Plan of Study
Detection of Microcystin with Strip Test

Goal
To determine the performance of the 10 ppb Abraxis Microcystin Strip Test with Quicklyse™ for recreational water. Toxin and non-toxin producing cultures of *Microcystis aeruginosa*, as well as samples collected from actual algal blooms, will be used to assess the kit's potential for correctly detecting the presence of microcystins.

Background
The cost associated with microcystin analytical testing can be reduced by not collecting samples that have not been screened for the presence of microcystins. ELISA kits and high performance liquid chromatography mass spectrometry (LC/MS-MS) have a high cost regardless of whether or not the analytes of interest are present. The strip test (low cost) can be used prior to submitting a sample to a chemistry laboratory for LC/MS-MS analysis thus avoiding the costs incurred by the testing of microcystin free water.

The strip test will also allow for a more rapid characterization of the water. Currently, a sample is collected for microscope identification of toxin producing species. This requires sample processing time, which can range from 15 minutes to several hours, and the added cost of experienced algal taxonomy staff.

The Bureau of Laboratories-Biology Section maintains six cultures of *M. aeruginosa*. Annually, the cultures are submitted to the Chemistry laboratory for toxin testing by LC/MS-MS to monitor for changes in their respective toxin profile. Each culture produces differing amounts of microcystin congeners with the exception of two of the cultures which are non-toxin producing cultures of *M.aeruginosa*. Two of the cultures are only known to produce microcystin-LR and another two are known to produce three congeners: microcystin-LR, microcystin-RR, and microcystin-YR. Coupling the annual LC/MS-MS culture testing with strip testing will provide an opportunity to analyze the performance of the strip test near their detection limit using diluted cultures and to determine if the strip test is more sensitive to a particular congener of microcystin over another.

Additionally, the strip test will be evaluated in the testing of samples from waterbodies with microcystin-producing and non-microcystin-producing bloom events if possible.

Materials
Abraxis Microcystin Strip Test with Quicklyse™ for Recreational Water (10ppb)-20 Strips
Sample Collection Bottles
Lysis Vials
Lab Cultures
Dilution Tubes
Graduated Pipettes (1 mL and 10mL)
Disposable Transfer Pipettes
Camera

Method
Schedule RQs for the lab cultures and the bloom sample.

Part I -Lab Cultures

1. Submit the cultures for LC/MS-MS testing per unpublished standard operating procedure AB-XX *Microcystis aeruginosa* culture submittal for toxin analysis-Version 3. See Appendix A.

2. Analyze the cultures using the Abraxis Microcystin Strip Test with Quicklyse™ for Recreational Water. See Appendix B for strip test instructions. Photograph the strip test and record results for each of the six cultures.
3. Obtain the results from the LC/MS-MS testing and perform a dilution for the Chapman culture (3 known congeners: microcystin-LR, microcystin-RR, and microcystin-YR) which would result in a microcystin level of 1-5 ppb. The Chapman culture was tested to have the highest amount of microcystin-YR. The Munson culture, which also has three congeners, has comparable amounts of microcystin-LR and microcystin-RR but a slightly lower level of microcystin-YR. This will help to determine if the strip tests have differing sensitivities to various congeners. Perform another dilution for the UTEX culture (1 known congener: microcystin-LR) that will also result in a microcystin level of 1-5 ppb. (Note: It may be difficult to dilute to 1-5 ppb because the LC/MS-MS testing does not test for all congeners of microcystin.)
4. Analyze the diluted cultures using the strip test following the instructions in Appendix B. Photograph the strip test and record results for each of the two dilutions.

Part II-Collected Samples

1. Locate waterbodies with a *M. aeruginosa* bloom and a non Microcystis bloom.
2. Confirm the dominant bloom species for each waterbody by microscope identification.
3. Collect a microcystin sample for chemistry and a microcystin sample for the Abraxis Microcystin Strip Test with Quicklyse™ for Recreational Water from each waterbody.
4. Submit the sample to chemistry and perform the strip test in the lab per the instructions in Appendix B. Photograph the strip test and record results for each of the waterbody.

Analysis:

Compare the LC/MS-MS results for all six of the cultures with the positive and negative results of the strip test.
Discuss how well the strip test performs with microcystin amounts <10 ppb.
Compare the sample results collected from the waterbody with a M. aeruginosa bloom and a non Microcystis bloom to the strip test results
Provide comments on using the strip tests.

