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 cyanobacterial blooms are an emerging issue worldwide due to increased source water nutrient pollution caused by eutrophication. Microcystins and Nodularins are cyclic toxin peptides. Microcystins (of which there are many structural variants, or congeners) have been found in fresh water throughout the world. To date, approximately 80 variants of Microcystin have been isolated. The most common variant is Microcystin-LR. Other common Microcystin variants include YR, RR, and LW. These toxins are produced by many types of cyanobacteria (blue-green algae), including *Microcystis, Anabaena, Oscillatoria, Nostoc, Anabaenopsis,* and terrestrial *Hapalosiphon.* Nodularins are produced by the genus Nodularia and they are found in marine and brackish water

Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms and, in several cases, has led to death. Human and animal exposure to these toxins occurs most frequently through the ingestion of water, 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 World Health Organization (WHO) has proposed a provisional upper limit for Microcystin-LR of 1.0 ppb (µg/L) in drinking water. For recreational bathing waters, the WHO has established 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

The U.S. Environmental Protection Agency (EPA) has also established guidelines for Microcystins in drinking water:

-For children below school age, 0.3 µg/L (ppb)
-For all other age groups, 1.6 µg/L (ppb)

Performance Data

Test sensitivity: The Abraxis Microcystins Strip Test for Recreational Water will detect Microcystins and Nodularins at 1 ng/mL or higher. At this level, the test line exhibits moderate intensity. At levels 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 Microcystin cyclic peptide toxin congeners tested to date.

Cell Lysing: When comparing samples lysed using the QuikLyse™ reagents and the 3 cycle freeze/ thaw method, average recovery obtained was 94%, SD = 16.7%.

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

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R102617

Microcystins Strip Test

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

QuikLyse™ reagents may be used in a method of U.S. Patent 9,739,777

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

1. General Description

The Abraxis Microcystins Strip Test for Recreational Water is a rapid immunochromatographic test, designed solely for the use in the qualitative screening of Microcystins and Nodularins in recreational water (freshwater samples only; please see the Brackish or Sea Water Sample Preparation technical bulletin for information on the screening of marine water samples). 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.

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 containing 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, 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 control line, Microcystins are present at a level > 2.5 ppb. Semi-quantitative results in the range of 0-10 ppb can be obtained by comparing the sample test strip appearance to the appearance of test strips from 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 include:
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 test is designed for use with freshwater recreational waters. The use of the test with brackish or seawater samples will produce inaccurate results. Please see the Brackish or Sea Water Sample Preparation technical bulletin for information on the preparation and screening of marine water samples using the Microcystins Strip Test for Finished Drinking Water. The Microcystins Strip Test 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. Warnings and Precautions

-The Microcystins Strip Test for Recreational Water is for the screening of freshwater recreational water samples for total Microcystins (free and cell-bound). Please see the Brackish or Sea Water Sample Preparation technical bulletin for the preparation and screening of marine water samples using the Microcystins Strip Test for Finished Drinking Water.

-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 (such as those which have undergone the freeze/thaw method), please use the Abraxis Microcystins Strip Test for Finished Drinking Water at 1 ppb, PN 520016 (5 Test) or PN 520017 (20 Test).

-Use only the Microcystins test strips and QuikLyse™ reagents from one kit lot, as they have been adjusted in combination.

-All reagents and samples should be allowed to reach room temperature before testing.

-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 desiccated 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.

-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. Diluted samples will have a cut-off of 20 ppb.

-Use reasonable judgment when interpreting the test results.

-Results should be interpreted within 5-10 minutes after completion of the test.

7. Sample Collection and Handling

-Collect water samples in glass or polyethylene terephthalate (PETG) containers only. The use of other types of plastic containers may result in adsorptive loss of Microcystins, producing inaccurate (falsely low) results.

-Samples can be stored refrigerated for up to 5 days. If samples must be held for greater than 5 days, samples should be stored frozen.

A. Materials Provided

1. Microcystins test strips in a desiccated container
2. Sample collection vials
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. Additional Materials (not provided with the test)

1. Timer
2. Microcystins Check Samples, Abraxis PN 422011, for the preparation of control solutions which can be analyzed with samples, to obtain semi-quantitative sample results (see Section C, Controls, below).

C. Controls

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

D. Test Preparation

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

E. Procedure

When analyzing for total Microcystins (dissolved, or free, and cell-bound), which may be present in recreational waters, a sample lysis is necessary before analysis. The Abraxis QuikLyse™ reagents provide a rapid option for cell lysis.

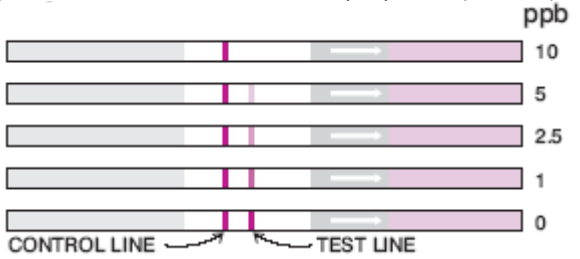
1. Using a new graduated disposable pipette for each sample, draw the sample to the 1 mL line (graduation mark slightly below bulb) and add 1 mL of sample to the lysis vial.
2. Cap the vial and shake for 2 minutes, then allow the sample in the vial to incubate at room temperature for 8 minutes, to begin the cell lysis.
3. Using the forceps provided, add 1 reagent paper to the lysis vial.
4. Cap the vial and shake for 2 minutes, then allow the sample in the vial to incubate at room temperature for 8 minutes.
5. Label conical test vials for each sample to be tested.
6. Using a new disposable transfer pipette for each sample, transfer 7 drops (approximately 200 µL) of the previously lysed water sample (Steps 1-4 above) to the appropriately labeled conical test vial.
7. Close the conical test vial and shake for 30 seconds. Examine the vial to ensure all dried reagents are completely dissolved (dried reagents will dissolve, turning the sample purple).
8. Insert test strip (arrows down) into the conical vial.
9. Allow the test to develop for 10 minutes.
10. Remove the test strip. Lay the strip flat and allow to continue developing for 5 minutes.
11. Read the results visually, as explained below in Section F, Interpretation of Results.

F. Interpretation of Results

Sample concentrations are determined by comparison of the intensity of the test line to the intensity of the control line on the same test strip. Although control line intensity may vary, a visible control line must be present for results to be considered valid. Test strips with a test line which is darker than or of equal intensity to the control line indicates a result which is below the limit of detection of the test. Test strips with a test line which is lighter than the control line indicates a result which is <10 ppb. Test strips with no test line visible (only the control line is visible) indicates a result which is ≥ 10 ppb. 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.

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 to equal intensity test line present	Between 0 and 10 ng/mL (ppb)

The appearance of test strips may also be compared to the illustration below to determine approximate sample concentration ranges. Please note that the illustration is intended for the demonstration of test line to control line intensity only. Results should not be determined by comparing the intensity of test lines from test strips to the test line intensity of the illustration, as the overall intensity of test strips may vary slightly with different lots of reagents. To obtain semi-quantitative results in the range of 0-10 ppb, solutions of known Microcystins concentration (control solutions) must be tested concurrently with samples. Sample test line intensities can then be compared with control solution 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.



Alternately, test strips can also be interpreted using the AbraScan test strip reader (PN 475025), which provides objective determination of line intensities for consistent interpretation of results as well as a digital photographic record of all test strips.

G. Additional Analysis

If necessary, positive samples can be confirmed by ELISA, HPLC or other conventional methods. These services are available from commercial analytical laboratories such as Green Water Labs (www.greenwaterlab.com).

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.
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