APPENDIX J

Standard Operating Procedures for Algal Samples

Procedure	Last Revised
Protocol for Algal Sample Collection (HAB-001)	05/10/22
Initial Processing of Algal Samples (HAB-002)	05/10/22
Microcystin Testing Using ELISA Coated Tube Kit (HAB-003)	05/10/22

PROTOCOL FOR ALGAL SAMPLE COLLECTION (HAB-001)

I. INTRODUCTION

A. Purpose

The following paragraphs describe the procedures for the collection, preservation, and transport of algal samples from water bodies with a validated HAB complaint. Exact collection bottles can vary, and the specifics of any instance will be communicated to the sampler.

B. Minimum Staff Qualifications

Personnel implementing this SOP should be an employee of KDHE or a partner agency who has had training/experience in ambient water sampling and field measurement. They must also receive training from KDHE personnel for HAB sample collection protocols.

C. Equipment/Accessories

1. Cubitainers – 1 per sample location
2. Amber Glass Preserved, Chilled Anatoxin-Saxitoxin Sampling Bottle – 1 per proxy sample station (“proxy” stations are those associated with raw water intakes for Public Water Supplies)
3. Small, plastic, Microcystin-Cylindrospermopsin Sampling Bottle – 1 per proxy sample station
4. Nutrient Bottle - 1 per HAB event (when nutrient samples are requested)
5. Waterproof permanent marker
6. Pole sampler with attached plastic beaker
7. GPS unit or smartphone with GPS capability
8. Camera or smartphone with camera
9. Paper Towels
10. Cooler
11. Cold packs or ice
12. Zip lock plastic bags for containment of concentrated samples
13. Tap or distilled water
14. Large trash bags

15. Map of area

II. PROCEDURES

A. Collection of Algal Samples - (one cubitainer per site)

NOTE: No samples should be taken early morning, if possible, as blooms often sink below the surface overnight and will not surface until late morning.

1. Take site photos, some close-up of the water and some facing out onto the open water;
2. Fill out Chain of Custody Form with necessary information;
3. Using a pole sampler with an attached beaker, either a “grab” or “composite” sample can be collected.

   a. *“Grab” Samples:*

   1) Verify that the designated sampling location(s) where a sample is to be collected is representative of a major public access area where the public will have full access to the water.

   2) Mark the cubitainer (clear, plastic, 1-liter cube shaped bottle, non-preserved) with the following information:

   a. Sample Station I.D. number

   b. Sample Collection Time

   c. Sample Collection Date

   d. Sample Collector’s Name or Initials

   3) Inflate the cubitainer. This can be done by removing the cap and gently exhaling into the opening until it completely inflates. (Do not contaminate the cube by expelling saliva into the container.)

   4) Submerge the pole sampler’s beaker open end down through the water until completely submerged. Slowly turn the sample pole until the beaker is sideways and approximately 1-2” under the water’s surface. Once the beaker is filled, pull the sample pole and attached beaker out of the water.

   5) Pour collected sample into the inflated cubitainer. It generally takes two of these processes to fill one cubitainer.

   6) Leave a headspace in the container of at least ½” for gas expansion.

   7) Secure the lid and wipe the cubitainer until it is clean and dry. Place in a plastic zip lock bag and then in a cooler.

   8) Move to your next sampling location.

   9) If sample collection is required in the future at that same public access area, then collect as close as possible to that exact point. (Do not “chase the

scum" that might appear a few feet away from your original sampling point.) This will maintain consistency of the sampling activities.

b. *"Composite" Samples:*

1) Verify that the designated sampling location(s) where a sample is to be collected is representative of a major public access area where the public will have full access to the water.

2) Mark the cubitainer (clear, plastic, 1-liter cube shaped bottle, non-preserved) with the following information:

a. Sample Station I.D. number

b. Sample Collection Time

c. Sample Collection Date

d. Sample Collector's name or initials

3) Inflate the cubitainer. Inflating the cubitainer can be done by removing the cap, gently exhaling into the opening until it completely inflates. (Do not contaminate the cube by expelling salvia into the container.)

