Importance of Microcystins/Nodularins Determination in Benthic Mats

Benthic mats are dense accumulations of algae that grow on the bottom of bodies of water and, under certain conditions, can become dislodged and rise to the surface. 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 that may be produced by blooms. 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. Microcystins (of which there are many structural variants, or congeners) have been found in freshwater throughout the world. To date, approximately 250 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 contact with or ingestion of contaminated water or benthic mats.

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 limit of detection (LOD) for Microcystins and Nodularins with the ABRAXIS® Microcystins Strip Test for Finished Drinking Water for benthic mats is 40 ppb (ng/g). At this level, the test line exhibits moderate intensity. At levels greater than 200 ppb (ng/g), the test line is not visible. When compared with samples of known Microcystins concentration, it is possible to obtain a semi-quantitative result. The limit of detection (LOD) with the ABRAXIS® Microcystins-ADDA test and the ABRAXIS® Microcystins-DM test for benthic mats is 15 ppb (ng/g).

Selectivity: The ABRAXIS® Microcystins test kits exhibit very good cross-reactivity with all Microcystin cyclic peptide toxin congeners tested to date.

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

References

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Worldwide Patenting PCT WO 01/18059 A2

U.S. Patent Number 6,967,240

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Version : 02

ABRAXIS® Benthic Mat Sample Extraction Kit for Microcystins

Product No. 529916

1. General Description

The ABRAXIS® Benthic Mat Sample Extraction Kit for Microcystins is for the collection of benthic mat samples and sample preparation/extraction of Microcystins and Nodularins from benthic mat samples prior to testing with the ABRAXIS® Microcystins Strip Test for Finished Drinking Water (PN 520016 or 520017). (*DO NOT use the Microcystins Strip Tests for Recreational Water or Source Drinking Water*). Benthic mat sample extracts can also be used for confirmatory testing with the ABRAXIS® Microcystins-ADDA ELISA (PN 520011) and DM-ELISA (PN 522015) test kits. Each extraction kit provides materials for 20 benthic mat samples.

2. Safety Instructions

Wear appropriate protective clothing (gloves, glasses, etc.) and avoid contact with skin and mucous membranes. The sample preparation procedure should be performed in a well-ventilated area. Avoid breathing aerosols. If contact with Microcystins occurs, wash with copious amounts of water. Discard samples according to local, state, and federal regulations.

3. Storage and Stability

The ABRAXIS® Benthic Mat Sample Extraction Kit for Microcystins should be stored between 2-30°C. The benthic mat samples to be extracted and analyzed should be at room temperature before use.

