISA
A for anatoxin ELISA
0614 month and day of the analysis.
 - c. Click Report to view the Sample Run file.
 - d. Click Select ALL.
 - e. Click Export to create the Sample.txt file and the Sample.xls file. The following are examples of both file names:
run20120901.txt

run20110901.xls.

- f. Click Print Preview to view the test report. Then click Print, ensure Microsoft Print to PDF is selected from the dropdown menu in the printer box. Then click OK.
- g. Save all four files (Standards, Sample run, Sample TXT, and Sample XLS) to the S: drive in the Cyanobacteria file folder on the computer under the year of analysis followed by the project subfolder and finally in the analysis set name folder. (Note: Periodically copy the year of analysis folder to the home drive for back-up storage purposes.)

B Analyze data in Abraxis Reader using the Chromate Module
(Note: Complete Steps 5. B 1. – 6. before the microtiter plate is ready to read, should take less than 30 minutes, and can be started any time after Step 1.)



1. Ensure the Chromate reader is connected to the computer with a USB cord. Turn on the Chromate reader. (Notes: The toggle switch is in back. The ON position is when the top half is flat and pushed in). Open the Abraxis Reader icon on the desktop.

2. Click on Assay Calibration on bottom of the Abraxis Reader screen to load the assay method onto the plate. From the drop-down menu on the top left of the screen, select either: Microcystin ADDA, Cylindrospermopsin 1X, Anatoxin-a ELISA, BMAA, or Saxitoxin. Go to top right and on the drop down and click ALL, to add the calibration test. Click OK. Choose two replicates for each calibration standard. Click on Add to Plates in the bottom right. Calibrants are now assigned to wells by row and column.

(Notes: When adding a new assay to the Chromate Manager, attach the report template for the new assay to a custom report format with an IDEM logo.

The steps below set the IDEM report template in the Chromate Manager for a new assay calibration report or to reset the templates for any of the assay calibration reports.)

In the Chromate Manager program:

- Go to Settings > Custom Report.
 - Click to highlight the assay to set the template (i.e., Anatoxin-a ELISA (2 rep)).
 - Click Set Template button.
 - Navigate, if needed, to the .rpt template file, should be in the RptCustom folder.
 - Select IDEM Assay Calibration Report rpt. or the template for the results, if setting the result report.
 - Click Open button.
3. If everything is connected correctly, the Chromate Reader ejects the tray. Click Tray In on left.
 4. Click Add Sample on bottom of screen. Click Add Sample ID on bottom left. Click Edit. Then add AB or DK numbers on bottom. The AB or DK number may remain from a different analysis. Use edit mode to replace with a new AB or DK number. Identify the sample, for example, if the sample is a field duplicate put FD in third column under List Name. (Note: AB or DK numbers remain in the database indefinitely unless deleted.)
 5. From the Sample Database, select the sample number to add to the analysis and in the bottom of first column click Choose. Sample numbers appear on the left side (top) ID column. Ensure all samples are listed and in the correct order. Highlight all. Close the Sample ID list.

6. Click on Test column and highlight either:
Microcystins ADDA, Cylindrospermopsin 1X,
Anatoxin-a ELISA, Saxitoxin, or BMAA
Click Add Test on third column at bottom of screen. See work list on right and check, whether highlighted.
Click Add to Plate. Leave the program here until plate is ready to be read.
7. Insert prepared microtiter plate into the Chromate reader.
Read the plate within 15 minutes of adding stop solution.
Position plate according to diagram on computer screen.
8. Click Read Plate. After analysis is read (Note: Takes approximately 2 minutes.), click Select All test results. Click Accept test results.
9. Save assay calibration and test reports to desktop of the computer. (Note: Do the same way for both Chromate, Abraxis Reader, and the CAAS Abraxis Manager.)
 - a. To observe results for standards and control, click Assay Calibration button on the bottom of the screen.
 - b. Click Print Preview to view the curve, CV%, and R2. Then click print (pull down) to make the PDF file for Standards. The following is an example of the naming:
st2012M0614.
st denotes a standard curve file
2012 denotes the year
Type of ELISA test is denoted by either:
M for microcystins ELISA
C for cylindrospermopsin ELISA
A for anatoxin ELISA
0614 denotes the analysis's month and day.
 - c. Click Report to view the Sample Run file.
 - d. Click Select ALL.
 - e. Click Export to create the Sample.txt file and the Sample.xls file. The following are examples of both file names:
run20120901.txt
run20110901.xls.
 - f. Click Print Preview to view the test report. Then click Print. Ensure Microsoft Print to PDF is selected from the dropdown menu in the printer box. Then click OK.

- g. Save all four files (Standards, Sample run, Sample TXT, and Sample XLS) to the S: drive under the Cyanobacteria file folder on computer under the year of analysis subfolder, and analysis set name. (Note: Periodically copy the year of analysis folder to the home drive for back-up storage purposes.)

Step 6. Quality Assurance Protocol, Reporting, and Electronic Data Interface for Importing ELISA Analytical Results into AIMS.