4) Submerge the pole sampler's beaker open end down through the water until completely covered. Slowly turn the sample pole until the beaker is sideways and approximately 1-2" under the water's surface. Once the beaker is filled pull the sample pole and attached beaker out of the water.

5) Pour collected sample into a clean bucket. Continue this process collecting samples from various locations around the dock, (*e.g.,* one from each side of the dock).

6) Once all samples have been collected and poured into the bucket, swirl the bucket to mix.

7) Pour the mixed sample into the inflated cubitainer.

8) Leave a headspace in the container of at least ½" for gas expansion. Secure lid, wipe cubitainer clean, and place it inside a plastic bag.

9) If sample collection is required in the future at that same public access area, then collect as close as possible to that exact initial point. (Do not "chase the scum" that might appear a few feet away from your original sampling point.) This will maintain consistency of the sampling activities.

B. How to Collect Water Samples for Drinking Water Analysis – (Anatoxin-Saxitoxin and Microcystin-Cylindrospermopsin for KHEL):

1. Using a pole sampler with an attached beaker or bucket with rope and swivel, a proxy sample can be collected.

2. Verify that the designated sampling location(s) is representative of where a public water supply system would be pulling their raw water samples.

3. Mark the sample bottles (amber glass preserved, chilled Anatoxin-Saxitoxin sampling bottle and a plastic, Microcystin-Cylindrospermopsin sampling bottle) with the following information:

   a. Sample location I.D. number

   b. Sample Collection Time

   c. Sample Collection Date

   d. Sample Collector's Name or Initials

4. Remove the cap.

5. Submerge the pole sampler's beaker open end down through the water until completely covered. Slowly turn the sample pole until the beaker is sideways and approximately 1-2" under the water's surface. Once the beaker is filled pull the sample pole and attached beaker out of the water. Do the same process for a bucket and rope.

6. Pour collected sample into the bottles, but be sure not to overfill as this will spill the preservatives.

7. Leave a headspace in the container of no less than ½" for gas expansion.

8. Secure the lid and wipe the bottle until it is clean and dry. Place in a cooler.

9. Move to your next sampling location.

10. If sample collection is required in the future at that same public access area, then collect as close as possible to that exact initial point. (Don't chase scum that might appear a few feet away from your original sampling point.) This will maintain consistency of the sampling activities.

C. How To Collect Nutrient Samples - (one Cubitainer and one Nutrient bottle per site):

1. Using a clean pole sampler with an attached beaker, collect a "grab" sample.

   a. Nutrient samples will be collected only from designated locations believed representative of the lake's normal ambient sampling station. Verify that the location where the sample is to be collected is at the normal ambient sampling station or as close as possible to that station location.

4. Mark both the nutrient container, (brown, opaque, plastic 250 milliliter bottle, Sulfuric Acid preserved) and a cubitainer with the following information:

   a. Sample Location I.D. number

   b. Sample Collection Time

   c. Sample Collection Date

   d. Sample Collector's name or initials

5. Submerge the beaker's open end down through the water until completely covered. Slowly turn the sample pole until the beaker is sideways and approximately 1-2" under the water's surface. Once the beaker is filled pull the sample pole and attached beaker out of the water.
6. Carefully open the Nutrient bottle. (The bottle contains a Sulfuric Acid preservative and will burn the skin or clothing.)
7. Slowly pour collected sample into the bottle making sure not to overfill, as a loss of preservative will affect the integrity of the sample.
8. Secure the lid tightly and wipe clean and dry. Place the sample container in a zip lock bag and secure bag. (Placing the container in its own individual bag will minimize the potential of cross contamination).
9. Follow the "How to Collect Phytoplankton Samples" directions to fill the cubitainer for additional nutrient parameters.
10. Place containers in a cooler.