4. Warnings and Precautions

-This procedure is intended for use with benthic mat samples. Other matrices should be thoroughly validated before use.
-Due to the high variability of compounds that may be found in benthic mat samples, test interferences caused by matrix effects cannot be completely excluded. Samples containing interferences that inhibit line development (producing extremely faint test and control lines) will require an additional 1:20 dilution (0.5 mL of sample extract into 9.5 mL of distilled or deionized water) to allow for full line development. Final sample concentrations will then be determined by multiplying the concentration of the corresponding test strip appearance (determined according to the criteria shown in section F, Interpretation of Results, below) by the dilution factor of 20. For example, test strips showing only a control line (no test line present) will correspond to the image of a test strip with a final result of ~200 ppb, which is then multiplied by the additional factor of 20 for final results of ~4000 ppb of Microcystins in the benthic mat sample.
-Please see the corresponding test kit Instructions for use for test limitations and interferences.
-The Microcystins Strip Test provides only a preliminary qualitative test result. An alternate, more quantitative analytical method, such as ELISA or instrumental analysis, should be used to obtain a confirmed quantitative analytical result. See step 9 in section D (Extraction Procedure), below.
-The cellular makeup of benthic mats can vary widely throughout the mat, which can therefore cause the toxin content of the mat to also vary widely throughout the mat. Because of this, whenever possible, the collected sample should be a composite, made up of pieces sampled from throughout the mat to provide the most accurate assessment of toxin concentration contained within the entire benthic mat. See section C, Sample Collection, for instructions on collecting a representative mat sample and ITRC HCB-2 and HCB-1 (https://hcb-2.itrcweb.org and https://hcb-1.itrcweb.org) for additional information.
-Distilled or deionized water **must** be used for sample preparation. Do **not** use tap water for sample preparation, as chlorine and other water treatment chemicals present in tap water will degrade Microcystins, causing inaccurate, biased low sample results.
-Re-usable equipment (scissors, tweezers, etc.) used for collection or extraction of benthic mat samples must be thoroughly cleaned between samples to avoid cross-contamination which may cause inaccurate sample results. Clean equipment after each use with a 10% bleach solution (1 part bleach in 9 parts water) and then rinse with clean water.
*Note: Tap water may be used for the preparation of the 10% bleach solution and for the rinsing of equipment after cleaning with the 10% bleach solution, but do **not** use tap water for sample preparation/extraction, as chlorine and other water treatment chemicals present in tap water will degrade Microcystins, causing inaccurate, biased low sample results.*
-Samples should be allowed to reach room temperature before testing.
-Microcystins have been found to adsorb onto the surface of many types of plastic, which may result in adsorptive loss of Microcystins with prolonged contact, producing inaccurate, biased low results. To minimize potential loss of Microcystins, samples should be transferred to glass vials immediately after processing in sample extraction tubes (do not store processed samples in sample extraction tubes).

A. Materials Provided

1. 40 mL amber glass Sample Collection Vials with caps and labels (20)
2. Disposable sample extraction tubes with disposable pestles (20)
3. Sample extraction tube holder (1)
4. Foam vial holder (1)
5. Disposable graduated pipettes (20, bags marked with blue sticker to indicate appropriate step for use)
6. Disposable transfer pipettes (20, bag marked with yellow sticker to indicate appropriate step for use)
7. 20 mL glass Dilution Vials (20)
8. 4 mL glass Final Sample Extract Vials with caps and labels (20)
9. Instructions for use

B. Additional Materials (not provided with the extraction kit)

1. Portable scale with two decimal place display (i.e., 0.00 g), PN 709049 or equivalent
2. Disposable gloves
3. Protective glasses
4. Scissors
5. Tweezers
6. 10% bleach solution
7. Tap water (for rinsing equipment, see section 4, Warnings and Precautions, above)
8. Distilled or deionized water
9. Paper towels
10. Disposable spatulas (optional), PN 705043 or equivalent
11. Permanent marker
12. Timer, PN 709055 or equivalent
13. ABRAXIS® Microcystins Strip Test for Finished Drinking Water, PN 520016 or 520017, or ABRAXIS® Microcystins-ADDA ELISA, PN 520011, or DM ELISA PN 522015, for confirmatory analysis

C. Sample Collection

1. Using clean scissors, remove pieces of mat material from throughout the benthic mat and place into a single clean, appropriately labeled amber 40 mL Sample Collection vial.
2. Clean scissors with 10% bleach solution and rinse with water. Remove and appropriately dispose of gloves.
3. If the sample is to be tested immediately, proceed to section D, Extraction Procedure. If the sample is to be stored for later extraction/testing, place tightly closed vial in a cooler with ice packs. Store samples refrigerated.