Data

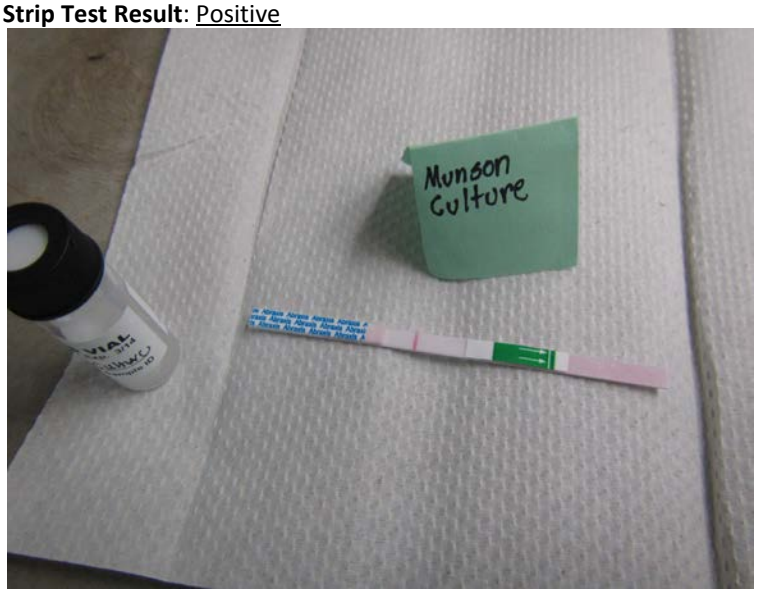
Per SOP AB-XX *Microcystis aeruginosa* culture submittal for toxin analysis-Version 3 dilutions of the cultures were performed to bring the amounts of Microcystin in the samples between 10 ppb and 50 ppb. The lowest concentration congener was not diluted below the calibration range. The results of the previous analysis (performed June 27, 2012) were consulted to determine the dilution factor. See each culture section for calculations. Nodularin was left out of the calculations as it has not been detected in the cultures.
The cultures had grown for 14 days at the time of dilution and sample submittal. These cultures were inoculated April 2, 2013 with 1 mL from cultures having seven days of growth. No comments were recorded in the algal culture logbooks to indicate culture density problems or other irregularities. The number '2' flask from each culture was used.

The minimum detection limit (MDL) is reported when the analyte was undetected (U-qualifier). Several of the results were detected between the MDL and the practical quantitation limit (PQL)(I-qualifier). The diluted Chapman culture microcystin-LR and the Southwood Pond nodularin results are estimated values (J-qualifier) due to quality control failures.

Munson Strip Test Result
04/16/13
Dilution Factor Calculation
Microcystin-LR 510 µg/L ÷50=10.2 µg/L
Microcystin-RR 2400 µg/L ÷ 50= 48 µg/L
50X dilution should yield 58.2 µg/L total microcystins
[1 mL culture: 49 mL DI H20]

LC/MS-MS Results: Microcystin LR 1.0 U µg/L
Microcystin RR 1.0 U µg/L Total: Undetected
Nodularin 1.0 U µg/L

Strip Test Result: Positive



Munson Culture Retest- No dilution
04/29/13

LC/MS-MS Results: Microcystin LR 6.8 µg/L
Microcystin RR 5.9 µg/L Total: 12.7 µg/L
Nodularin 1.0 U µg/L

A strip test was not performed a second time.

Chapman Strip Test Result
04/16/13
Dilution Factor Calculation
Microcystin-LR 620 µg/L ÷50=12.4 µg/L
Microcystin-RR 5500 µg/L ÷ 50= 110 µg/L
50X dilution should yield 122.4 µg/L total microcystins
[1 mL culture: 49 mL DI H20]

LC/MS-MS Results: Microcystin LR 13 µg/L
Microcystin RR 29 µg/L Total: 42 µg/L
Nodularin 1.0 U µg/L

Strip Test Result: Positive

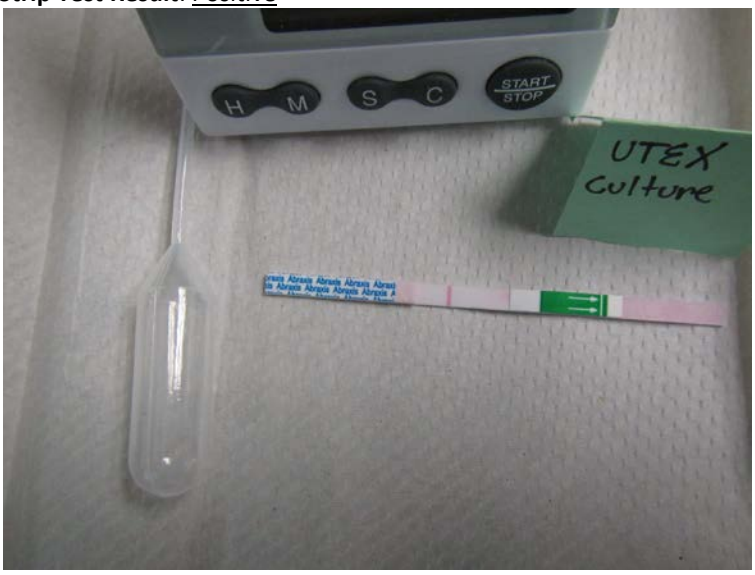
UTEX Strip Test Result

04/16/13

Dilution Factor Calculation
Microcystin-LR 1000 µg/L ÷50=20 µg/L
50X dilution should yield 20 µg/L total microcystins
[1 mL culture: 49 mL DI H20]

LC/MS-MS Results: Microcystin LR 13 µg/L
Microcystin RR 1.0 U µg/L Total: 13 µg/L
Nodularin 1.0 U µg/L

Strip Test Result: Positive



St. Johns Strip Test Result
04/16/13
Dilution Factor Calculation
Microcystin-LR 1000 µg/L ÷50=20 µg/L
50X dilution should yield 20 µg/L total microcystins
[1 mL culture: 49 mL DI H20]