1. Review results following the Quality Assurance Protocol checklist in Appendix 9.3. and answer the questions of the decision tree in the Cyanotoxin Run Log. Document instrumentation issues in the run comments section in the Cyanotoxin Run Log. Give details about QC failures in the QA comments section in the Cyanotoxin Run Log.
2. Report critical QC failures and any instrument issues to the project leader and QAO, usually via email, if the issue is not urgent.
3. Prepare final reports for the Cyanobacteria and Cyanotoxin program manager designee and the QAO assigned to the project. Put together a PDF report per cyanotoxin with Adobe Acrobat DC. The report includes the Standard calibration file, the Sample Run file with LS's signature on bottom; and an Excel summary report with sample name, sample number, collection date of samples, and results reported to two significant figures. Send the PDF report and the Excel result summary report to the Cyanotoxin program manager or designee by Friday at noon through email. An example of the PDF file name is Microcystins ELISA Results for August 8, 2012. Store the PDF file in designated location. Appendix 9.4 contains an example final report.
4. Save the reports developed in Step 6. 3. on the shared drive at S:IGCN/OWQ/ WSP/OWM/Algae/Cyanobacteria/YYYY Lab Results/Analysis Set#/ . The assigned QAO uses the file to review data uploaded into Assessment Information Management System (AIMS).
5. The electronic folder in the branch shared drive at S:IGCN/OWQ/ WSP/OWM/Algae/Cyanobacteria/YYYY Lab Results/Analysis Set#/ now contains all documents created by the analysis including COC and Cyanotoxin Run Log for

the QAO's review and building an electronic data interface (EDI) to import ELISA analytical results into AIMS. The template for creating an EDI is stored in an .xlsm file named cyanotoxin edi. Specific instructions for building the EDI are in the Index sheet.

2.3. Related Technical Issues:

A. Health and Safety Warnings

- Whenever handling samples, standards, solutions, and reagents wear gloves, safety glasses, and a lab coat.
- SDSs for all chemicals are in the SDS binder stored in the Algae Lab in room 124.
- The ELISA assay kits contain tetramethylbenzidine (TMB) (substrate solution) and diluted sulfuric acid (stop solution). Review SDS for each before using the ELISA kits.
- Do not open WASH, RINSE, or WASTE containers while the CAAS is powered on. The containers are pressurized.

B. Cautions

- Store original raw samples in the freezer after analysis to prevent degradation of the toxins per EPA Method 546.
- Update the Abraxis Reader and Abraxis Manager computer modules when updates become available.

C. Interferences

- Always add reagents to the titer wells in the same order to ensure consistent incubation time periods between all wells on the plate ensuring accuracy.
- Always vortex samples (a few seconds) and standards before pipetting to obtain a homogeneous aliquot.
- Bubbles left in plate wells after a wash step may cause analysis errors.
- Failure to mix original sample before collecting a subsample can result in nonrepeatable results.
- Do not touch the top of microtiter cells containing solution with the analyst's gloves. This may ruin the cells affected.
- When not in use, store standards and solutions in the refrigerator to prevent degradation.

- Do not mix caps of the reagents – Label with colored dot stickers. Otherwise, the reagents could become contaminated.

D. Calibration

- Calibration standards are provided in the ELISA kits and must be analyzed at the beginning of each batch.
- The calibration curve is validated by evaluating the %CV of the absorbance values for the well replicates representing each calibration level, and the correlation coefficient (r^2) of a four-parameter logistic curve. Appendix 9.3, Checklists for QA Review of Data for Microcystins, Cylindrospermopsin, Anatoxin-a, Saxitoxin, and BMAA questions 1. – 2. act as a decision tree.

E. Troubleshooting:

1. Contact customer service representatives from Abraxis for advice and opinions on problems occurring with analysis or with the CAAS.
2. Abraxis CAAS Troubleshooting Tips are in the Abraxis Training Manual located in the Algae Lab, room 142. The Abraxis CAAS Operators Manual also contains troubleshooting tips.
3. For chromate computer module problems, contact Awareness Technology with Model 4300 Serial Number 4300-1315 Tech Support mstandt@awaretech.com.
4. A second LS may observe analyst or run analysis to identify operator error.
5. When adding a new assay to the Chromate Manager, the report template for the new assay should be attached to a custom report format with an IDEM logo. Step 5. B. 2 describes the steps to set the IDEM report template in Chromate Manager for a new assay calibration report or to reset the templates for any of the assay calibration reports.

3.0. Roles

3.1 Responsibilities

A. Sample collector

1. Collect water samples for the analysis of microcystins, cylindrospermopsin, anatoxin-a, saxitoxin, and BMAA in ambient or drinking water.

B. Laboratory staff

1. Extract and quantify microcystins, cylindrospermopsin, anatoxin-a, saxitoxin, and BMAA from water samples.
 2. Check in toxin samples brought into the laboratory from the field.
 3. Evaluate laboratory procedures for quality control and quality assurance compliance with this TSOP.
- C. Project leader
1. Make final decisions on problems and changes to procedures concerning the cyanobacteria toxin sample preparation and ELISA.
 2. File a report to the Targeted Monitoring Section chief.
- D. Targeted Monitoring Section chief
1. Responsible for the algae project and approves results for release to the public.
- E. Quality assurance officer
1. Prepares data entry forms, validates the quality of ELISA results in AIMS, and releases data for use in AIMS.