D. Extraction Procedure

1. Unfold the cardboard vial holder from the sample extraction kit and place on the portable scale. Place one sample extraction tube into the vial holder and press the "tare" button to bring displayed weight to "0.00 g".
2. Remove the benthic mat sample from the amber vial and place on paper towels. Blot to remove excess moisture.
3. Using tweezers or a disposable spatula, remove small pieces of the mat material from throughout the sample and place in the sample extraction tube. Transfer a total of 0.25 g of benthic mat to the tube.
   Note: Tweezers must be cleaned with 10% bleach solution and rinsed with water after each use and gloves must be cleaned or changed if they come into contact with benthic mat sample or water that is removed during blotting of the sample to prevent cross-contamination of extraction/testing materials or samples which can cause inaccurate test results.
4. Using a clean disposable graduated pipette from bag 1 (bag with blue sticker), add 0.5 mL of distilled or deionized water to the tube, being careful to avoid touching the sample or tube with the pipette to prevent cross-contamination.
5. Using a clean disposable pestle, thoroughly grind the sample and water solution for 4 minutes, being careful to avoid causing the sample or water to overflow or spill from the tube. When thoroughly ground, the sample will have a thin, mud-like appearance, with no large mat pieces visible in the solution.
   Note: If sample or water overflows from the tube during extraction, wipe the tube with a paper towel saturated with 10% bleach solution and clean or change gloves to prevent contact with Microcystins or cross-contamination of extraction/testing materials that can produce inaccurate results.
6. Using the same disposable graduated pipette used in step 4, transfer 9.5 mL of distilled or deionized water to a clean, appropriately labelled Dilution Vial.
7. Using the same disposable graduated pipette used in step 6, transfer all of the sample extract from step 5 to the Dilution Vial. Discard pipette after use. Cap vial tightly and shake for 30 seconds to thoroughly mix. Allow to settle for 10 minutes.
8. Using a clean transfer pipette from bag 2 (yellow sticker), transfer the top of the upper liquid portion of the extract from the Dilution Vial into a clean, appropriately labeled amber Final Extract vial.
9. Analyze the diluted benthic mat sample extract (from step 8, above) as described in section E, Test Strip Sample Analysis Procedure. This extract may also be used for confirmatory testing of positive results using the ABRAXIS® Microcystins-ADDA or DM ELISA kits if desired. Sample extracts require an additional 1:2.5 dilution of the final extracts (0.4 mL of the sample from the final extract vial into 0.6 mL of Microcystins sample diluent) prior to analysis. The diluted sample should be analyzed as described in the Assay Procedure section of the appropriate plate kit Instructions for use. Final results for samples are determined by multiplying the ELISA results by a factor of 100.

E. Test Strip Sample Analysis Procedure (Analysis using ABRAXIS® Microcystins Strip Test for Finished Drinking Water, PN 520016 or 520017, not included with Benthic Mat Sample Extraction Kit)

1. Label conical test vials for each sample to be tested.
2. Using a new disposable exact volume transfer pipette for each sample, transfer 200 µL of the benthic mat sample extract to the appropriate labeled conical test vial (see pipette package for usage instructions).
3. 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).
4. Incubate the conical test vial at room temperature for 20 minutes.
5. Insert test strip (arrows down) into the conical vial.
6. Allow the test to develop for 10 minutes.
7. Remove the test strip. Lay the strip flat and allow to continue developing for 5 minutes.
8. Read the results visually, as explained below in section F, Interpretation of Results.

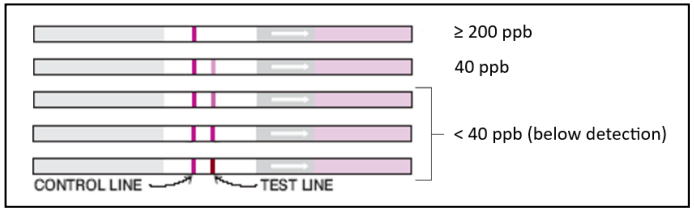
F. Interpretation of Results

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 (<40 ppb). Test strips with a test line which is lighter than the control line indicates a result which is ≥ 40 ppb. Test strips with no test line visible (only the control line is visible) indicates a result which is ≥ 200 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	≥200 ppb
Control line present	Test line present	Between 0 and <200 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, 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.

Visual Interpretation:



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. Please note that benthic mat sample results interpreted using the AbraScan test strip reader are raw results that must be multiplied by 40 to obtain final sample concentrations. For example, the AbraScan test strip reader result of ~1 ppb x 40 = ~40 ppb of Microcystins in a benthic mat sample. (See section 4 for results calculation for samples requiring additional dilution.)