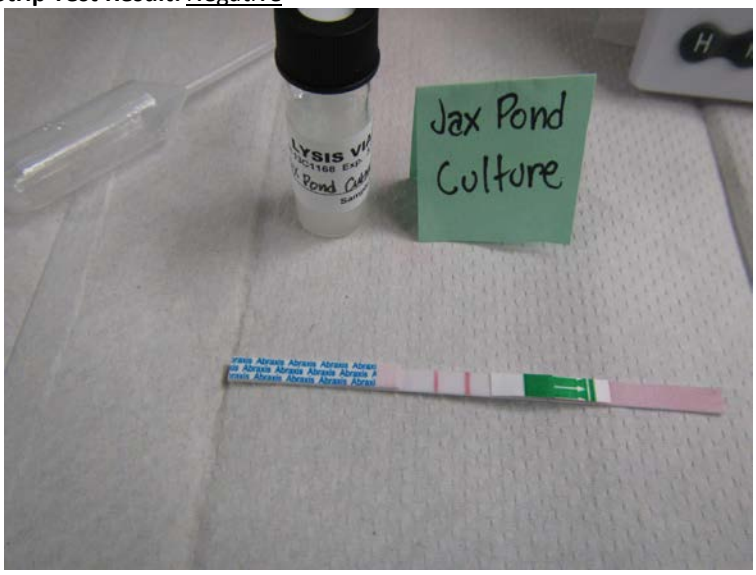
LC/MS-MS Results: Microcystin LR 13 µg/L
Microcystin RR 1.0 U µg/L Total: 13 µg/L
Nodularin 1.0 U µg/L

Strip Test Result: Positive

Jax Pond Strip Test Result
04/16/13
Dilution Factor Calculation
No toxin. 10X dilution to reduce number of cells to prevent column clogging.
[1 mL culture: 9 mL DI H20]

LC/MS-MS Results: Microcystin LR 1.0 U µg/L
Microcystin RR 1.0 U µg/L Total: Undetected
Nodularin 1.0 U µg/L

Strip Test Result: Negative



Killearney Strip Test Result
04/16/13
Dilution Factor Calculation
No toxin. 10X dilution to reduce number of cells to prevent column clogging.
[1 mL culture: 9 mL DI H20]

LC/MS-MS Results: Microcystin LR 1.0 U µg/L
Microcystin RR 1.0 U µg/L Total: Undetected
Nodularin 1.0 U µg/L

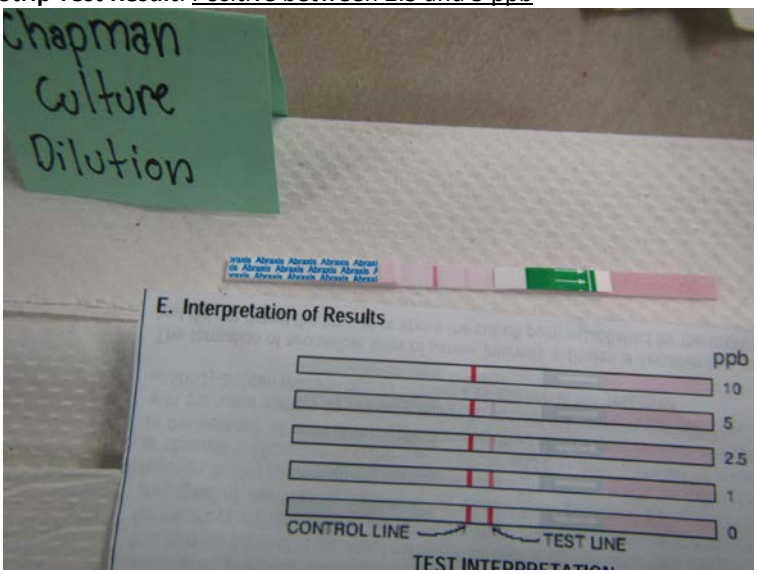
Strip Test Result: Negative

Diluted Chapman Strip Test Result
04/29/13

Dilution Factor Calculation
Dilution Target: 1-5 ppb
Total Microcystin 2100µg/L ÷500=4.2 µg/L
500 X dilution should yield 4.2 µg/L total microcystins
[0.25 mL culture: 124.75 mL DI H20]

LC/MS-MS Results: Microcystin LR 1.0 UJ µg/L
Microcystin RR 2.4 I µg/L Total: 2.4 µg/L
Nodularin 1.0 U µg/L

Strip Test Result: Positive between 2.5 and 5 ppb

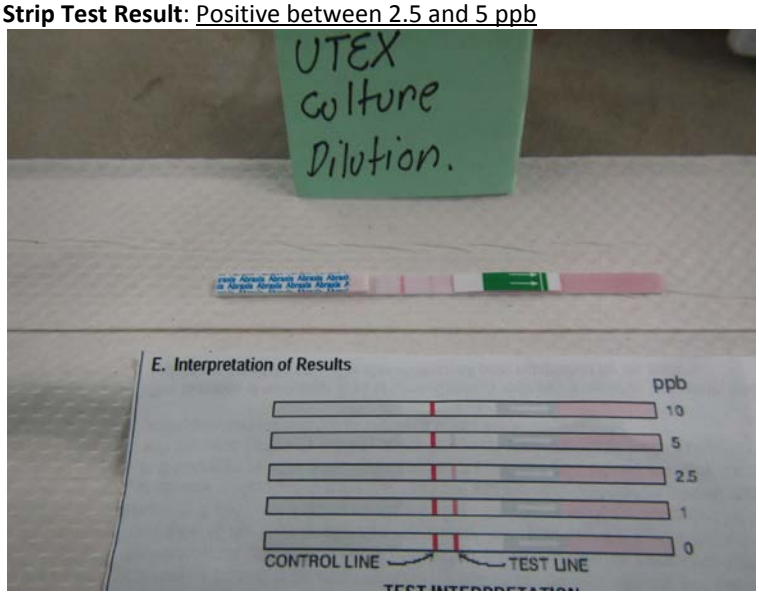


Diluted UTEX Strip Test Result
04/29/13

Dilution Factor Calculation
Dilution Target: 1-5ppb
Total Microcystin 650 µg/L ÷150=4.3 µg/L
150X dilution should yield 4.3 µg/L total microcystins
[0.5 mL culture:74.5 mL DI H20]

