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 5 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 114%, SD = 22.7%.

Samples: A sample correlation between the Abraxis Strip Test and ELISA methods showed a good correlation.

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Microcystins Strip Test

Immunochromatographic Strip Test for the Detection
of Microcystins and Nodularins in Source Drinking Water at 1 ppb

Product No. 520019 (5 Test), 520020 (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 source drinking 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 (>1 ppb). Semi-quantitation is also possible by comparing the test line intensity to the control line and to the chart provided. Available Microcystins controls may be used to approximate the quantity of toxin present in water samples.

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 source drinking water. 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.

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 source drinking water, 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. Transfer pipettes (calibrated at 1 mL)
5. Forceps
6. Reagent papers
7. Conical Test vials
8. Disposable pipettes
9. User's guide

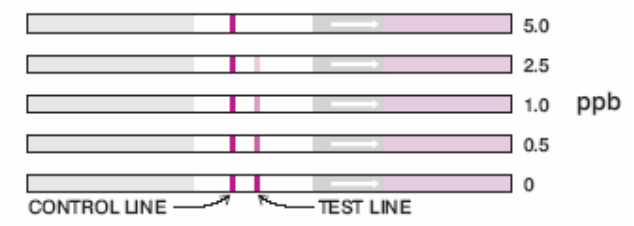
B. Test Preparation

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 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. 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.
5. Incubate the sample for 20 minutes (dried reagents will dissolve turning the sample purple).
6. Insert test strip (arrows down), into the conical vial containing the sample/antibody mixture.
7. Allow the test to develop for 10 minutes.
8. Remove the test strip. Lay it flat and allow to continue the development for 5 minutes.
9. Read the results visually as explained below under Interpretation of Results.

D. 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	>5 ng/ml (ppb)
Control line present	Moderate intensity test line present	Between 0 and 5 ng/ml (ppb)

E. Addtional Materials (not provided with the test)

1. Timer

F. 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.

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.