3.2 Training requirements

- A. Algae sample collection
1. Sample collector
- B. Microcystin, cylindrospermopsin, anatoxin-a, saxitoxin, and BMAA extraction, analysis with either the CAAS or Chromate Reader, and reporting
1. Laboratory staff
- C. Algae sample receiving and check in to the laboratory
1. Laboratory staff

4.0. List forms, equipment, or software to be used

4.1 Forms

- A. IDEM OWQ Chain of Custody Form – Printed from AIMS database for the beach monitoring program.
- B. Cyanotoxin Run Log – MS excel files containing information recorded about each analysis, such as, preparation work and QA/QC monitoring. A new file is used on an annual basis. Inside the files, are worksheets serving as a maintenance log for the CAAS, a calibration log for the Sartorius balance, and a log to monitor pipet precision and accuracy checks amongst others. The Cyanotoxin Run Log includes the following worksheets:

- Maintenance Log
- QA Checklists
- Sartorius Log – Used to record and track analytical Sartorius balance calibrations.
- Pipet Log – Used to record and check pipet accuracy using the balance.
- Method detection limit (MDL) and initial demonstration of capability (IDC) Logs – Used to record each time an MDL is performed and on a new worksheet.
- Run logs – Used to record the date of analysis and is titled by date followed by the first letter of the toxin ELISA.
- Reagents Log – Used to record and for traceability of reagents.
- Spike Log – Used to record and for traceability of working spike solutions.
- Unacceptable Analyses – Used to record analyses failing batch requirements.

4.2 Equipment

- A. Adjustable and fixed micropipette (sizes 10-100 μ L, 20-200 μ L, 1000 μ L, 0.5-5.0mL)
- B. Multichannel pipette (25-300 μ L)
- C. Multichannel pipette reagent reservoirs
- D. Parafilm
- E. Vortex mixer
- F. Plastic disposable Luer-lock syringes: preferably 5 mL volume
- G. Syringe filters: 0.45 μ L pore size (glass fiber)
- H. Pipette tips (1-250 μ L, 100-1000 μ L, 0.5-5.0 mL)
- I. Light protective cover
- J. Laptop or desktop with software in 4.3 installed
- K. Glass 4.0 mL vials
- M. Borosilicate glass 20 mL vials, Teflon-lined screw cap vials
- N. Titer-Tops clear adhesive film
- O. Chromate Reader
- P. CAAS an automated assay analysis system
- Q. ELISA photometer
- R. Refrigerator capable of maintaining 4°C

- S. Freezer
- T. Class 'A' volumetric flasks and beakers for making and storing reagents and standards
- U. Tray for water bath capable of maintaining 35°C
- V. Abraxis ELISA kits (anatoxin-a, BMAA, cylindropspermopsin, microcystins, and saxitoxin)
- W. 1 N hydrochloric acid
- X. 70% isopropyl alcohol
- Y. Sodium thiosulfate tablets

4.3 Software

- A. Abraxis Reader (Chromate)
- B. Abraxis Manager (CAAS)

5.0. Records Management

- Completed original COC forms copies are stored in the top drawer of lab file cabinet by the LS. Electronic copies are stored on the shared drive. (S:\IGCN\OWQ\WSP\OWM\Algae\Cyanobacteria\YYYY Lab Results\)
- Analysis and comments summaries are stored in worksheets named by analysis date in the Cyanotoxin Run Log on the shared drive by the LS. (<S:\IGCN\OWQ\WSP\OWM\Algae\Cyanobacteria\YYYY Lab Results>)
- Final reports and QA reports are stored electronically on the shared drive by the LS and QAO respectively. (<S:\IGCN\OWQ\WSP\OWM\Algae\Cyanobacteria\YYYY Lab Results>)
- Analysis results are stored electronically on the shared drive by the LS. (<S:\IGCN\OWQ\WSP\OWM\Algae\Cyanobacteria\YYYY Lab Results>)
- Chromate reader accepted analyses are stored electronically by the LS in the Abraxis program module on a designated secured computer stored in the Algae Lab, room 142.
- The QAO uploads and stores results in AIMS.
- Electronic record retention periods are indefinite. COC hard copies are retained for 5 years and then disposed.

6.0. Definitions

- 6.1. "AA/AB number" – A number assigned to each individual watershed sampling event conducted by Indiana Department of Environmental Management (IDEM) field crews. The number is used to identify the

sampling event in the Assessment Information Management System database (AIMS database).

- 6.2. “Acid” – A chemical substance commonly found in laboratories. Reacts with bases to form salts. From a safety perspective, chemicals characterized by their corrosive effects on skin and lungs.
- 6.3. “Adjustable micropipette” –Volume of liquid contained in the tip is variable and controlled via a dial or other mechanism, depending on the model.
- 6.4. “Agency staff” – Any employee or representative of IDEM including regular employees, temporary employees, contractors, and interns.
- 6.5. “Analysis batch” – Consists of standards, samples, and quality control elements assayed in a single 96-well microtiter plate using identical lots of reagents and wells.
- 6.5. "ASTM International (ASTM)" – Originally known as the American Society for Testing and Materials. An international standards organization which develops and publishes voluntary consensus technical standards for a wide range of materials, products, systems, and services.
- 6.6. “Assessment Information Management System database (AIMS database)” – IDEM database containing information related to water chemistry, aquatic habitat, macroinvertebrate and fish communities, fish tissue analyses, sediments, algae, and *E. coli* bacteria data collected by agency staff from watershed sampling events.
- 6.7. “Chain of custody (COC)” – The records documenting the possession of samples from the time obtained until disposed or shipped off-site.
- 6.8. “Deionized water” – Water from which the mineral ions are removed.
- 6.9. “DK Number” – A number assigned to each individual drinking water sample taken by the Drinking Water Branch program.
- 6.10. “Millipore filtration system” – A system developed by Millipore to deliver ASTM type 1 water.
- 6.11. “Millipore water” – Water meeting ASTM type 1 quantitative specifications for purity.

