APPENDIX J

Standard Operating Procedures for Algal Samples

Procedure	Last Revised
Protocol for Algal Sample Collection (HAB-001)	03/20/20
Initial Processing of Algal Samples (HAB-002)	02/27/15
Microcystin Testing Using ELISA Qualitube Kit ET-022 or Equivalent (HAB-003)	04/01/19

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.

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 (TOC), Non-Preserved Bottle – 1 per BA sample station (“BA” stations are those associated with raw water intakes for Public Water Supplies)
3. Nutrient Bottle - 1 per HAB event (when nutrient samples are requested)
4. Waterproof permanent marker
5. Pole sampler with attached plastic beaker
6. GPS unit
7. Paper Towels
8. Cooler
9. Cold packs or ice
10. Zip lock plastic bags for ice if needed
11. Tap or distilled water
12. Large trash bags
13. Map of area
14. Personal protective supplies such as gloves, wash water, wading boots, etc.

II. PROCEDURES

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

1. 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.

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. Collection of Samples for Drinking Water Analysis – (one glass non-preserved TOC bottle):

1. Using a pole sampler with an attached beaker or bucket with rope and swivel, a BA 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 bottle (amber, glass, TOC bottle, non-preserved) 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 glass bottle.

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

C. Collection of 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.

2. 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

3. 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.
4. Carefully open the Nutrient bottle. (The bottle contains a Sulfuric Acid preservative and will burn the skin or clothing.)
5. Slowly pour collected sample into the bottle making sure not to overfill as a loss of preservative will affect the integrity of the sample.
6. 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).
7. Follow the "How to Collect Phytoplankton Samples" directions to fill the cubitainer for nutrients.
8. Place containers in a cooler.

*****REMINDER: All 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 collect the sample at 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 at a different, but nearby, 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.

   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 cubitainers.
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-WPMAS HAB 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-WPMAS.

If samples are shipped by carrier, to minimize shipping costs, send all samples to BOW/BEFS, including nutrient samples and we will carry the nutrient samples to the lab.

Further questions? Contact:

2020 HAB response team and sample analysis team:

Elizabeth Smith – (785) 296-4332
William Blair – (785) 296-0079
Britini Jacobs – (785) 296-5576
Layne Knight – (785) 291-3885
Tony Stahl – (785) 296-5578

Policy:

Tom Stiles 785-296-5500

HAB Hotline:

(785) 296-1664

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-WPMAS 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.

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 place on the black shelving unit in middle of Algal ID lab room in the order in which they will be processed.

2. In the drawer directly to the left of the sink, remove the yellow, 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. 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 yellow 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. A portion of the 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.
8. Continue this process with all the remaining samples.
9. Place the preserved samples on the table with the algal taxonomist's microscope.
10. Place the cubitainers with the saved water into the freezer in the main lab (middle) room. Make sure the lid to the freezer is closed tightly.

MICROCYSTIN TEST USING ELISA QUALITUBE KIT ET-022 OR EQUIVALENT (HAB – 003)

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.

B. Minimum Staff Qualifications

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

C. Equipment/Accessories

1. Envirologix qualitube kit for Microcystin.
2. Wooden block or 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. HACH Pocket Colorimeter™ II Analysis Systems 450 nm, 59530-45; with a 1 cm tube cell adapter
8. Kimwipes
9. Adjustable pipette (0.1 – 1.0 mL) and disposable tip

II. PROCEDURES

A. Preliminary procedures:

1. Remove frozen samples from freezer to begin thawing.
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 1 and 2 can be performed in 3 minutes or less.
5. Do not run more than 6 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. Place tubes in wooden tube rack. The Calibrator tubes are the two tubes behind the six in front. These will be the tubes used to compare color intensity between 0.5 ppb and 3.0 ppb results. See Photo below.

