MISSOURI DEPARTMENT OF NATURAL RESOURCES
DIVISION OF ENVIRONMENTAL QUALITY
ENVIRONMENTAL SERVICES PROGRAM
Standard Operating Procedure

SOP#: MDNR-ESP-360 
EFFECTIVE DATE: June 19, 2017

SOP TITLE: Qualitative Screening of Algal Toxins in Drinking and Recreational Waters Using Strip Tests by Abraxis, Inc.

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REVISED BY: N/A – This is a new SOP.

APPROVED BY: Original signed by Brian Allen, Director, ESP

SUMMARY OF REVISIONS: N/A – This is a new SOP.

APPLICABILITY: This test applies to ESP personnel who conduct screening for algal toxins in drinking and recreational waters.

DISTRIBUTION: MoDNR Intranet
SOP Coordinator

RECERTIFICATION RECORD:

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<th>Date Reviewed</th>
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1.0 SCOPE AND APPLICABILITY

This standard operating procedure (SOP) describes the procedure to be followed by Environmental Services Program (ESP) personnel and regional office field staff when conducting field analysis of algal toxins present in recreational water, source drinking water, and finished drinking water.

Cyanobacteria are photosynthetic bacteria that naturally occur in lakes, ponds, and other surface waters. When excessive phosphorus and nitrogen is present, an algal bloom can occur. Cyanobacteria blooms are usually green or blue in color, but can be other colors such as red or brown. They can look very scummy or like paint on the surface of the water. Algal blooms are considered harmful when they present a threat to the environment or human and animal health. Cyanobacteria are capable of producing extremely dangerous toxins that can cause sickness or even kill animals and humans. The most common toxins produced by cyanobacteria are microcystin, cylindrospermopsin, anatoxin-a, and saxitoxin. These toxins are dermatotoxic, hepatotoxic (liver toxins), and neurotoxic. Symptoms can include skin rashes, abdominal pain, vomiting, diarrhea, liver inflammation, convulsions, and respiratory paralysis.

2.0 SUMMARY OF METHOD

The Abraxis Strip Test provides only preliminary, qualitative test results. This test is based on the recognition of algal toxins by specific antibodies. The toxin conjugate competes for antibody binding sites with the toxin that may be present in the water sample. Positive results should be confirmed by a quantitative test method such as Enzyme Linked Immunosorbent Assay (ELISA) analysis or another instrumental method. See MDNR-ESP-365 *Analysis of Cyanotoxins by Enzyme-Linked Immunosorbent Assay (ELISA) Using the Abraxis Cyanotoxin Automated Assay System (CAAS)* and MDNR-ESP-370 *Sample Collection of Cyanotoxins* for details regarding laboratory analysis.

3.0 DEFINITIONS AND ABBREVIATIONS

- CAAS – Cyanotoxin Automated Assay System
- COC – Chain of custody
- ELISA – Enzyme Linked Immunosorbent Assay
- ESP – Environmental Services Program
- MoDNR – Missouri Department of Natural Resources
- ng/ml - Nanograms per milliliter
- ppb - Parts per billion
- PPE – Personal protective equipment
- SOP – Standard Operating Procedures
- WQMS – Water Quality Monitoring Section
- 1 ng/ml = 1 ppb

4.0 HEALTH AND SAFETY REQUIREMENTS

4.1 Algal toxins include neurotoxins, hepatotoxins, and dermatotoxins that have been known to cause illness and death in humans and wildlife. Personal protective equipment (PPE), such as disposable gloves and waders, should always be worn. Glove length should be determined based upon sampling requirements. If sampling is conducted from a boat, a personal floatation device should be worn at all times while in the boat.
4.2 Sampling locations may include streams and lakes where the sample collector should maintain awareness of their surroundings, such as weather and stream conditions and boat traffic.

4.3 Skin and eye contact should be avoided. If eye exposure occurs, immediately flush eyes with clean water for at least 15 minutes. Immediately wash any areas of skin exposure with soap and clean water.

4.4 Any equipment, such as waders or secondary sampling devices, should be washed as soon as possible to prevent spreading of scums and future contact by staff. If decontamination cannot be conducted onsite, contaminated equipment should be bagged and sealed until decontamination is performed.

4.5 Studies have shown that toxins can become aerosolized through wave action created by wind and/or boating. Inhalation of aerosolized toxins may result in allergy or asthma like symptoms. Whenever possible, waterbodies experiencing a bloom should be approached from the windward (upwind) direction to minimize contact with any fine mists. If using a boat, "no wake" speed should be used in the affected areas. Individuals with respiratory illnesses such as asthma or other respiratory diseases are more susceptible to breathing difficulties and may experience more severe symptoms. Such individuals should consider avoiding exposure if possible. If one uses a rescue inhaler, they should ensure it is readily available. Although wearing an N95 mask or respirator may reduce respiratory exposure, there is no documented evidence that it will completely eliminate the risks.

