Evaluation of Two Test Kits for Measurement of Microcystin Concentrations

Prepared for

Nova Scotia Department of the Environment

By

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I. Introduction

The objective of this study was to determine the suitability, feasibility and potential cost savings of using two commercially available test kits, selected by personnel of the Nova Scotia Department of Environment, which could potentially be used in the field to provide an immediate estimate of microcystins levels present within a water body. The trade names and product numbers of the test kits evaluated were the ABRAXIS Microcystin Test Strip (PN 520022) and the ENVIROLOGIX QualiTube Kit for Microcystins (PN ET 022).

The trials were conducted between the 14th and 25th of August 2011 during water quality surveys carried out at ten lakes being monitored within the Carleton River area of Digby and Yarmouth counties. The results were compared to those obtained for duplicate water samples collected and sent to a commercial laboratory (ALS Environmental) located in Winnipeg, MB.

II. Methodologies

Both the ABRAXIS and ENVIRLOGIX test kits use the ELISA (Enzyme-Linked-Immunosorbent Assay) protocol for measuring microcystins. This protocol is commonly used in immunology to detect the presence of an antibody or antigen in a sample. In this procedure, an unknown amount of antigen (in this case microcystin) is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and a substance is added that the enzyme can convert to a detectable signal, most often a colour change in a chemical substrate. As a result, the more of the antigen present, the lesser is the amount of free enzyme present to react with the chemical substrate and the lesser is the degree of colour development.

The ALS laboratory, to which the results of the test kits are compared, uses the ENVIROLOGIX Microcystin Plate Kit procedure to measure microcystins. This is also based on the ELISA protocol, but is a more sophisticated procedure involving color development within a series of test wells that is measured spectrophotometrically using a microtiter plate reader.

Details of the procedure involved in using the ABRAXIS and ENVIROLOGIX kits are contained in APPENDICES I and II respectively.

Water samples for analysis of microcystins were collected in pre-cleaned amber glass bottles, stored in a cooler and analyzed on the same day they were collected. Water samples for the ALS lab analyses were collected in clear glass sample containers provided by ALS, stored refrigerated in the dark until all lakes were sampled and then couriered to ALS in a styrofoam cooler containing ice packs.

III. Results

Of the ten lakes sampled and analyzed for microcystin concentrations using each of the two test kits, only one site had a result that was above the detection limits of the each test procedure (Table 1). This was Nowlans Lake in which the ABRAXIS test kit exhibited a microcystin concentration >10 µg/L. This result was confirmed by repeating the test three times. The ENVIROLOGIX test kit did not produce a positive result. The difference is most likely due to the cell lysing step used in the ABRAXIS procedure but absent in the ENVIROLOGIX procedure (see Section IV). The ALS laboratory analysis results also indicated a microcystin concentration above 10 µg/L for Nowlans Lake.

Table 1. Comparison of microcystin analyses carried out using the field test kits with results obtained by the ALS Environmental laboratory.

LAKE	ABRAXIS Test Strip Kit Total Microcystins (µg/L)	ENVIROLOGIX QualiTube Kit Free Microcystins (µg/L)	ALS Environmental	
			Free Microcystins (µg/L)	Total Microcystins (µg/L)
Hourglass	< 1	< 0.5	<0.20	<0.20
Placides	< 1	< 0.5	<0.20	<0.20
Porcupine	< 1	< 0.5	<0.20	<0.20
Parr	< 1	< 0.5	<0.20	<0.20
Ogden	< 1	< 0.5	<0.20	<0.20
Fanning	< 1	< 0.5	<0.20	<0.20
Sloans	< 1	< 0.5	<0.20	<0.20
Vaughan	< 1	< 0.5	<0.20	<0.20
Nowlans	> 10	< 0.5	<0.20	11.82
Provost	< 1	< 0.5	<0.20	<0.20

IV. Discussion

Although the principle is the same for both the ABRAXIS and ENVIRLOGIX test kits, there are important differences between the two. The former uses a membrane test strip and the latter an antibody coated test tube in which to visualize the degree of color development. In addition, and most importantly, the ABRAXIS procedure involves an initial step in which the water sample

being tested is subjected to a cell lysing agent that releases intracellular microcystins and the results represent total (i.e., free or dissolved plus intracellular) microcystin concentration. In contrast, the ENVIRLOGIX test procedure does not include a cell lysing step and the results represent only the concentration of microcystins free within the water sample. The ENVIROLOGIX Microcystin Plate Kit used by ALS Environmental, like the ENVIRLOGIX QualiTube Kit, does not routinely include an initial cell lyses step and also measures only free, as opposed to total, microcystins. However, ALS claims that freezing samples prior to analysis is a suitable means for lysing *Microcystis* and this was requested in addition to the usual un-lysed cell analyses.