LC/MS-MS Results: Microcystin LR 2.6 I µg/L
Microcystin RR 1.0 U µg/L Total: 2.6 µg/L
Nodularin 1.0 U µg/L

Strip Test Result: Positive between 2.5 and 5 ppb



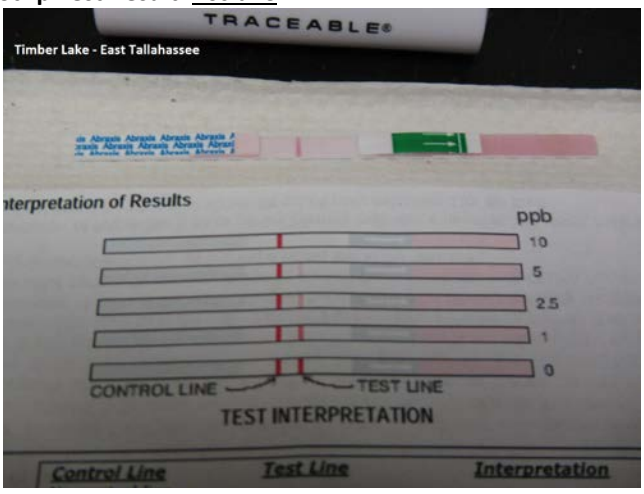
Waterbody Microcystis Bloom Sample Results
Timber Lake Retention Pond East Tallahassee (corner of Timber Ridge Rd and Red Oak Rd)
Samples collected 04/15/13 1330

Microscope Identification Description:
Algae collected from a bloom in Timber Lake located in the Timberlake Residential Community was dominated by *Microcystis aeruginosa*. *Pseudanabaena mucicola* was also abundant in the mucilaginous sheaths of the *M. aeruginosa* colonies.

LC/MS-MS Results: Microcystin LR 140 µg/L
Microcystin RR 440 µg/L Total: 580 µg/L
Nodularin 1 U µg/L

Site Photo:

Strip Test Result: Positive



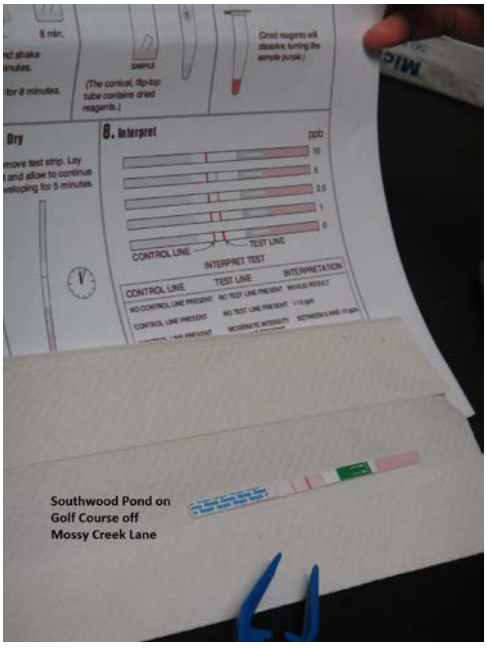
Waterbody Non-Microcystis Bloom Sample Results
Southwood Pond on Golf Course off Mossy Creek Lane
Samples collected 04/11/13 0950

Microscope Identification Description:
Algae collected from a bloom in a pond located on the Southwood golf course was dominated by *Euglena* sp. Many of the *Euglena* sp. were in the encystment stage which means there was a large amount of gelatinous mucilage in the scum.

LC/MS-MS Results: Microcystin LR 1.0 U µg/L
Microcystin RR 1.0 U µg/L Total: Undetected
Nodularin 1.0 UJ µg/L

Site Photo:

Strip Test Result: Negative
NOTES: Initially the strip test was read backwards; Arrows on the strip must be facing to the right. The control line is lighter and thinner than the test line. The interpretation diagrams provided with the kit do not demonstrate the difference in the control and test lines.



Results:

Part I-Lab Cultures

The results of the strip test agreed with the results of the LC/MS-MS except for the Munson culture. Microcystins were undetected in the 50X dilution submitted for LC/MS-MS analysis but were detected in an amount greater than 10 µg/L on the strip test. An additional undiluted sample was tested again by LC/MS-MS, and the result was 12.7 µg/L. This low amount of microcystins is atypical for this culture. The cells in flask 2 from the undiluted aliquot were examined and compared with the cells from flask 1. Flask 2 contained an uncontaminated culture of *Microcystis aeruginosa* but the appearance of the cells differed in that they had fewer aerotopes (gas vacuoles) than the cells in flask 1. A likely explanation for the discrepancy in the strip test result and the LC/MS-MS analysis is the Munson culture is now producing microcystin congeners other than microcystin-LR and microcystin-RR. Currently, these are the only two congeners tested in the LC/MS-MS analysis. Based on previous LC/MS-MS analysis, the Munson culture also produces microcystin-YR in low amounts. Additional testing is planned for the Munson culture.

Diluted Cultures

The strip tests were able to detect microcystins below 10 µg/L. The test line was visible and noticeably lighter than a result greater than 10 µg/L. While observing the strip test, staff estimated the amount to be between 2.5 µg/L and 5.0 µg/L for both the diluted Chapman culture and the diluted Utex culture. The LC/MS-MS result of the diluted Chapman culture was 2.4 µg/L, slightly below the estimated strip test value range.