4.6 At any time during a bloom investigation or sampling event, if staff experience adverse side effects such as headaches, skin irritation, respiratory irritation, etc., the staff should vacate the area and notify their supervisor. If sampling is required, the event shall then be conducted by staff equipped with additional PPE.

5.0 PERSONNEL QUALIFICATIONS

Field personnel must have a working knowledge of field sample collection procedures. Staff shall have, at a minimum, attended a basic sampling workshop or received training from another MoDNR employee knowledgeable on proper sample collection procedures.

6.0 SUPPLIES AND EQUIPMENT

The following supplies are needed to perform the strip test on recreational water, source drinking water, and finished drinking water samples.

- Sample collection vials (the collection vials do not have to be the ones provided in the kit. The samples have to be collected in a clean, amber glass container).
- Algal toxin strip test kit (specific to the toxin of interest, desired detection level, and waterbody type, e.g., recreational or drinking water)
- Timer
- Sodium thiosulfate for neutralization of chlorine in microcystin and cylindrospermopsin samples
- Ascorbic acid for neutralization of chlorine in anatoxin-a samples
• Disposable gloves
• Waders
• Respiratory protection such as N95 mask or respirator – see section 4.5

Kits may include the following materials.

• Graduated disposable pipettes (not included in all kits)
• Lysis vials (not included in all kits)
• Reagent papers (not included in all kits)
• Sample preservation vials (only included in Anatoxin-a kits)
• Forceps
• Disposable transfer pipettes
• Conical test vials
• Test strips

7.0 PROCEDURE

7.1 Sample Collection and Handling

7.1.1 In order to collect a sample that represents a potential risk, the water should be gently mixed from the surface to approximately 6 inches below. Fill the sample container within the mixed 6-inch water column, cap, treat and preserve (if required) according to section 7.1.5-7.1.7. The heaviest scums should be avoided, if possible, as they may inhibit the cell lysing process and the capillary action required for the test strip.

7.1.2 If samples are to be analyzed on-site, samples may be collected in the provided sample vial in the kit. If samples are to be analyzed at the laboratory, samples shall be collected in an amber glass container and preserved according to 7.1.3 - 7.1.5.

7.1.3 Recreational water samples or drinking water samples where an analytical report is desired, shall be numbered according to MDNR-ESP-003 Sample Numbering and Labeling and recorded on a chain of custody (COC) according to MDNR-ESP-002 Field Sheet and Chain of Custody Record. The completed COC shall be submitted to the ESP laboratory, for entry into the Laboratory Information Management System.

7.1.4 Sample(s) should be analyzed on-site and as soon as possible. If this is not possible, samples can be stored refrigerated for up to five days. If samples are to be held for longer than five days, samples must be frozen. If samples are to be frozen, care must be taken to allow sufficient headspace to prevent breaking of sample container as sample freezes.

7.1.5 Chlorinated drinking water samples for microcystin and cylindrospermopsin analysis, must be treated with sodium thiosulfate (10 mg per 100 mL of sample) to quench the chlorine.

7.1.6 Chlorinated drinking water samples for anatoxin-a analysis, must be treated with ascorbic acid (0.01 mg per 100 mL of sample) to quench the chlorine.
7.1.7 Anatoxin-a samples must be preserved at time of collection to prevent loss of toxin. Using a new graduated pipette for each sample, transfer 3 mL of the sample to a labeled amber glass Sample Preservation Vial. Shake the vial for 30 seconds, and then allow the vial to sit at room temperature for 5 minutes. The sample is now ready for analysis or storage according to section 7.1.2.

7.1.8 If quantitative analysis is desired, refer to MDNR-ESP-370 *Sample Collection of Cyanotoxins* for a comprehensive guide to sample collection.

7.2 Microcystin Strip Test for Recreational Water

7.2.1 Allow the test kit and water samples to be analyzed to come to room temperature before use. Label lysis vials and conical test vials for each sample collected.

7.2.2 Using a new graduated pipette for each sample, transfer 1 mL of the sample to the lysis vial.

7.2.3 Cap the vial and shake for two minutes, then let rest for eight minutes.

7.2.4 Use the forceps provided to add one reagent paper to the lysis vial.

7.2.5 Cap the vial and shake for two minutes then let rest for eight minutes.

7.2.6 Using a new transfer pipette for each sample, transfer seven drops of sample to the conical test vial, which contains a dried reagent.

7.2.7 Close the conical test vial and shake for 30 seconds. Examine the vial to ensure all dried reagents are dissolved, turning the sample purple.

7.2.8 Insert the test strip in the conical test vial with the arrow pointing down (sample pad down), and allow test to develop for ten minutes.