2. Rapidly add **5 drops** of Microcystin Assay ***diluent*** to each tube in the assay.
3. Using the sample pipette provided, immediately add **2 drops of 0.5 ppb Microcystin *calibrator*** to the first tube. Add 2 drops of 3.0 ppb **Microcystin *calibrator*** into the second tube. Add 2 drops of sample to each of the subsequent

tubes up to a total of 4 samples. **Do Not Add Microcystin-enzyme conjugate in this step.**

4. Thoroughly mix the contents of the tubes by moving the tube holder in a rapid circular motion on flat surface for a full 20 - 30 seconds.

5. Incubate tubes at ambient temperature for 5 minutes.

6. Add **5 drops of Microcystin-enzyme *conjugate*** to each tube. Do not empty the tub contents or wash the tubes at this time. Thoroughly mix the contents of the tubes as in step 3.

7. Incubate tubes at ambient temperature for 20 minutes.

8. After incubation, vigorously shake the contents of the tubes into a sink or other suitable container. Flood the tubes completely with cool tap water, and then shake to empty. Repeat this wash step 3 times. Invert the tubes on a paper towel and tap to remove as much water at possible.

9. Add **10 drops of *Substrate*** to each tube. Thoroughly mix the contents of the tubes, as in Step 3. Incubate substrate in tubes for 10 minutes at ambient temperature.

NOTE: If blue color does not develop in the 0.5 ppb Calibrator tube, the assay is invalid and should be repeated.

10. This assay is designed to be read visually with un-stopped tubes (blue solution). If tubes are to be read using the tube colorimeter, pipette 0.7 mL of Stop Solution into each tube and mix thoroughly. This will turn the tube contents yellow.

    a. Tubes should be read **within 30 minutes** of the addition of Stop Solution.

    b. Proceed to step E to interpret the results using a colorimeter.

11. Interpret the results of un-stopped tubes immediately following the 10-minute substrate incubation.

D. Interpreting the results visually.

1. Compare the intensity of the blue color of each sample tube to the intensity of the blue color in the 0.5 and 3.0 ppb calibrator tubes.

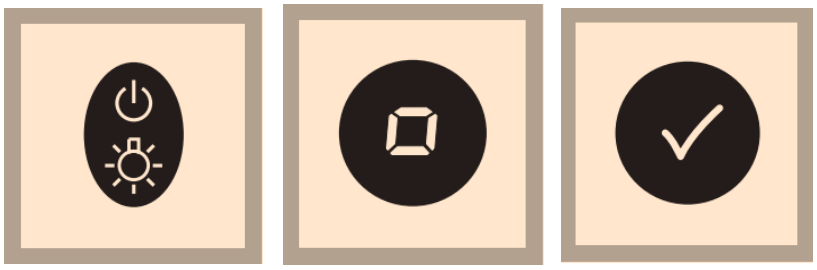
2. Score each sample tube as having less than, more than or equal color to the two calibrator tubes.

3. Use the Table below to determine the level of microcystin in the samples.

Samples with Optical Density (OD) values…..	**Contain…..**
Greater than OD of 0.5 ppb Calibrator	Less than 0.5 ppb Microcystin
Between OD of 0.5 ppb and 3.0 ppb Calibrator	Between 0.5 and 3.0 ppb Microcystin
Less than OD of 3.0 ppb Calibrator	More than 3 ppb Microcystin

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.