Part II-Collected Samples

The strip tests detected microcystins from a *Microcystis aeruginosa* bloom and did not detect microcystins in another non-Microcystis bloom. It is important to note that the strip test for the non-Microcystis bloom was initially read backwards , which would yield an incorrectly positive result. The control line is lighter and thinner than the test line. The interpretation diagrams provided with the kit do not demonstrate the difference in the control and test lines. It would be helpful if the manufacturer added the green section of the strip on the interpretation diagram to better represent the strip test.

Appendix A

Effective Date: 06/01/2012
Revision Date: 06/01/2012
Revision Author: C. Swanson
AB-XX

Note: GreenWater Labs will be contracted to perform future toxin analysis. Coordination with chemistry and the use of borosilicate tubes as part of culture toxin submittal will no longer be necessary.
JW 06/29/12

Microcystis aeruginosa culture submittal for toxin analysis

1. SCOPE AND APPLICATION

   1.1 This SOP describes the process for submitting stock cultures of *Microcystis aeruginosa* for toxin analysis. Toxin analysis can check for possible contamination that may occur during routine maintenance or document any changes in amounts of toxin produced that may occur as a result of culturing.

2. SUMMARY OF METHOD

   2.1 To accommodate workload as best as possible, the date of submittal is coordinated with the Chemistry Organic subsection. Aliquots of diluted or undiluted 10 to 17 day old stock culture are transferred to sample containers. Samples are submitted for analysis through Receiving. Results are compared with previous results to check for changes in the toxin profile of each culture.

3. APPARATUS AND EQUIPMENT

   3.1 Erlenmeyer flasks (250 mL, autoclaved)
   3.2 Disposable glass pipettes (10 mL)
   3.3 Pipettor (10 mL)
   3.4 Graduated cylinder (100 mL)
   3.5 Labeling tape, 3/4-in wide or waterproof label sheet for computer printing
   3.6 Black, fine-tip, permanent marker
   3.7 250 mL glass amber bottles with Teflon lined screw caps (from Receiving)
   3.8 16 x 125mm Borosilicate glass culture tubes (from Chemistry)
   3.9 Aluminum foil
   3.10 *Microcystis Culture Logbook*

4. REAGENTS AND CHEMICALS

   4.1 *Microcystis aeruginosa* cultures from different sources (Shaker Racks in B246A).
   4.2 De-ionized water

5. CULTURE COLLECTION AND PRESERVATION

   5.1 Samples used for isolating a *Microcystis aeruginosa* culture are collected from blooms where it has been confirmed that *Microcystis aeruginosa* is the overwhelming dominant in the bloom and, in some cases, the algal toxin, microcystin, is being produced. Samples are delivered directly or shipped overnight to the laboratory without chemical preservation.

6. CULTURE MAINTENANCE PROCEDURES

   6.1 Cultures are maintained per SOP AB-13-1.4.

7. CULTURE SUBMITTAL PROCEDURES

   7.1 Coordinate with the Chemistry Organic subsection on sample submittal. Depending on workload, sample analysis (per SOP LC-038) may be performed immediately. If this is the case, Chemistry will provide 16 x 125 mm borosilicate culture tubes for sample submittal. If Chemistry is waiting

to run the culture samples in a batch with other incoming samples, culture samples will be placed in screw cap vials for storage in the refrigerator or freezer. Ask if Chemistry requires any duplicates or blanks to be submitted along with the culture samples.

7.2 In LIMS, have an RQ created and approved for test W-MCYST-AA with the appropriate number of samples. Print the sample submittal form.

7.3 Select a flask series (1A, 1B, 2A, or 2B) between 10 and 17 days old from the culture shaker room (B246A). Only one flask from each culture will be used for toxin analysis.

7.4 Label all 40 mL glass amber Teflon lined screw cap top vials or borosilicate tubes and the dilution containers (250mL clean glass culture flasks) with the culture name, date and time of culture aliquot collection for toxin analysis, and field ID which is the flask series selected and the dilution if applicable. (Example: Munson Culture, 2B 10x Dilution).

7.5 Review past toxin results for cultures. Transfer aliquots of undiluted or diluted cultures to the vials or tubes as follows:

Note: Precautions must be taken to avoid cross-contamination of the cultures. Perform the steps for one culture before performing the steps for another culture. Perform the transfers in a separate place in the lab from the stock cultures, and discard the pipettes used for one culture before using a pipette for the other culture.

7.5.1 Cultures that have not historically produced high levels of toxins (i.e. toxin levels less than 50 ug/L) do not need dilution. For each culture that does not need dilution, homogenize the culture and then pour 40 mL into the corresponding labeled 40 mL vial or the amount specified by the Chemistry Organic subsection into the corresponding labeled borosilicate tube. Cap vials or cover the top of the tube with aluminum foil.

7.5.2 For cultures that need to be diluted before transferring to a vial or tube (i.e. historical toxin levels greater than 50 ug/L), perform a dilution to reach a 20-50 ug/L concentration of the highest microcystin congener. Some cultures usually produce high levels of Microcystin-LR,dilutions produce more reliable results. Furthermore, high cell concentrations can damage the equipment used for analysis (Shimadzu HPLC system and Applied Biosystems LC-MSMS system). A 10x dilution is typically performed for the Munson, Utex, Chapman, and St. Johns cultures. Other dilutions can be performed as needed. For each, homogenize the culture flask by gently swirling until any settled cells are suspended in the media. Using a clean 10 mL pipette for each culture, transfer 4 mL of culture into a 250 mL clean glass culture flask (dilution). In a graduated cylinder, measure 36 mL of deionized water and pour it into the culture flask (dilution). Homogenize the 10x dilution by gently swirling the culture flask. Pour the diluted culture into the corresponding labeled 40 mL vial or pour the amount specified by the Chemistry Organic subsection into the corresponding labeled borosilicate tube. Cap vials or cover the top of the tube with aluminum foil.