7.2.9 Remove the test strip, lay flat, and allow it to continue developing for five minutes.

7.2.10 Interpret the test results visually by examining the control line and test line. See section 7.7, Interpretation of Results, for an explanation of results.

7.3 Microcystin Strip Test for Source Drinking Water

7.3.1 Allow the test kit and water samples to be analyzed to come to room temperature before use. Label lysis vials and conical test vials for each sample collected.

7.3.2 Using a new graduated pipette for each sample, transfer 1 mL of the sample to the lysis vial.

7.3.3 Cap the vial and shake for two minutes, then let rest for eight minutes.
7.3.4 Use the forceps provided to add one reagent paper to the lysis vial.

7.3.5 Cap the vial and shake for two minutes then let rest for eight minutes.

7.3.6 Using a new transfer pipette for each sample, transfer seven drops of sample to the conical test vial, which contains a dried reagent.

7.3.7 Close the conical test vial and shake for 30 seconds. Examine the vial to ensure all dried reagents are dissolved, turning the sample purple.

7.3.8 Allow the conical test vial to incubate at room temperature for 20 minutes.

7.3.9 Insert the test strip in the conical test vial with the arrow pointing down (sample pad down) and allow test to develop for ten minutes.

7.3.10 Remove the test strip, lay flat, and allow it to continue developing for five minutes.

7.3.11 Interpret the test results visually by examining the control line and test line. See section 7.7, Interpretation of Results, for an explanation of results.

7.4 Microcystin Strip Test for Finished Drinking Water

7.4.1 Ensure chlorinated samples were treated according to section 7.1.5.

7.4.2 Allow the test kit and water samples to be analyzed to come to room temperature before use. Label lysis vials and conical test vials for each sample collected.

7.4.3 Using a new transfer pipette for each sample, transfer seven drops of sample to the conical test vial, which contains a dried reagent.

7.4.4 Close the conical test vial and shake for 30 seconds. Examine the vial to ensure all dried reagents are dissolved, turning the sample purple.

7.4.5 Allow the conical test vial to incubate at room temperature for 20 minutes.

7.4.6 Insert the test strip in the conical test vial with the arrow pointing down (sample pad down) and allow test to develop for 10 minutes.

7.4.7 Remove the test strip, lay flat and allow it to continue developing for five minutes.

7.4.8 Interpret the test results visually by examining the control line and test line. See section 7.7, Interpretation of Results, for an explanation of results.

7.5 Cylindrospermopsin Strip Test for Drinking and Recreational Waters

7.5.1 Ensure chlorinated samples were treated according to section 7.1.5
7.5.2 Allow the test kit and water samples to be analyzed to come to room temperature before use. Label lysis vials and conical test vials for each sample collected.

7.5.3 Using a new graduated pipette for each sample, transfer 1 mL of the sample to the lysis vial.

7.5.4 Cap the vial and shake for two minutes, then let rest for eight minutes.

7.5.5 Use the forceps provided to add one reagent paper to the lysis vial.

7.5.6 Cap the vial and shake for two minutes then let rest for eight minutes.

7.5.7 Using a new transfer pipette for each sample, transfer seven drops of sample to the conical test vial, which contains a dried reagent.

7.5.8 Close the conical test vial and shake for 30 seconds. Examine the vial to ensure all dried reagents are dissolved, turning the sample purple.

7.5.9 Allow the conical test vial to incubate at room temperature for ten minutes.

7.5.10 Insert the test strip in the conical test vial with the arrow pointing down (sample pad down) and allow test to develop for ten minutes.

7.5.11 Remove the test strip, lay flat, and allow it to continue developing for five minutes.

7.5.12 Interpret the test results visually by examining the control line and test line. See section 7.7, Interpretation of Results, for an explanation of results.

7.6 Anatoxin-a Strip Test in Drinking and Recreational Waters

7.6.1 Ensure samples were treated and preserved according to section 7.1.6 and 7.1.7 respectively.

7.6.2 Allow the test kit and water samples to be analyzed to come to room temperature before use. Label lysis vials and conical test vials for each sample collected.

7.6.3 Using a new transfer pipette for each sample, transfer seven drops of the preserved sample to the conical test vial, which contains a dried reagent.

7.6.4 Close the conical test vial and shake for 30 seconds. Examine the vial to ensure all dried reagents are dissolved, turning the sample purple.

7.6.5 Allow the conical test vial to incubate at room temperature for ten minutes.

7.6.6 Insert the test strip in the conical test vial with the arrow pointing down (sample pad down) and allow test to develop for ten minutes.
7.6.7 Remove the test strip, lay flat, and allow it to continue developing for five minutes.

7.6.8 Interpret the test results visually by examining the control line and test line. See section 7.7, Interpretation of Results, for an explanation of results.

7.7 Interpretation of Results

7.7.1 Qualitative results are determined by comparing the intensity of the test line to the intensity of the control line on the test strip.