7.0. Quality Assurance / Quality Control

- 7.1. The QA protocol adheres to EPA Method 546 for the analysis of total microcystins by Adda ELISA. The QA Protocols for the other toxins are also modeled after EPA Method 546, because EPA methods do not exist for analysis of the other cyanotoxins by ELISA. (Appendix 9.3)

- 7.2. Analysis batch QC requirements follow Appendix 9.3 and recommendations from Abraxis labs. If a critical QC element is out of specification and cannot be fixed, rerun the analysis or document why the analysis was not rerun in the Cyanotoxin Run Log in the QA comments section.
- 7.3. LS analyzes MDLs annually for each cyanotoxin ELISA. An acceptable MDL must be less than or equal to half the current MRL. For total microcystins and nodularins, the LS must pass an initial demonstration of capability (IDC) per EPA requirements (EPA Method 546 pg. 8-10) prior to analyzing field samples. After completing an acceptable IDC for total microcystins and nodularins, the LS then analyzes MDLs annually starting the following year. Record all MDL and IDC results in the Cyanotoxin Run Log.
- 7.4. Order annual proficiency testing (PT) samples from an outside vendor. Currently PT samples include the following toxins: microcystins, anatoxin-a, and cylindrospermopsin.

8.0. References

- 8.1. (Method 546), [EPA Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay](#). EPA 815-B-16-013, United States Environmental Protection Agency, Office of Water (MS-140) September 2016.
- 8.2. (Abraxis 2015), [Abraxis CAAS Operators Manual. Doc. 2925 09/2015.Rev A](#).

9.0. Appendices

- | | |
|---------------------|---|
| Appendix 9.1 | Chain of Custody Form |
| Appendix 9.2 | Copy of Cyanotoxin Run Log Worksheet |
| Appendix 9.3 | Checklists for QA Review of Data for Microcystins, Cylindrospermopsin, Anatoxin-a, Saxitoxin, and BMAA |
| Appendix 9.4 | Example of Final Report Formats. |
| Appendix 9.5 | Sample Preservation, Storage, and Handling Quick Reference Guide. |

Appendix 9.1 OWQ Chain of Custody Form



Indiana Department of Environmental Management **OWQ Chain of Custody Form**

Project:
OWQ Sample Set or Trip #:

I Certify that the sample(s) listed below was/were collected by me, or in my presence. Date: _____

Signature: _____ Section: _____

Sample Media (Water, Algae, Fish, Macro, Cyanobacteria/Microcystin, Sediment)

Lab Assigned Number / Event ID	IDEM Control Number	Sample Type	ID	1000 ml P.N.M.	1000 ml G.N.M.	40 ml Vial	120 ml P (Bact)	2000 ml Nalgene	250 ml Nalgene	125 ml Glass	Date and Time Collected		One check per bottle present
											Date	Time	
P = Plastic		G = Glass		N.M. = Narrow Mouth		Bact = Bacteriological Only		Should samples be iced?		Y		N	
M = MS/MSD		B = Blank		D = Duplicate		R = Revisit							

Carriers

I certify that I have received the above sample(s).

Signature	Date	Time	Seals Intact		Comments
Relinquished By:			Y	N	
Received By:					
Relinquished By:			Y	N	
Received By:					
Relinquished By:			Y	N	
Received By:					
IDEM Storage Room # _____					

Lab Custodian

I certify that I have received the above sample(s), which has/have been recorded in the official record book. The same sample(s) will be in the custody of competent laboratory personnel at all times, or locked in a secured area.

Signature: _____ Date: _____ Time: _____

Lab: _____ Address: _____

Appendix 9.2 Sample Copy of a Cyanotoxin Run Log Worksheet

Analyst: David Jordan
Instrument: **Abraxis CAAS**
 Run Date: 6/19/2019 **Time:** 8:35
Toxins Analyzed: Microcystins
 19HAB006 & 19LAW008 &
 19LAW009
Analysis Set(s) Analyzed:
Pipet Used for Prep P2 Finnpiquette F2 (1000µL) QU01360
 P5 Finnpiquette F1 (10-100µL)
 GH11578
 P3 Finnpiquette F2 (0.5-5mL)
 QU02133

Lot# of ELISA Kit(s) Used 18C6063 exp. 8/2019 (MCT)
 18J7972 exp. 7/2020 (Abraxis PN
 520013)
Lot# of 546 accessory kit Used

Freeze/Thaw Cycle

	Freeze Date & Time	Thaw Date & Time
1	6/18/2019 1407	6/18/2019 1430
2	6/18/2019 1502	6/18/2019 1522
3	6/18/2019 1547	6/18/2019 1611

Run Comments:

No problems to report.

Reagents Made:

QA Checklist Decision Tree

Toxin:	Microcystins
1) %Coefficient of Variance	Yes
2) Calibration R ²	Yes
3) 2 LRBs < 0.15 ug/L (546) or < MRL	Yes
4) Low-CV >= 50% & <=150% True Value	Yes
5) Abs. %CV > 15% for Field or QC Sample	Yes
6) %Rec for ea. LFB >= 60% & <= 140%	Yes
7) Mean %Rec for LFSM & LFSMD >= 60% & <= 140%	Yes
8) RPD for LFSM & LFSMD <= 40%	Yes
9) %Rec for QCS >=70% & <= 130% or within Spec. range	N/A
10) All results within calibration range	Yes

QA Comments:

Samples were spiked at 0.60 ppb.