*****REMINDER: All nutrient samples collected should be taken 1 to 2 inches under the water surface. Samples do not need to be taken at deeper depths when sampling for blue-green algae. Do not "skim" from the surface (the air/water interface).**

D. Cleaning Equipment Between Samples:

1. Between Sampling Locations at the Same Waterbody
   - **a.** After the sample has been collected, discard any remaining water from sampling equipment.
   - **b.** Wipe all equipment dry with paper towel
   - **c.** Once at the next sampling location, rinse sampling equipment with ambient water from a location that is different from but near the location where the sample is to be collected.
   - **d.** Discard rinse water, then move to sampling location to collect sample.
2. Between Different Water Bodies
   - **a.** Clean all sampling equipment with tap water. Be especially diligent to wash thoroughly if the equipment was used in a water body known or suspected to have zebra muscles.
   - **b.** Once at sampling location, rinse sampling equipment several times with ambient water at a location that is different from but near the location where the sample is to be collected.
   - **c.** Discard rinse water, then move to sampling location to collect sample.
   - **d.** Follow the above directions if additional samples are to be collected at the same waterbody.

E. Preparing Samples for Shipment:

1. Wipe dry the exterior of all containers and ensure that lid is secured tightly. Place container inside zip lock plastic bag if the sample is being shipped via commercial courier, or if the sample is sufficiently concentrated that could contaminate the cooler or harm the recipient if it leaks

2. Place all containers in a cooler along with a cold pack(s). If ice is used, double bag it first before placing in the cooler. This will minimize the potential of cross-contamination or making a mess when the ice melts. Plastic pop bottles filled with water and then frozen can also be used in place of cold packs. Place enough cold packs or ice in the cooler to keep the samples cool. **DO NOT FREEZE THE SAMPLES.**

3. Fill out the algae and/or the KHEL PWS submission/chain-of-custody form (see **Appendix E**). When filling out the algae sampling form, include environmental conditions at time of sampling, *e.g.,* water and air temperature, wind speed/direction, cloud cover, any unusual conditions.

4. If the samples are to be delivered by a carrier company, then place the submission form(s) inside a plastic bag and tape to the inside the lid of the cooler.

5. If samples are to be transported to the lab(s) by the collector or handed off to another partner, then place the submission form(s) inside a plastic bag and tape to the OUTSIDE the cooler so it is readily accessible for additional chain of custody signatures.

6. Secure the cooler.

D. Shipment of Samples:

1. Samples must be shipped no later than overnight.

2. At any time that the samples exchange to a different handler, then the chain-of-custody must be signed. Exception is if shipment is sent by commercial carrier, then note which company the samples were “relinquished to …”

3. All algae samples are to be delivered or sent to:

Kansas Dept of Health and Environment
BOW-MASS HAB Response Program
1000 SW Jackson
Suite 420
Topeka, Kansas 66612-1367

4. All nutrient samples are to be sent to:

KHEL Laboratories
Forbes Field, Building 740
Topeka, Ks. 66620-0001

5. If necessary, Public Drinking Water samples will be taken to the Kansas Health and Environmental Lab by BOW/PWS staff, for those not analyzed by BOW-MASS.

If samples are shipped by commercial carrier, minimize shipping costs by sending all samples to BOW/BEFS, including nutrient samples, and program staff will carry the nutrient samples to the lab. Send emails at shipping time to confirm this arrangement.

Further questions? Contact:

HAB response team:
Josh Cullum – (785) 296-0079
Katlynn Decker (785) 296-5580
Elizabeth Smith – (785) 296-4332
Taxonomy and toxin analysis:
Britini Jacobs – (785) 296-5576
Tony Stahl – (785) 296-5578
Policy:
Tom Stiles 785-296-5500

INITIAL PROCESSING OF ALGAL SAMPLES (HAB – 002)

I. INTRODUCTION

A. Purpose

The following paragraphs describe the procedure for the collection, preservation, and transport of algal samples from water bodies with a validated HAB complaint.