7.6 If Chemistry requires duplicates or blanks, fill extra labeled vials or tubes as necessary using clean equipment and de-ionized water for blanks. Follow Steps 7.5.1 or 7.5.2, as appropriate, for culture duplicates.

7.7 In the *Microcystis Culture Logbook*, record amount (mL) and culture age for each culture, along with the date and time each culture was submitted for toxin testing.

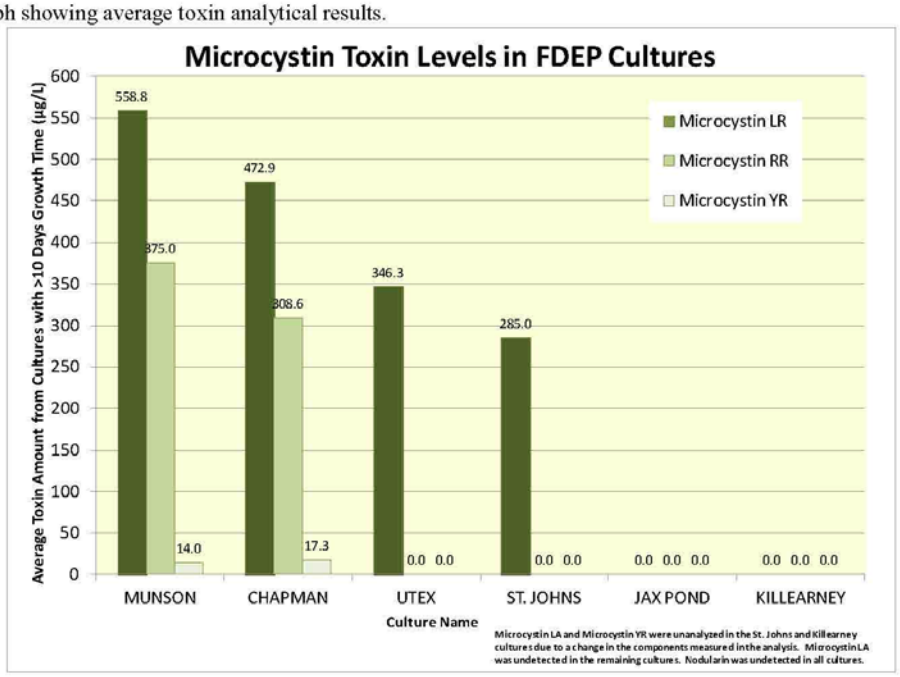
7.8 Fill out the submittal paperwork. The sample location is the culture name with the word 'culture' to separate it from water grab samples, and the field ID is the flask series and dilution if applicable (example: Munson Culture, 2B 10x Dilution). In the comments section, record the age of the culture.

7.9 Samples need to be logged into LIMS through Receiving. If submitting vials to Receiving, immediately place vials on ice and deliver submittal form and vials; complete chain of custody. If submitting tubes directly to Chemistry, take submittal form and tubes to Receiving to verify chain of custody but then deliver tubes to Chemistry. Tubes do not need to be iced because they will be immediately placed in an autoclave.

7.10 The 10 to 17 day old cultures should be returned to the culture shaker room (B246A) and will be discarded at 21 days old. The remainder of the diluted samples can be disposed of down the sink. Thoroughly rinse all glassware used. The dilution flasks should be washed in the dishwasher during the next routine culture maintenance, and the graduated cylinder should be cleaned in wash party per SOP BG-05. Dispose of used pipettes in the glass disposal.

7.11 When the analysis is complete, obtain the results from LIMS. For diluted samples, calculate the final result by multiplying each diluted sample's result by 10. To check for contamination and/or changes in toxin production, compare the final result to the culture's historical profile located in \\tlhlab5\Biology\AlgalCommon\Culture. For example, Munson has had the highest microcystin-LR content whereas all microcystin congeners should be undetected in the Killearney culture. If the results differ from the toxin profile below, inform a supervisor. Record the changes in the *Microcystis Culture Logbook*.

Graph showing average toxin analytical results.



8. QUALITY CONTROL AND DOCUMENTATION

8.1 Use a pen with black ink for all logbook entries.

8.2 *Microcystis Culture Logbook*

8.3 To correct raw data entries, place a single line through the incorrect entry, write the corrected entry near the error and initial the correction. If the correction requires an explanation, write a comment and reference the error.

9. SAFETY

9.1 Lab coat, latex or vinyl gloves, and eye protection. Proper laboratory protection is required when working with chemicals and unprocessed samples.

9.2 Avoid breathing fumes or exposing co-workers to them. Use proper safety equipment, a lab hood or chemical ventilation system, and keep face away from open containers of this chemical. Work in a well ventilated area.