There are also important differences in the range of microcystin levels measured by the two kits. The ABRAXIS kit is designed for microcystin concentrations more suited for evaluation with reference to recreational water quality guidelines while the ENVIROLOGIX kit is designed for concentrations more suited to drinking water guidelines.

There are other differences between the two test kits (Table 2), but these are mostly minor with respect to their suitability as a field test kit.

Table 2. Comparison between test kits evaluated.

Parameter	**ABRAXIS Test Strip Kit (520022)**	**ENVIROLOGIX QualiTube Kit (ET 022)**
Protocol	ELISA	ELISA
Units Measured	Total microcystins	Free microcystins
Assay Range	1 - 10 µg/L	0.5 – 3 µg/L
Limit of detection	1 µg/L	0.3 µg/L
Time to Complete Test	30-40 min.	40-50 min.
Number of water samples that can be processed per kit.	20	12 to 24
Cost/Sample	US$30	$20 to $40
Shelf Life/Recommended storage	One year/Room temperature	One year/Refrigerated
Suggested maximum sample storage time prior to analysis	Five days if refrigerated; longer if frozen.	Unspecified
Availability of control samples	Available for $150/kit	Provided with kit
Optional Visualization Aids Available	Test strip reader	Portable photometer

It should be noted that it has been estimated that there are as many as 80 variants (congeners) in the chemical forms of microcystins which is thought to be a result of the numerous strains of *Microcystis* known to occur in nature. For the test kits evaluated in this study, it is thought that they will detect more than just the Microcystin-LR form, which is the form specified in the World Health Organization (WHO) and Health Canada (HC) guidelines. For both test kits the manufactures indicate that it is not known exactly how many forms each will detect. Although both WHO and HC guidelines specify Microcystin-LR levels, it appears that all microcystin variants are toxic, although to various degrees, so this difference may not be of much relevance.

V. Recommendations

As a field based test kit that can provide a rapid assessment, the ABRAXIS Test Strip Kit is the one I would recommend. It requires minimal technical skill to use, measures total microcystins which is the form most applicable to WHO and HC guidelines, and the results are available within 30 minutes. Its only real shortcoming is that without modification of the procedure, the maximum range it can detect is 10 µg/L whereas the WHO and HC guideline for recreational water use is <20 µg/L. However, this limitation can easily be overcome by dilution of a sample prior to testing. For example, if the sample were diluted 1:1 with clean water (preferably deionized) prior to analysis, and the results were still greater than 10 µg/L, this would indicate that the concentration of total microcystins is ≥20 µg/L.

In comparison, although the ENVIROLOGIX QualiTube Kit is only slightly more difficult to use, it has greater shortcomings. Most importantly, because it does not contain a lysing agent the measurement of total microcystins requires that the water sample be subjected to a freeze/thaw cycle for a recommended minimum of three times. This results in a much longer time to achieve the result as well as making it impractical for use in the field.

If the ABRAXIS kit is chosen, I would suggest evaluation and potential adoption of one or both of two options that are available at an additional cost. The first is the use of calibration solutions containing known amounts of microcystin that are run concurrently with the water sample being tested. In addition to providing a reference for comparing color development, use of the calibration solutions ensures that the test kit is working properly. Calibration solutions are available for US$150 if purchased with a kit, or for US$300 if purchased separately. The second option is the use of a test strip reader that would make estimating colour development of the test strip more objective than just using a visual determination. This is available from ABRAXIS at a cost of US$1800. Appendix III contains a description of this instrument.

I would also recommend, as suggested by the manufacturer, that if a sample shows results >20 µg/L, this be confirmed by a more precise and accurate test such as the ENVIROLOGIX Microcystin Plate Kit procedure on lysed cells as used by ALS laboratory.