DK34203 had a %CV above 15% and will be re-tested again next week.

No other issues to report.

Appendix 9.3 Checklists for QA Review of Data

Total Microcystins and Nodularins in Drinking Water and Ambient Water by ADDA ELISA

Checklist for QA Review of Data in Compliance with EPA Method 546

Decision Tree

1. Is % Coefficient of Variance (% CV) of the replicate absorbances of each calibration standard <10%?

Note: one pair is allowed to exceed 10% but must be < or = 15%.

2. Is the R^2 of the calibration curve **0.98** or better?

Note: If 1. or 2. is no, then the run is **rejected**.

3. Are the results for the 2 LRB(s) in the batch < 0.15 ppb ($\frac{1}{2}$ the MRL of 0.3 ppb)?

If no, then all samples that yielded a positive result in the Analysis Batch are **invalid**.

4. Is the Low-CV (0.3 ppb) greater than, or equal to 50% (0.15) and less than, or equal to 150% (0.45) of its true value?

If no, then the entire Analysis Batch is **invalid**.

5. Does the %CV exceed 15% for a field sample or QC sample?

If yes, then those samples are **rejected**.

6. Are the percent recoveries for each of the LFB(s) (at least 2 are required with each batch) greater than, or equal to 60%, and less than, or equal to, 140% of their true value?

If No for any LFB, then the entire Analysis Batch is **invalid**.

7. Are the mean % rec. for each LFSM and LFSMD greater than, or equal to, 60% and less than, or equal to, 140% of the true value?

8. Is the RPD for each LFSM and LFSMD (or MS/MSD) less than, or equal to, 40%?

Note: If 7. or 8. is no, but 6. is yes, then the precision may be matrix based.

Qualify the result for the sample from which the LFSM was prepared as "**suspect-matrix**".

9. Is the percent recovery for the QCS (**Note: this is only for IDC and once with each new lot of calibration standards**) greater than, or equal to 70% and less than, or equal to 130%?

If no, the Analysis Batch is **rejected**.

BMAA-L, Saxitoxin, Anatoxin-a, and Cylindrospermopsin in Drinking Water and Ambient Water by ELISA

Checklist for QA Review of Data (based off of EPA Method 546)

Decision Tree

1. Is % Coefficient of Variance (% CV) of the replicate absorbances of each calibration standard <10%?

Note: one pair is allowed to exceed 10% but must be < or = 15%.

2. Is the R^2 of the calibration curve **0.98** or better?

Note: If 1. or 2. is no, then the run is **rejected**.

3. Are the results for each LRB in the batch < MRL?

If no, then all samples that yielded a positive result in the Analysis Batch are **invalid**.

4. N/A

5. Does the %CV exceed 15% for a field sample or QC sample?

If yes, then those samples are **rejected**.

6. Are the percent recoveries for each of the LFB(s) greater than, or equal to 60%, and less than, or equal to, 140% of their true value?

If No for any LFB, then the entire Analysis Batch is **invalid**.

7. Are the mean % rec. for each LFSM and LFSMD greater than, or equal to 60% and less than, or equal to 140% of the true value?

8. Is the RPD for each LFSM and LFSMD (or MS/MSD) less than, or equal to, 40%?

Note: If 7. or 8. Is no, but 6. is yes, then the precision may be matrix based.

Qualify the result for the sample from which the LFSM was prepared as "**suspect-matrix**".

9. Is the result for the QCS within kit range specifications?

If no, the Analysis Batch is **rejected**.

Appendix 9.4 Examples of Final Reports



Microcystins ELISA Summary Report

Office of Water Quality - Watershed Assessment and Planning Branch

Sample #	Location	Date Collected	Date Analyzed	Conc. (ppb)	Percent Recovery (%)
MCT 546 LRB 1	Lab Reagent Blank 1	5/14/2019	5/16/2019	< 0.30	
MCT 546 LFB 1	Lab Fortified Blank 1	5/14/2019	5/16/2019	0.491	81.8
AB34808	Raccoon Lake SRA	5/13/2019	5/16/2019	< 0.30	
AB34809	Fairfax SRA	5/13/2019	5/16/2019	< 0.30	
AB34811	Starve Hollow SRA	5/13/2019	5/16/2019	< 0.30	
AB34812	Whitewater Memorial SP	5/14/2018	5/16/2019	< 0.30	
AB34813	Quakertown SRA	5/14/2018	5/16/2019	< 0.30	
AB34814	Deam Lake SRA	5/14/2018	5/16/2019	< 0.30	
AB34815	Hardy Lake SRA	5/14/2018	5/16/2019	< 0.30	
AB34816	Mounds SRA	5/14/2018	5/16/2019	< 0.30	
AB34817	Fairfax SRA (Field Duplicate)	5/13/2019	5/16/2019	< 0.30	
AB34818	Field Blank	5/13/2019	5/16/2019	< 0.30	
MCT 546 LFB 2	Lab Fortified Blank 2	5/14/2019	5/16/2019	0.555	92.5
MCT 546 LRB 2	Lab Reagent Blank 2	5/14/2019	5/16/2019	< 0.30	



Test Report (by Request)