B. Minimum Staff Qualifications

Personnel implementing this SOP should meet requirements for State of Kansas Environmental Associate job class and be experienced in the measurement of the physicochemical and microbiological properties of surface water and the performance of environmental field investigations.

C. Equipment/Accessories

1. Lugol's solution

2. Sample bottles with lids

3. Yellow round labels

4. Chain of Custody sheet

II. PROCEDURES

A. Receiving samples

1. Coolers containing samples will be directed to BOW-MASS HAB Response Team or Analytical Support staff. This is usually around 10:00 to 10:30 a.m. on Tuesday or Wednesday, depending upon the carrier bringing in the samples.

2. Upon receiving the coolers, remove the samples and paperwork.

3. Place samples in refrigerator in the Algal ID lab until ready to process. Make sure the refrigerator door closes completely.

4. On the back of the Algae Sample Submission Form (see **Appendix E**) and under the Chain of Custody section, date and sign by the Received By line. Also note whether the sample was cold when received.

5. The Algae Sample Submission Forms are to be kept with the samples and are placed by the algal taxonomist's scope. Once sample analysis is completed, then the Sample Submission Form is to be filed by year with the lake reports.

B. Algal Preservation

1. When ready to process samples, remove them from the refrigerator and arrange on the lab bench in the order in which they will be processed. Take a photo of the samples from each waterbody.

2. In the drawer directly to the left of the sink, remove the round stickers and a pencil to mark each bottle used to preserve samples.

3. In the chlorophyll lab room (small room) and directly to the left of the door on a cart, remove one bottle for each sample to be preserved (or two bottles, if live samples are also to be retained). Lids to the bottles are in the far-right bottom cabinet and on the bottom shelf.

4. Line the bottles and lids up on the counter by the refrigerator in the Algal ID lab. Write on the stickers the lake number and sampling point (for example – Milford would be 190 as the lake number and AA is one of the sample points). Place the stickers on top of the lids prior to preserving sample.

5. In the same room and under the sink is the brown Lugol's container. It has a small needleless syringe attached to it. Place 5 drops of Lugol's in each bottle. Be careful with this as Lugol's does stain just about everything it comes in contact with, including the counter, hands, and clothes. Place bottle back under the sink.

6. Take the first sample and shake cubitainer vigorously to thoroughly mix the water and contents. Over the sink, carefully pour sample water into one of the small bottles containing the Lugol's until it is about to the line where the neck begins (about ¼ inch from the top). Double check that the lid you put on the bottle matches the sample you poured into it. Screw that lid onto the bottle securely.

7. Optional step: along with each preserved sample, aliquot another sample into a second vial, label it the same, and place into the refrigerator so that live sample can be compared to preserved.

8. A portion of the remaining sample will need to be frozen to conduct the ELISA toxicity test, so pour a good portion of the sample down the drain but retain at least a ½ inch in the bottom of the cubitainer.

9. Continue this process with all the remaining samples.

10. Place the preserved samples on the table with the algal taxonomist's microscope.

11. Place the cubitainers with the saved water into the ultracold freezer..

12. Scan the chain of custody forms (in IT office or upstairs, or through OneDrive scan to PDF function on an agency mobile hone) for electronic records retention, and return hard copy form to the algal taxonomists' bench.

MICROCYSTIN TEST USING ABRAXIS ELISA COATED TUBE TESTS OR EQUIVALENT (HAB – 003)

I. INTRODUCTION

A. Purpose

The following paragraphs describe the procedures to test water samples for the presence and quantification of microcystins.

B. Minimum Staff Qualifications

Personnel implementing this SOP should be experienced in the measurement of the physicochemical and microbiological properties of surface water.