APPENDIX I

ABRAXIS TEST STRIP PROCEDURE

Microcystins Strip Test

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

ABRAXIS

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.

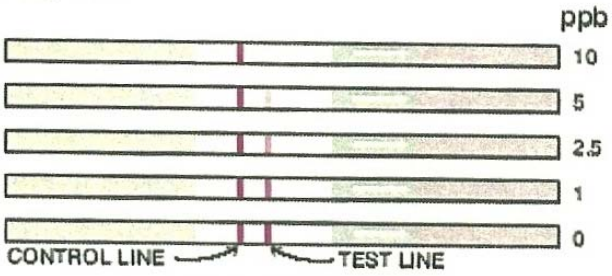
C. Assay Controls

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. Addtional 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.

General Limited Warranty/Disclaimer: Abraxis LLC warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis LLC makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.** The ETV verifies the performance of commercial ready technologies under specific criteria, testing conditions, and quality assurance. ETV does not imply approval or certification of this product, nor does it make any explicit or implied warranties or guarantees as to product performance. www.epa.gov/etv.

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R1111410

Algal Toxin Strip Test (Microcystins) Recreational Water

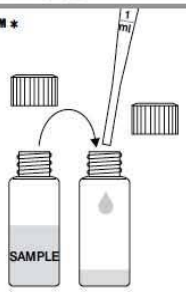
1. Collect Sample

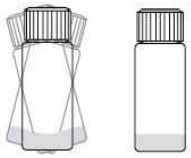
Collect 1 to 2 mL of sample.

2. Transfer/QuikLyse™ *

Using the graduated pipette provided, transfer 1 mL of SAMPLE to the lysis vial containing the dried lysis reagent.

*Patent Pending



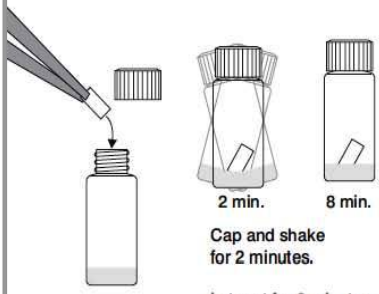


Cap and shake for 2 minutes.

Let rest for 8 minutes.

3. Add Reagent Paper/QuikLyse™ *

Using the forceps provided, add 1 reagent paper to the lysis vial.

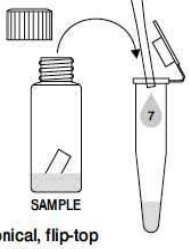


Cap and shake for 2 minutes.

Let rest for 8 minutes.

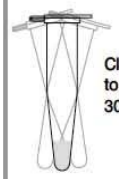
4. Transfer

Using the pipette provided, add 7 drops of SAMPLE to the conical, flip-top tube.



(The conical, flip-top tube contains dried reagents.)

5. Shake and incubate



Close the conical, flip-top tube and shake for 30 seconds.

(Dried reagents will dissolve, turning the sample purple.)

6. Test

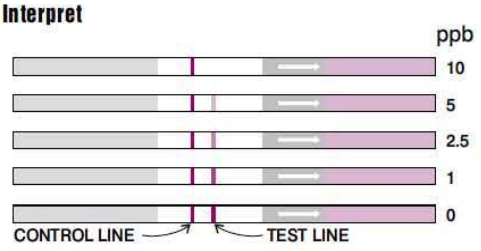
Insert test strip into conical, flip-top tube with arrow pointing down. (sample pad down).

Incubate for 10 minutes.

7. Dry

Remove test strip. Lay flat and allow to continue developing for 5 minutes.

8. Interpret



INTERPRET TEST

CONTROL LINE	TEST LINE	INTERPRETATION
NO CONTROL LINE PRESENT	NO TEST LINE PRESENT	INVALID RESULT
CONTROL LINE PRESENT	NO TEST LINE PRESENT	>10 ppb
CONTROL LINE PRESENT	MODERATE INTENSITY TEST LINE PRESENT	BETWEEN 0 AND 10 ppb

For Ordering or Technical Assistance Contact:
ABRAXIS, LLC 54 Steamwhistle Drive, Warminster, PA 18974 Phone: 215-357-3911 Fax: 215-357-5232 www.abraxiskits.com