7.7.2 Although control line intensity may vary, a visible control line must be present for results to be valid.

7.7.3 A test line which is darker than or of equal intensity to the control line indicates a result that is below the limit of detection for the test.

7.7.4 A test line which is lighter than the control line represents a result that is within range of the test.

7.7.5 A test line which is not visible (only control line is visible) indicates a result that is greater than the range of the test.

7.7.6 The range for each test is as follows:

<table>
<thead>
<tr>
<th>Test</th>
<th>Range (ng/mL)</th>
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<tbody>
<tr>
<td>Microcystins (Recreational Water)</td>
<td>0 – 10</td>
</tr>
<tr>
<td>Microcystins (Source Drinking Water)</td>
<td>0 – 5</td>
</tr>
<tr>
<td>Microcystins (Finished Drinking Water)</td>
<td>0 – 5</td>
</tr>
<tr>
<td>Microcystins (Low Level Drinking Water)</td>
<td>0.3</td>
</tr>
<tr>
<td>Cylindrospermopsin</td>
<td>0 – 10</td>
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<tr>
<td>Anatoxin-a</td>
<td>0 – 2.5</td>
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</tbody>
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7.7.7 Refer to the respective flow chart and user guide for each test, found in Appendix A, for illustrated test strip examples and more information.

7.7.8 Results should be determined within 5 – 10 minutes after the final step in each test’s procedure.

8.0 SPECIAL CONSIDERATIONS

8.1 The only way to determine which toxin may be present is to identify the type of cyanobacteria causing the bloom. If a field microscope is available, this could be done on site. If not, it is recommended that tests be conducted for all three toxins. Please contact the ESP/ Water Quality Monitoring Section (WQMS) regarding laboratory identification of cyanobacteria.

8.2 The strip test kits should be stored between 4 – 30°C.
8.3 The test strips, test vials, and water samples to be analyzed should be at room temperature before use.

8.4 Avoid cross-contamination of water samples by using a new sample vial and disposable pipettes for each sample.

8.5 Samples containing unusually large amounts of algal blooms or very thick algal scums should be diluted 1:1 with deionized or distilled water prior to lysis, as overly viscous samples may not allow for uniform cell lysis or proper capillary flow up the test strip.

8.6 Only use the test strips, test vials, and reagents from one kit lot, as they have been adjusted in combination.

8.7 Use of the test strips without the QuikLyseTM* reagents (when required) will adversely affect the performance of the test, producing inaccurate results. Refer to the respective user guide for each test, found in Appendix A, for more information.

9.0 QUALITY ASSURANCE/QUALITY CONTROL

9.1 MoDNR only utilizes the strip tests to determine presence/absence of toxins, and if present, the relative concentration. Each lot of test strips will be tested against a positive and negative control to document the strip’s ability to detect toxins. The positive control will consist of either a purchased, known standard or an environmental sample-testing positive through the ELISA test method. Documentation of accuracy checks will be maintained by the ESP/WQMS. Quality control information will be made available upon request.

9.2 Confirmation of positive samples by ELISA or other instrumental methods should be considered. If desired, contact the ESP/WQMS and see MDNR-ESP-365 Analysis of Cyanotoxins by Enzyme-Linked Immunosorbent Assay (ELISA) Using the Abraxis Cyanotoxin Automated Assay System (CAAS) and MDNR-ESP-370 Sample Collection of Cyanotoxins.

10.0 REFERENCES

Algal Toxin Strip Test Anatoxin-a Drinking and Recreational Waters. 2016. Abraxis, Inc.


MDNR-ESP-002. 2016. Field Sheet and Chain of Custody Record. Missouri Department of Natural Resources. Division of Environmental Quality, Environmental Services Program.

MDNR-ESP-003. 2015. Sample Numbering and Labeling. Missouri Department of Natural Resources. Division of Environmental Quality, Environmental Services Program.


MDNR-ESP-370. 2017. Sample Collection of Cyanotoxins. Missouri Department of Natural Resources, Division of Environmental Quality, Environmental Services Program.
APPENDIX A

User’s Guides (Links) and Flow Charts for Toxin Strip Test Kits
Microcystins in Recreational Water User’s Guide


Microcystins in Recreational Water Flow Chart


Microcystins in Source Water User’s Guide


Microcystins in Source Water Flow Chart


Microcystins in Finished Water User’s Guide


Microcystins in Finished Water Flow Chart


Cylindrospermopsin in Drinking and Recreational Water User’s Guide


Cylindrospermopsin in Drinking and Recreational Water Flow Chart


Anatoxin-a in Drinking and Recreational Water User’s Guide


Anatoxin-a in Drinking and Recreational Water Flow Chart

http://www.abraxiskits.com/wp-content/uploads/2016/05/AnatoxinADrinkRecreat_PN520042_DS.pdf