C. Equipment/Accessories

1. ABRAXIS ELISA Coated Tube Test
2. Test tube rack to place tubes in for analysis
3. Timer
4. Use of water in sink area
5. Thawed water samples from freezer of lakes that are under investigation
6. Paper and Pencil
7. ABRAXIS Model 6+ Colorimeter
8. Kimwipes
9. Adjustable pipette (0.1 – 1.0 mL) and disposable tips

II. PROCEDURES

A. Preliminary procedures:

1. Remove frozen samples from freezer to begin thawing, ensuring that all samples have returned to room temperature before starting the process.
2. Read all the instructions before running the kit.
3. Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed tubes and reagents at room temperature – do not remove tubes from bag with desiccant until they have warmed up).
4. Organize all samples and reagents so that steps can be performed with as few delays as possible.
5. Do not run more than 12 tubes at a time.

B. Dilutions

1. To complete the assay in a timely manner, it may be helpful to dilute samples that are potentially high in toxin before testing them (*i.e.* based on previous week's analysis results, or based on visual appearance of sample). A dilution of 1:5 is often a good starting place; samples that do not fall within the absorbance range of the calibrators will need to be diluted at a different level.
2. A list of common dilutions is printed in the ELISA book, and include:

Ratio	Sample (mL)	DI Water (mL)	Dilution multiplier
1:1	0.5	0.5	x 2
1:2	0.5	1	x 3
1:3	0.5	1.5	x 4
1:4	0.5	2	x 5
1:5	0.5	2.5	x 6
1:6	0.5	3	x 7
1:7	0.5	3.5	x 8

3. Multiply the resultant concentration by the dilution multiplier to find the actual concentration of the sample.

C. Completing the assay.

1. Remove the required number of coated test tubes from the re-sealable aluminum pouch. Place the tubes in a rack capable of holding 12 mm test tubes securely and label appropriately.
2. Label appropriately so as not to mix up samples.
3. Make sure to run a set of standards for calibration curve.
4. Add 500 uL of enzyme conjugate solution to the coated tubes successively using a stepping pipette.
5. Add 500 uL of the standard solutions, controls, or samples into the appropriate coated tubes.
6. Add 500 uL of antibody solution to the coated tubes successively using a stepping pipette. Carefully vortex the tubes at a low speed for 1 to 2 seconds or swirl the tubes rapidly allowing the contents to mix and being careful not to spill or splash contents.
7. Incubate the tubes for 20 minutes at room temperature.
8. Decant the contents of the tubes by vigorously shaking into a sink. Blot the inverted tubes on absorbent paper towels. Flood the tubes with 5 mL of diluted (1x) washing