ABRAXIS

APPENDIX II

ENVIROLOGIX QUAITUBE KIT INSTRUCTIONS

ENVIROLOGIX™

QualiTube™ Kit For Microcystin

Highlights:

- *Semi-quantitative field screening of Microcystin toxin in surface water*
- *Detects from 0.5 to 3 ppb*

Contents of Kit:

- *36 antibody-coated test tubes*
- *1 vial of 0.5 ppb Microcystin LR Calibrator*
- *1 vial of 3 ppb Microcystin LR Calibrator*
- *1 dropper bottle of Assay Diluent*
- *1 dropper bottle of Microcystin-enzyme Conjugate*
- *1 dropper bottle of Substrate*
- *1 bottle of Stop Solution*
- *36 sample pipettes*

Optional Accessory Item:

- *ACC 062 – 1 vial of 1.5 ppb Microcystin LR Calibrator*

Precision

Intra-Assay Precision (n=7)	
	%CV (OD)
Negative Control	8.4%
1.0 ppb Control	8.1%
Inter-Assay Precision (n=8)	
	%CV (B/B_0)
1.0 ppb Control	9.6%

Cross-Reactivity

Compound	50% B_0	81.5% B_0 LOD
Microcystin LR	0.94	0.30
Microcystin LA	0.78	0.43
Microcystin RR	1.53	0.65
Microcystin YR	2.53	0.69
Nodularin	1.44	0.53

Catalog Number ET 022

Intended Use

The EnviroLogix QualiTube Kit for Microcystin is designed for semi-quantitative field screening of Microcystin toxin in surface water samples. The kit is supplied with calibrators at 0.5 and 3 ppb. The assay range can easily be extended.

How the Test Works

The QualiTube Kit for Microcystin is a competitive Enzyme-Linked ImmunoSorbent Assay (ELISA). In the test, Microcystin toxin in the sample competes with enzyme (horseradish peroxidase)-labeled Microcystin for a limited number of antibody binding sites on the inside surface of the test tubes.

After a simple wash step, the outcome of the competition is visualized with a color development step. As with all competitive immunoassays, sample concentration is inversely proportional to color development.

Darker color = Lower concentration
Lighter color = Higher concentration

Limit of Detection

The Limit of Detection (LOD) of the EnviroLogix Microcystin Tube Kit is 0.3 ppb. The LOD was determined by interpolation at 81.5% B_0* from a standard curve. 81.5% B_0 was determined to be 2 standard deviations from the mean of a population of negative water samples.

*100% B_0 equals the maximum amount of Microcystin-enzyme conjugate that is bound by the antibody in the absence of any Microcystin in the sample (i.e. negative control). %Bo = (OD of Sample or Calibrator/OD of Negative Control) x 100.

Precision

Microcystin-fortified control solutions were repetitively analyzed within a single assay. The data is expressed as %CV for absorbance (OD) and $\%B_0$.

False Positive/False Negative Rate

Six surface water samples were fortified with Microcystin to a concentration of one half and twice the 0.5 ppb low calibrator. All six samples (6/6) fortified with 0.25 ppb resulted in absorbances greater than the 0.5 ppb microcystin calibrator, for a 0% false positive rate. All six samples (6/6) fortified with 1.0 ppb produced absorbances between 0.5 and 3.0 ppb in microcystin concentration, for a 0% false negative rate.

Cross-Reactivity

The QualiTube Kit for Microcystin does not distinguish between the Microcystin toxin variants, but detects their presence to differing degrees. The table (left) shows the value for 50% B_0 and the value for the 81.5% B_0 limit of detection for four microcystin toxin variants and nodularin toxin. Concentration is in ppb.

Humic acid did not interfere in the assay up to a concentration of 100 ppm.

Materials Not Provided

- marking pen (indelible)
- timer (5, 20 and 10 minutes)
- cool tap or distilled water for rinsing tubes, in a wash bottle
- photometer for reading tubes (optional)
- test tube rack that can hold at least 6 tubes securely enough to flick out water after wash step (Contact EnviroLogix for information on obtaining an appropriate rack)
- disposable tip, adjustable air-displacement pipette which will measure 0.7 mL (optional)

How to Run the Assay

- Read all of the instructions before running the kit.
- 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).
- Organize all samples and reagents so that steps 1 and 2 can be performed in 3 minutes or less.
- Do not run more than 6 tubes at a time.