9.3 Review Laboratory Safety Manual. See http://depnet/burlabs/safety.htm

10. POLLUTION PREVENTION AND WASTE MANAGEMENT

10.1 Review Laboratory Safety Manual. See http://depnet/burlabs/safety.htm

11. REFERENCES

11.1 SOP AB-13-1.4 Culture of *Microcystis aeruginosa* Stocks
11.2 SOP BG-05 Biology Wash Party Procedures

Appendix of Changes

Appendix B

Microcystins Strip Test

Immunochromatographic Strip Test for the Detection
of Microcystins and Nodularins in Recreational Water at 10 ppb

Product No. 520023 (5 Test), 520022 (20 Test)

1. General Description

The Abraxis Microcystins Strip Test is a rapid immunochromatographic test, designed solely for the use in the qualitative screening of Microcystins and Nodularins in recreational water. A rapid cell lysis step (QuikLyse* ™) performed prior to testing is required to measure total microcystins (dissolved or free, plus cell bound). The Abraxis Microcystins Strip Test provides only preliminary qualitative test results. If necessary, positive samples can be confirmed by ELISA, HPLC or other conventional methods.

* Patent Pending

2. Safety Instructions

Discard samples according to local, state and federal regulations.

3. Storage and Stability

The Microcystins Strip Kit should be stored between 4–30°C. The test strips, test vials and water samples to be analyzed should be at room temperature before use.

4. Test Principle

The test is based on the recognition of microcystins, nodularins and their congeners by specific antibodies. The toxin conjugate competes for antibody binding sites with microcystins/nodularins that may be present in the water sample. The test device consists of a vial with specific antibodies for microcystins and nodularins labeled with a gold colloid and a membrane strip to which a conjugate of the toxin is attached. A control line, produced by a different antibody/antigen reaction, is also present on the membrane strip. The control line is not influenced by the presence or absence of Microcystins in the water sample, and therefore, it should be present in all reactions. In the absence of toxin in the water sample, the colloidal gold labeled antibody complex moves with the water sample by capillary action to contact the immobilized microcystins conjugate. An antibody-antigen reaction occurs forming a visible line in the 'test' area.

The formation of two visible lines of similar intensity indicates a negative test result, meaning the test did not detect the toxin at or above the cut-off point established for the toxin.

If Microcystins are present in the water sample, they compete with the immobilized toxin conjugate in the test area for the antibody binding sites on the colloidal gold labeled complex. If a sufficient amount of toxin is present, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the toxin conjugate, therefore preventing the development of a colored line. If a colored line is not visible in the Test Line Region, or if the Test Line is lighter than the negative Control Line, Microcystins is present at the levels of concern (>10 ppb). Semi-quantitative results in the range of 0-10 ppb can be obtained by comparing the test line intensity to those produced by solutions of known Microcystins concentrations (control solutions). Microcystins controls are available through Abraxis (PN 422011).

5. Limitations of the Microcystins Strip Test, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects can't be completely excluded.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be:
Inadequate storage conditions of the test strip, too long or too short incubation times, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The assay is designed for use with recreational waters. The Microcystins Test Strip provides only a preliminary qualitative test result. Use another more quantitative analytical method such as ELISA or instrumental analysis to obtain a confirmed quantitative analytical result. Apply good judgement to any test result, particularly when preliminary positive results are observed.

6. Warning and Precautions

-Use reasonable judgment when interpreting the test results.
-Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
-For test strips packaged in a dessicant vial, the vial should be kept completely closed except for opening to remove test strips. When re-closing, snap lid firmly.
-Avoid cross-contamination of water samples by using a new sample vial and disposable pipette for each sample.
-Use only the Microcystins Test Strips & QuikLyse™ reagents from one kit lot, as they have been adjusted in combination. Use of the Microcystins Test Strips without the QuikLyse™ reagents will adversely affect the performance of the test, producing inaccurate results. To test samples without using QuikLyse™ reagents for cell lysis, such as when testing for free Microcystins only or when testing samples which have been previously lysed (i.e. frozen/thaw 3X), please use the Abraxis Microcystins Strip Test for Finished Drinking Water at 1 ppb, PN 520016 (5 Test) or PN 520017 (20 Test).
-It is recommended that samples containing unusual amounts of algal blooms or very thick algal scums be diluted 1:1 with distilled water prior to lysis, as overly viscous samples may not allow for uniform cell lysis or proper capillary flow on the dipstick. Diluted samples will then have a cut-off of 20 ppb.

7. Sample Collection and Handling

Collect water samples in glass containers and test within 24 hours. If samples must be held for longer periods (5 days), samples should be refrigerated. For longer storage periods, samples should be kept frozen.

7.1 Total Microcystins

When analyzing for total microcystins (dissolved or free microcystins plus cell bound), such as might be present in recreational waters, a sample lysis is needed before analysis. The Abraxis QuikLyse™ reagents provide a rapid option for cell lysis:

- Using the graduated disposable pipette, draw sample to the 1 mL line (graduation mark slightly below bulb) and add 1 mL of sample to the lysis vial.
- Cap and shake for 2 minutes then let the sample in vial rest for 8 minutes to start the cell lysing.
- After the 8 minute incubation, add 1 reagent paper (using the forceps provided) to the lysis vial. Shake for 2 minutes and then incubate for 8 minutes.
- After the final incubation step, proceed to Assay Procedure step.

A. Materials Provided

1. Microcystins Test Strips in a dessicated container
2. Sample collection vessels
3. Lysis vials
4. Graduated disposable pipettes (calibrated at 1 mL)
5. Forceps
6. Reagent papers
7. Conical Test vials
8. Disposable transfer pipettes
9. User's guide

B. Test Preparation

1. Adjust the test strip and water sample to room temperature before use.
2. Remove the number of test strips required from the package. The remaining strips are stored in the tightly closed storage container.