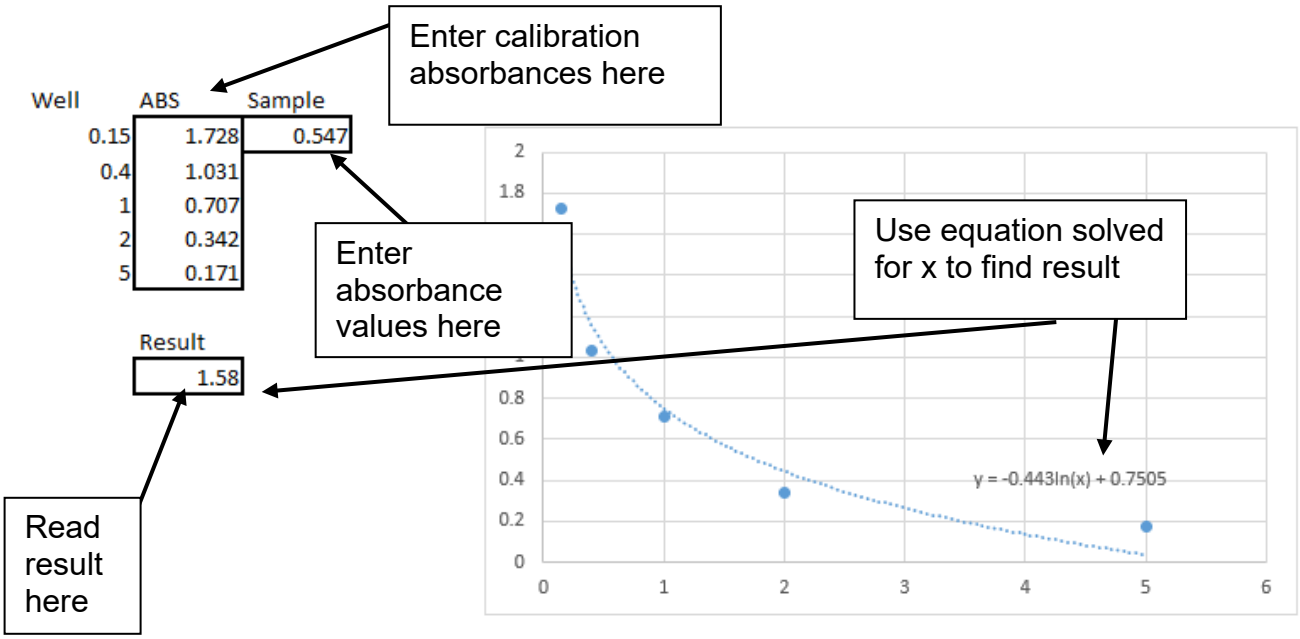
solution, decant by shaking vigorously into a sink, and blot the inverted tubes on absorbent paper towels. Repeat four times for a total of five washes.

9. Add 500 uL of substrate (Color) solution to the tubes. Carefully vortex the tubes at a low speed for 1 to 2 seconds or swirl the tubes rapidly, being careful not to spill or splash the contents.
10. Incubate the tubes for 20 minutes at room temperature. Protect the tubes from direct or indirect sunlight.
11. Add 500 uL of stop solution to the tubes in the same sequence as for the substrate solution.
12. Read the absorbance at 450 nm using a test tube photometer within 15 minutes after the addition of stopping solution.

D. Interpreting the results using a colorimeter.

1. Fill a blank tube half way with DI water.
2. Plug in the Colorimeter and wait for it to turn on and warm up (5 minutes).
3. Remove the meter cap. Wipe excess liquid and finger prints off tube using a Kimwipe. Place the tube in the reader.

4. Press the SEL/ESC button and ensure [1]BLANK is selected.
5. Check the blank by pressing the READ/ENTER button. The instrument will show "- - - -" followed by the results.
6. Remove the blank and place the samples/calibration tubes into the reader.
7. Press the SEL/ESC button and navigate to [4]SAMPLE. Agree to wipe previous data if asked.
8. Press the READ/ENTER button. The instrument will show "- - - -" followed by the results. Record the absorbance values in the Laboratory ELISA Assay Work Sheet.
9. Repeat steps 6-7 for the calibrator tubes prepared and all the samples. Record the absorbance values in the Laboratory ELISA Assay Work Sheet. Make sure to have all tubes and measurements properly labeled to their correct site.
10. To find the results, enter the absorbance values of the calibration tubes and the sample tubes into the Envirologix Interpolation excel spreadsheet. Calibration values do not change, but simply change out the "Sample" cell to see all values.
11. NOTE: the results cell must be updated every week using a reversed equation from the calibration curve. Once calibration ABS values are entered, the best fit curve on the graph will have a formula. Solve for x,and put this equation into the "Results" cell, with Y being the sample cell. This will allow you to input ABS and receive an answer back in ug/L of Microcystin.



	Lw Acc	Up Acc
Control = 0.75+/- 0.188	0.562	0.938
Std. 5 = 5.0 +/- 1.25	3.75	6.25

NOTE: For all diluted samples, the test results must be multiplied by the appropriate dilution factor to yield the final concentration of the original sample.