Test Information

Request: 5/16/2019 8:51:55 AM
 Date: 5/16/2019 - 5/16/2019

Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference
MCT Std 0	MICROCYSTINS ADDA 546	1.527 Abs	0.000 µg/L	R ² =0.99769	0.000
MCT Std 0	MICROCYSTINS ADDA 546	1.406 Abs [1.4665] (5.8 CV)	0.019 µg/L [0.009] (14.1)	R ² =0.99769	0.000
MCT Std 1	MICROCYSTINS ADDA 546	1.148 Abs	0.146 µg/L	R ² =0.99769	0.150
MCT Std 1	MICROCYSTINS ADDA 546	1.178 Abs [1.1630] (1.8 CV)	0.126 µg/L [0.136] (10.4)	R ² =0.99769	0.150
MCT Std 2	MICROCYSTINS ADDA 546	0.898 Abs	0.381 µg/L	R ² =0.99769	0.400
MCT Std 2	MICROCYSTINS ADDA 546	0.810 Abs [0.8540] (7.3 CV)	0.512 µg/L [0.447] (20.7)	R ² =0.99769	0.400
MCT Std 3	MICROCYSTINS ADDA 546	0.610 Abs	1.017 µg/L	R ² =0.99769	1.000
MCT Std 3	MICROCYSTINS ADDA 546	0.618 Abs [0.6140] (0.9 CV)	0.988 µg/L [1.002] (2.0)	R ² =0.99769	1.000
MCT Std 4	MICROCYSTINS ADDA 546	0.497 Abs	1.574 µg/L	R ² =0.99769	2.000
MCT Std 4	MICROCYSTINS ADDA 546	0.442 Abs [0.4695] (8.3 CV)	2.005 µg/L [1.789] (17.0)	R ² =0.99769	2.000
MCT Std 5	MICROCYSTINS ADDA 546	0.265 Abs	> 5.000 µg/L		5.000
MCT Std 5	MICROCYSTINS ADDA 546	0.280 Abs [0.2725] (3.9 CV)	> 5.000 µg/L		5.000
MCT 546 LRB 1	MICROCYSTINS ADDA 546	1.347 Abs	0.041 µg/L		
MCT 546 LRB 1	MICROCYSTINS ADDA 546	1.319 Abs [1.3330] (1.5 CV)	0.052 µg/L [0.047] (16.7)		
MCT 546 Low-CV	MICROCYSTINS ADDA 546	0.980 Abs	0.286 µg/L		
MCT 546 Low-CV	MICROCYSTINS ADDA 546	0.950 Abs [0.9650] (2.2 CV)	0.318 µg/L [0.302] (7.5)		
MCT 546 LFB 1	MICROCYSTINS ADDA 546	0.882 Abs	0.402 µg/L		
MCT 546 LFB 1	MICROCYSTINS ADDA 546	0.772 Abs [0.8270] (9.4 CV)	0.581 µg/L [0.491] (25.8)		
AB38408	MICROCYSTINS ADDA 546	1.286 Abs	0.068 µg/L	LOW	0.300 - 5
AB38408	MICROCYSTINS ADDA 546	1.309 Abs [1.2975] (1.3 CV)	0.057 µg/L [0.063] (12.4)	LOW [LOW]	0.300 - 5
AB38409	MICROCYSTINS ADDA 546	1.344 Abs	0.042 µg/L	LOW	0.300 - 5
AB38409	MICROCYSTINS ADDA 546	1.306 Abs [1.3250] (2.0 CV)	0.058 µg/L [0.050] (22.6)	LOW [LOW]	0.300 - 5
AB38411	MICROCYSTINS ADDA 546	1.390 Abs	0.024 µg/L	LOW	0.300 - 5
AB38411	MICROCYSTINS ADDA 546	1.348 Abs [1.3690] (2.2 CV)	0.040 µg/L [0.032] (35.4)	LOW [LOW]	0.300 - 5
AB38412	MICROCYSTINS ADDA 546	1.400 Abs	0.021 µg/L	LOW	0.300 - 5
AB38412	MICROCYSTINS ADDA 546	1.352 Abs [1.3760] (2.5 CV)	0.039 µg/L [0.030] (42.4)	LOW [LOW]	0.300 - 5
AB38413	MICROCYSTINS ADDA 546	1.237 Abs	0.092 µg/L	LOW	0.300 - 5
AB38413	MICROCYSTINS ADDA 546	1.360 Abs [1.2985] (6.7 CV)	0.035 µg/L [0.064] (63.5)	LOW [LOW]	0.300 - 5
AB38414	MICROCYSTINS ADDA 546	1.316 Abs	0.054 µg/L	LOW	0.300 - 5
AB38414	MICROCYSTINS ADDA 546	1.172 Abs [1.2440] (8.2 CV)	0.130 µg/L [0.092] (58.4)	LOW [LOW]	0.300 - 5
AB38415	MICROCYSTINS ADDA 546	1.298 Abs	0.062 µg/L	LOW	0.300 - 5
AB38415	MICROCYSTINS ADDA 546	1.167 Abs [1.2325] (7.5 CV)	0.133 µg/L [0.097] (51.5)	LOW [LOW]	0.300 - 5
AB38416	MICROCYSTINS ADDA 546	1.482 Abs	0.000 µg/L	LOW	0.300 - 5
AB38416	MICROCYSTINS ADDA 546	1.497 Abs [1.4895] (0.7 CV)	0.000 µg/L [0.000]	LOW [LOW]	0.300 - 5
AB38417	MICROCYSTINS ADDA 546	1.372 Abs	0.031 µg/L	LOW	0.300 - 5
AB38417	MICROCYSTINS ADDA 546	1.410 Abs [1.3910] (1.9 CV)	0.017 µg/L [0.024] (41.2)	LOW [LOW]	0.300 - 5
AB38418	MICROCYSTINS ADDA 546	1.435 Abs	0.009 µg/L	LOW	0.300 - 5
AB38418	MICROCYSTINS ADDA 546	1.421 Abs [1.4280] (0.7 CV)	0.014 µg/L [0.012] (30.7)	LOW [LOW]	0.300 - 5
MCT 546 LFB 2	MICROCYSTINS ADDA 546	0.794 Abs	0.540 µg/L		0.300 - 5
MCT 546 LFB 2	MICROCYSTINS ADDA 546	0.778 Abs [0.7860] (1.4 CV)	0.570 µg/L [0.555] (3.8)		0.300 - 5
MCT LRB 2	MICROCYSTINS ADDA 546	1.322 Abs	0.051 µg/L	LOW	0.300 - 5
MCT LRB 2	MICROCYSTINS ADDA 546	1.391 Abs [1.3565] (3.6 CV)	0.024 µg/L [0.038] (50.9)	LOW [LOW]	0.300 - 5