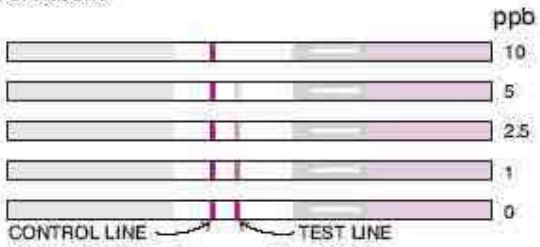
C. Assay Controls

It is good laboratory practice to use positive and negative controls to ensure proper test performance. Water samples containing known quantities of Microcystins (positive controls) should be analyzed with each lot of test strips to provide a reference for line intensity to be expected.

D. Assay Procedure

1. Test strip and water sample(s) should be at room temperature before conducting any testing.
2. Label conical test vials for each sample to be tested.
3. Using the disposable transfer pipette, transfer 7 drops (approximately 200 uL) of the previously lysed water sample (Step 7.1) to the previously labeled conical test vial.
4. Close the conical test vial and shake for 30 seconds (dried reagents will dissolve turning the sample purple).
5. Insert test strip (arrows down), into the conical vial containing the sample/antibody mixture.
6. Allow the test to develop for 10 minutes.
7. Remove the test strip. Lay it flat and allow to continue the development for 5 minutes.
8. Read the results visually as explained below under Interpretation of Results within 5-10 minutes after completion of test.

E. Interpretation of Results



TEST INTERPRETATION

Control Line	***Test Line***	***Interpretation***
No control line present	No test line present	Invalid result
Control line present	No test line present	> 10 ng/ml (ppb)
Control line present	Moderate intensity test line present	Between 0 and 10 ng/ml (ppb)

Illustration is for demonstration of test line intensity range only, since overall intensity may vary slightly with different lots of reagents, etc. Results should be determined within 5-10 minutes after completion of the strip test procedure. Determination made using strips which have dried for more or less than the required time may be inaccurate, as line intensities may vary with drying time. To obtain semi-quantitative results in the range of 0-10 ppb, solutions of known Microcystins concentration must be tested concurrently with samples. Sample test line intensities can then be compared with known (control) test line intensities, yielding approximate sample concentrations. Do not use strips run previously to determine semi-quantitative sample concentrations, as test line intensities may vary once strips are completely dry.

F. Additional Materials (not provided with the test)

1. Timer
2. Microcystins Check Samples, Abraxis PN 422011 (If a semi-quantitative determination between 1-10 ppb is desired).

G. Additional Analysis

If necessary, positive samples can be confirmed by ELISA, HPLC or other conventional methods. Commercial Analytical Labs such as Green Water Labs [www.greenwaterlab.com] offer such services.

H. References

(1) W. J. Fischer, I. Garthwaite, C.O. Miles, K.M. Ross, J.B. Aggen, A.R. Chamberlain, N.A. Towers, and D.R. Dietrich, Congener-Independent Immunoassay for Microcystins and Nodularins. Environ. Sci. Technol. 35, 2002, 4849-4858.
(2) Worldwide Patenting PCT WO 01/18059 A2.
(3) U.S. Patent Number 6,967,240.

Importance of Microcystins/Nodularins Determination

Most of the world's population relies on surface freshwaters as its primary source for drinking water. The drinking water industry is constantly challenged with surface water contaminants that must be removed to protect human health. Toxic cyanobacteria (blue-green algae) blooms are an emerging issue worldwide because of increased source water nutrient pollution caused by eutrophication. Microcystins and Nodularins are cyclic toxin peptides. Microcystins (several structural variants or congeners are found) have been found in fresh water throughout the world. They are produced by the genus *Microcystis, Anabaena, Oscillatoria, Nostoc, Anabaenopsis,* and terrestrial *Hapalosiphon.* Nodularins are produced by the genus Nodularia and they are found in marine and brackish water. To date, approximately 65 variants of microcystins have been isolated, the most common variant is microcystin-LR. Other common microcystin variants include YR, RR, and LW.

Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms, and in several cases has lead to death. Human and animal exposure to these toxins occurs most frequently through the ingestion of water i.e. through drinking or during recreational activities in which water is swallowed. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of the serine/threonine protein phosphatases, and therefore they may act as tumor promoters.

To protect consumers from adverse health effects caused by these toxins, the WHO has proposed a provisional upper limit for microcystin-LR of 1.0 ppb (ug/L) in drinking water. For recreational bathing waters, the WHO has set up the following guidelines:

-Relatively low risk of exposure effect at 4 ng/mL (ppb)
-Moderate probability of exposure effect at 20 ng/mL
-High probability of exposure effect- scums

Performance Data

Test sensitivity:	The Abraxis Microcystins Test Strip will detect microcystins and nodularins at 1 ng/mL or higher. At this level the test line exhibits moderate intensity. At greater than 10 ng/mL the test line is not visible. When compared with samples of known microcystins concentration, it is possible to obtain a semi-quantitative result.
Selectivity:	The assay exhibits very good cross-reactivity with all cyanobacterial cyclic peptide toxin congeners tested to date.
Cell Lysing:	When comparing samples lysed using the QuikLyse*™ reagents and samples lysed using the freeze and thaw method (3 times), average recovery obtained was 94%, SD = 16.7%.
Samples:	A sample correlation between the Abraxis Strip Test and ELISA methods showed a good correlation.

For ordering or technical assistance contact:

Abraxis LLC
54 Steamwhistle Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

R1111410