E. Interpreting the results using a colorimeter.



POWER ZERO/SCROLL READ/ENTER

1. Fill a sample cell to the 10 mL line with DI water.

2. Press the POWER button to turn the meter on.

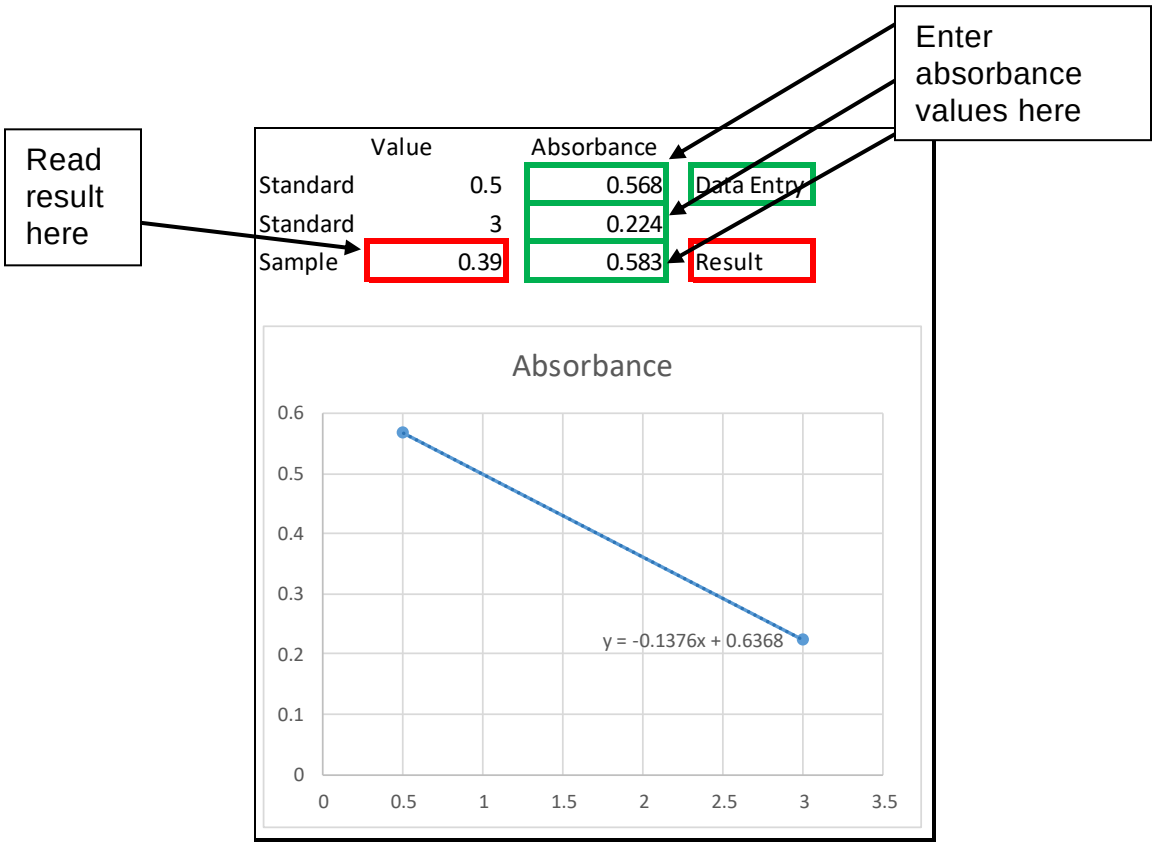
3. Remove the meter cap. Wipe excess liquid and finger prints off sample cells using a Kimwipe. Place the blank in the cell holder with the diamond mark facing the keypad. Fit the meter cap over the cell compartment to cover the cell.

4. Press ZERO/SCROLL button. The display will show “- - - -“, then “0.000”.

5. Check the blank by pressing the READ/ENTER button. The instrument will show “- - - -“ followed by the results.

6. Remove the meter cap. Wipe excess liquid and finger prints off sample cells using a Kimwipe. Place the 0.5 calibrator in the cell holder with the diamond mark facing the keypad. Fit the meter cap over the cell compartment to cover the cell.

7. Press the READ/ENTER button. The instrument will show "- - - -" followed by the results. Record the absorbance values in the Laboratory ELISA Assay Work Sheet.
8. Repeat steps 6-7 for the 3.0 calibrator and all the samples. Record the absorbance values in the Laboratory ELISA Assay Work Sheet.
9. To find the results, enter the absorbance values of the calibration cells, and the sample cell into the Envirologix Interpolation excel spreadsheet.



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.

EnviroLogix® ELISA QualiTube assays were used according to manufacturer's specifications to measure total microcystin (detection limit 0.5 µg/L), viewable online at: http://www.envirologix.com/wp-content/uploads/2015/05/ET022-Microcystin-101215.pdf. EnviroLogix, Portland, Maine.

F. If this test kit is not available, then the HAB Response Team will use best professional judgment to identify and perform an equivalent assay.