Note

Signature *David Jordan*
 Date: 5/16/2019



MICROCYSTINS ADDA 546 - Assay Calibration Report

Assay Information

Assay Name: MICROCYSTINS ADDA 546
 Version: 1
 Temperature: Room Temperature
 Last Modified By: Security disabled
 Units: µg/L
 Assay Description:

Assay Mode: 4-Parameter Logistic Weight by:None
 Well Type: Flat bottom
 Last Modified On: 5/9/2019 11:43:40 AM
 Normal: 0.300 - 5.000
 # of decimals: 3

Assay Substances:

Controls:
 MCT 546 LRB 1
 MCT 546 Low-CV
 MCT 546 LFB 1
 Standards:
 MCT Std 0, Concentration = 0.000, Minimum number to use: 2
 MCT Std 1, Concentration = 0.150, Minimum number to use: 2
 MCT Std 2, Concentration = 0.400, Minimum number to use: 2
 MCT Std 3, Concentration = 1.000, Minimum number to use: 2
 MCT Std 4, Concentration = 2.000, Minimum number to use: 2
 MCT Std 5, Concentration = 5.000, Minimum number to use: 2
 Curve valid interval: 7 days 0 hours
 Axis Mode: Y = Abs, X = Log(Conc)

Assay Calibration

Current Calibration Status: "

Name	Absorbance	Concentration	Interpretation	Position
5/16/2019 8:51:55 AM				
MCT Std 0	1.527 Abs	0.000 µg/L	R ² =0.99769	RK1:23--A01@2
MCT Std 0	1.406 Abs [1.4665] (5.8 CV)	0.019 µg/L [0.009] (141.4 CV)	R ² =0.99769	RK1:23--B01@2
MCT Std 1	1.148 Abs	0.146 µg/L	R ² =0.99769	RK1:24--C01@2
MCT Std 1	1.178 Abs [1.1630] (1.8 CV)	0.126 µg/L [0.136] (10.4 CV)	R ² =0.99769	RK1:24--D01@2
MCT Std 2	0.898 Abs	0.381 µg/L	R ² =0.99769	RK1:25--E01@2
MCT Std 2	0.810 Abs [0.8540] (7.3 CV)	0.512 µg/L [0.447] (20.7 CV)	R ² =0.99769	RK1:25--F01@3
MCT Std 3	0.610 Abs	1.017 µg/L	R ² =0.99769	RK1:26--G01@3
MCT Std 3	0.618 Abs [0.6140] (0.9 CV)	0.988 µg/L [1.002] (2.0 CV)	R ² =0.99769	RK1:26--H01@3
MCT Std 4	0.497 Abs	1.574 µg/L	R ² =0.99769	RK1:27--A02@2
MCT Std 4	0.442 Abs [0.4695] (8.3 CV)	2.005 µg/L [1.789] (17.0 CV)	R ² =0.99769	RK1:27--B02@2
MCT Std 5	0.265 Abs	> 5.000 µg/L		RK1:28--C02@2
MCT Std 5	0.280 Abs [0.2725] (3.9 CV)	> 5.000 µg/L		RK1:28--D02@2

5/16/2019 8:51:55 AM				
MCT 546 LRB 1	1.347 Abs	0.041 µg/L		RK1:29--E02@2
MCT 546 LRB 1	1.319 Abs [1.3330] (1.5 CV)	0.052 µg/L [0.047] (16.7 CV)		RK1:29--F02@3
MCT 546 Low-CV	0.980 Abs	0.286 µg/L		RK1:30--G02@3
MCT 546 Low-CV	0.950 Abs [0.9650] (2.2 CV)	0.318 µg/L [0.302] (7.5 CV)		RK1:30--H02@3
MCT 546 LFB 1	0.882 Abs	0.402 µg/L		RK1:31--A03@2
MCT 546 LFB 1	0.772 Abs [0.8270] (9.4 CV)	0.581 µg/L [0.491] (25.8 CV)		RK1:31--B03@2

Statistic				
MCT Std 0 [MEAN]	1.4665	0.0095		
MCT Std 0 [SD]	0.0856	0.0134		
MCT Std 0 [%CV]	5.8343	141.4214		
MCT Std 1 [MEAN]	1.1630	0.1360		
MCT Std 1 [SD]	0.0212	0.0141		
MCT Std 1 [%CV]	1.8240	10.3986		
MCT Std 1 [%DIFF]		-9.3333		
MCT Std 2 [MEAN]	0.8540	0.4465		
MCT Std 2 [SD]	0.0622	0.0926		
MCT Std 2 [%CV]	7.2863	20.7460		
MCT Std 2 [%DIFF]		11.6250		
MCT Std 3 [MEAN]	0.6140	1.0025		
MCT Std 3 [SD]	0.0057	0.0205		
MCT Std 3 [%CV]	0.9213	2.0455		
MCT Std 3 [%DIFF]		0.2500		
MCT Std 4 [MEAN]	0.4695	1.7895		

*Generated by software version (B.4.1.943/1065/1.000.95) 5/16/2019 12:46:51 PM

Appendix 9.5 Sample Preservation, Storage, and Handling Quick Reference Guide.