1. Rapidly add **5 drops of Microcystin Assay Diluent** to each tube in the assay.

2. Using the sample pipette provided, immediately add two drops of **0.5 ppb Microcystin Calibrator** to the first tube. Add two drops of **3.0 ppb Microcystin Calibrator** to the second tube. Add **two 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.**

3. 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.

4. Incubate tubes at ambient temperature for 5 minutes.

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

6. Incubate tubes at ambient temperature for 20 minutes.

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

8. 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.

TABLE 1
The following table illustrates results interpretation of water samples read visually:

Samples with blue color ...	Contain ...
Darker than the blue color of 0.5 ppb Calibrator	Less than 0.5 ppb Microcystins
Between the blue color of 0.5 ppb and 3.0 ppb Calibrator	Between 0.5 and 3.0 ppb Microcystins
Lighter than the blue color of 3.0 ppb Calibrator	More than 3 ppb Microcystins

TABLE 2
The following table illustrates results interpretation of water samples using a tube photometer:

Samples with OD values ...	Contain ...
Greater than OD of 0.5 ppb Calibrator	Less than 0.5 ppb Microcystins
Between OD of 0.5 ppb and 3.0 ppb Calibrator	Between 0.5 and 3.0 ppb Microcystins
Less than OD of 3.0 ppb Calibrator	More than 3 ppb Microcystins

Caution: Stop Solution is 1.0 N Hydrochloric acid. Handle carefully.

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

NOTE: Read the tubes within 30 minutes of the addition of Stop Solution.

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

How to Interpret the Results

Reading Tubes 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 Table 1 (left) to determine the level of microcystin in the samples.

Spectrophotometric Measurement

1. Set the wavelength of your photometer to 450 nanometers (nm). (If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.)

2. If the photometer does not auto-zero on air, zero the instrument against 1 mL water in a blank tube. Measure and record the optical density (OD) of each tube's contents. Alternatively, measure and record the OD in every tube, then subtract the OD of the water blank from each of the readings.

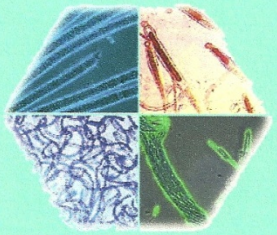
3. Use Table 2 (left) to determine the level of Microcystin in the sample.

4. For information on a field portable differential photometer contact EnviroLogix Technical Support. Contact information is at the end of these instructions.

Figure 1. Illustrative results interpretation using tube photometer

Well Contents	OD	Microcystin Concentration (ppb)
0.5 ppb Calibrator	0.984	NA
3.0 ppb Calibrator	0.306	NA
Sample	1.332	< 0.5 ppb
Sample	0.604	> 0.5 ppb, < 3.0 ppb

*Actual values may vary; this data is for demonstration purposes only.



Precautions and Notes

- **While dropping solutions into tubes from dropper bottles, hold the top of each tube between your thumb and index finger. This will prevent the drops from adhering to the sides of the tube, allowing the drops to fall to the bottom of the tube.**
- **Hold pipette bulbs and dropper bottles vertically over the tube opening while dropping.**
- Store all Tube Kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not expose Tube Kit components to temperatures greater than 37°C (99°F) or less than 2 °C (36°F).
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before use.
- Do not use kit components after the expiration date.
- Do not use reagents or test tubes from one Tube Kit with reagents or test tubes from a different Tube Kit.
- Do not expose **Substrate** to **sunlight** during pipetting or while incubating in the test tubes.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure.
- As with all tests, it is recommended that results be confirmed by an alternate method if necessary.
- Microcystin LR in aqueous solution will stick to plastics such as polypropylene. Collect and process samples in glass containers.
- Observe any applicable regulations when disposing of samples and kit reagents.

For Technical Support Contact Us At:

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APPENDIX III

ABRASCAN DIPSTICK READER

ABRASCAN DIPSTICK READER

AbraScan provides an easy means to objectively read, store and interpret results from the three Abraxis dipstick Microcystin test kits. It enhances results by removing the subjectivity of visual interpretation of the dipsticks. It also eliminates the need to manual record results.

PN 475025 AbraScan Dipstick Reader

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