Cyanotoxins in Water

Sample Collection Quick Reference Guide for ELISA Plate Assays

Analyte	Collection/Storage Container <small>Use with PTFE lined screw caps</small>	Sample Preservation**	Storage & Handling
Anabaenopeptins	<ul style="list-style-type: none"> Glass – clear or amber Polyethylene terephthalate glycol (PETG) 	Treated water: <i>Immediately upon collection</i> , quench chlorine with ascorbic acid or sodium thiosulfate* (up to 1 mg/mL; recommended concentration 0.1 mg/mL)	Analyze immediately, refrigerate (4 °C) for up to 5 days, or freeze (-20 °C) Ship overnight on ice
Anatoxin-a	<ul style="list-style-type: none"> Glass – amber only, do NOT use clear glass <i>Anatoxin-a is light sensitive – avoid exposure to light, as this will degrade the toxin and produce inaccurate results</i> 	<p>Treated water: <i>Immediately upon collection</i>, quench chlorine with ascorbic acid* (up to 1 mg/mL; recommended concentration 0.1 mg/mL). Do NOT use sodium thiosulfate, as it will degrade the toxin and produce inaccurate results</p> <p>Freshwater (treated & raw): <i>Immediately upon collection</i>, preserve the sample by diluting 9 parts sample to 1 part 10x concentrated sample diluent & adjust pH to between 5-7 using 1N NaOH or HCl‡</p> <p>Marine water: Adjust pH to between 5-7 using 1N NaOH or HCl‡</p> <p>‡Avoid exposure to high pH conditions, as this will degrade the toxin and produce inaccurate results</p>	Analyze immediately, refrigerate (4 °C) for up to 28 days, or freeze (-20 °C) if greater than 28 days Ship overnight on ice
BMAA	<ul style="list-style-type: none"> Glass – clear only, do NOT use amber glass <i>Toxin will adsorb to amber glass, producing inaccurate results</i> Polyethylene terephthalate glycol (PETG) High density polyethylene (HDPE) Polycarbonate (PC) Polypropylene (PP) Polystyrene (PS) 	All samples: Analyze samples <i>immediately upon collection</i> or freeze (-20 °C) to avoid degradation of the toxin, which will produce inaccurate results	Analyze or freeze (-20 °C) immediately upon collection Ship overnight on ice
Cylindrospermopsin	<ul style="list-style-type: none"> Glass – clear or amber Polyethylene terephthalate glycol (PETG) High density polyethylene (HDPE) Polycarbonate (PC) Polypropylene (PP) Polystyrene (PS) 	Treated water: <i>Immediately upon collection</i> , quench chlorine with ascorbic acid or sodium thiosulfate* (up to 1 mg/mL; recommended concentration 0.1 mg/mL)	Analyze immediately, refrigerate (4 °C) for up to 5 days, or freeze (-20 °C) Ship overnight on ice
Microcystins/ Nodularins	<ul style="list-style-type: none"> Glass – clear or amber Polyethylene terephthalate glycol (PETG) <i>Avoid plastic containers other than PETG, as toxin will adsorb to container surfaces, producing inaccurate results</i> 	Treated water: <i>Immediately upon collection</i> , quench chlorine with sodium thiosulfate* (up to 1 mg/mL; recommended concentration 0.1 mg/mL). Do NOT use ascorbic acid, as this may degrade the toxin and produce inaccurate results	Analyze immediately, refrigerate (4 °C) for up to 5 days, or freeze (-20 °C) Ship overnight on ice
Saxitoxins	<ul style="list-style-type: none"> Glass – clear or amber Polyethylene terephthalate glycol (PETG) High density polyethylene (HDPE) Polycarbonate (PC) Polypropylene (PP) Polystyrene (PS) 	<p>Treated water: <i>Immediately upon collection</i>, quench chlorine with ascorbic acid or sodium thiosulfate* (up to 1 mg/mL; recommended concentration 0.1 mg/mL)</p> <p>Freshwater (treated & raw): <i>Immediately upon collection</i>, preserve the sample by diluting 9 parts sample to 1 part 10x concentrated sample diluent</p> <p>Marine water: None required</p>	Analyze immediately, refrigerate (4 °C) for up to 5 days, or freeze (-20 °C) Ship overnight on ice

*The use of solid quenching reagents is recommended. Note: EPA Method 545 prohibits the use of liquid reagent solutions for sample quenching.

**Additional sample preparation such as cell lysis and filtration may be required prior to analysis depending on analytical objectives (e.g. "total" versus "free" toxin). Refer to specific kit user's guide for additional information.
 DISCLAIMER: Current recommendations by applicable regulatory agencies should always take precedence and should be followed in the event of a conflict.