

Importance of Anatoxin-a Determination

Anatoxin-a is an alkaloid neurotoxin produced by some species of cyanobacteria (blue-green algae). It is one of the most toxic of the cyanobacterial toxins. In humans and other animals, the skeletal neuromuscular junction constitutes a primary target for Anatoxin-a (Anatoxin-a can also cross the blood-brain barrier). The neuromuscular junction is specialized for the rapid transmission of neuronal information from the pre-synaptic nerve terminal to the post-synaptic muscle fiber. This transmission is mediated by the synchronous release of the neurotransmitter acetylcholine (ACh), which activates nicotinic acetylcholine receptors (nAChRs) in the muscle endplate, triggering a series of events that lead to muscle contraction. Most ACh molecules are hydrolyzed by acetylcholinesterases, which are highly concentrated at the neuromuscular junction. Anatoxin-a functions as an agonist of nAChRs, like ACh, but is about 20 times more potent. Unlike ACh, it is not degraded by acetylcholinesterases and produces sustained depolarization of the muscle endplate, causing overstimulation of the muscles, leading to muscle fatigue and ultimately paralysis. Symptoms begin within 5 minutes of ingestion of Anatoxin-a and progress rapidly, resulting in cyanosis, convulsions, cardiac arrhythmia, and respiratory paralysis, which ultimately results in death due to suffocation.

Humans and other animals may be exposed to Anatoxin-a through ingestion of contaminated water, through drinking or during recreational activities in which water is swallowed. Due to the potential for serious harm and even death, many countries are expanding monitoring programs to include Anatoxin-a and are establishing regulations regarding the amount of Anatoxin-a in drinking and recreational waters. New Zealand is among those taking regulatory action, establishing a 6.0 µg/L provisional maximum acceptable value (MAV) for Anatoxin-a, and the U.S. Environmental Protection Agency (EPA) will be announcing drinking and recreational water health advisories.

Performance Data

Test sensitivity: The limit of detection (LOD) with the ABRAXIS® Anatoxin-a Strip Test for freshwater is 0.4 ppb. At this level, the test line exhibits moderate intensity. At levels greater than 2.5 ppb, the test line is not visible. When compared with samples of known Anatoxin-a concentration, it is possible to obtain a semi-quantitative result.

Selectivity: The assay exhibits very good cross-reactivity with (+) Anatoxin-a and Homoanatoxin-a.

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

*The monoclonal antibody and conjugate (Patent Application P201531661) included in the ABRAXIS® Anatoxin-a Strip Test has been licensed from the Spanish National Research Council (CSIC) and the University of Valencia (UVEG).

Additional Analysis

If necessary, positive samples can be confirmed by ELISA, HPLC, or other conventional methods. These services are available from commercial analytical laboratories (list of analytical laboratories available upon request).

References

C. Bumke-Vogt, W. Mailahn, W. Rotard, and I. Chorus, A Highly Sensitive Analytical Method for the Neurotoxin Anatoxin-a, Using GC-ECD, and First Application to Laboratory Cultures. *Phycologia*. 35, 1996, 51-56.

M.W. Heath, S.A. Wood, R.F. Barbieri, R.G. Young, and K.G. Ryan, Effects of Nitrogen and Phosphorus on Anatoxin-a, Homoanatoxin-a, Dihydroanatoxin-a and Dihydrohomoanatoxin-a Production by *Phormidium autumnale*. *Toxicon*. 92, 2014, 179-185.

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ABRAXIS® Anatoxin-a Strip Test

Immunochemical Strip Test for the Detection of Anatoxin-a*
in Drinking and Recreational Waters
Product No. 520042 (5 Test), 520043 (20 Test)

1. General Description

The ABRAXIS® Anatoxin-a Strip Test for Water is a rapid immunochemical test, designed for use in the qualitative screening of Anatoxin-a in freshwater (please see the Gold Standard Diagnostics Horsham website for information on sample preparation for benthic mat samples using the ABRAXIS® Benthic Mat Extraction Kit for Anatoxin-a Strip Test). The ABRAXIS® Anatoxin-a 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 ABRAXIS® Anatoxin-a Strip Kit should be stored between 2-30°C. The test strips, test vials, and water samples to be analyzed should be at room temperature before use. Reagents may be used until the last day of the month as indicated by the expiration date on the box.

4. Test Principle

The test is based on the recognition of Anatoxin-a by specific antibodies. The toxin conjugate competes for antibody binding sites with Anatoxin-a that may be present in the water sample. The test device consists of a vial containing specific antibodies for Anatoxin-a 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 Anatoxin-a 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 Anatoxin-a 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 Anatoxin-a is present in the water sample, it competes 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, Anatoxin-a is present at a level of concern. Semi-quantitative results can be obtained by comparing the sample test strip appearance to the appearance of test strips from solutions of known Anatoxin-a concentrations (control solutions). Concentrated Anatoxin-a standards which can be used to prepare controls are available through Gold Standard Diagnostics (PN300620).

5. Limitations of the ABRAXIS® Anatoxin-a 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 cannot 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), use of the test with water samples which have not been preserved properly (see section 6, Warnings and Precautions, and section 7, Sample Collection and Handling).

The test is designed for use with freshwater samples. Please see the ABRAXIS® Benthic Mat Extraction Kit for Anatoxin-a Strip Test Instructions for use for sample preparation and testing procedures for benthic mat samples. The ABRAXIS® Anatoxin-a 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

-Use only the ABRAXIS® Anatoxin-a test strips and reagents from one kit lot, as they have been adjusted in combination.

-Water samples must be preserved using the Sample Preservation Vials included in the kit before analyzing with the ABRAXIS® Anatoxin-a strip test. Use of the ABRAXIS® Anatoxin-a test strips with water samples which have not been treated with the reagents contained in the Sample Preservation Vials **will produce inaccurate results** (see section 7, Sample Collection and Handling, for information on collecting water samples for evaluation of Anatoxin-a content). Use of sample preservation reagents other than those described in this Instructions for use may also produce inaccurate results. Please see the ABRAXIS® Benthic Mat Extraction Kit for Anatoxin-a Strip Test Instructions for use for information on sample preparation for benthic mat samples.

-Test strips and conical test vials should be kept sealed in their original packaging with desiccant when not in use. Exposure to humidity during storage may adversely impact test performance and cause inaccurate results. After initial use in high humidity conditions, remaining kit components should be stored tightly closed with desiccant and refrigerated (2-8°C) when not in use. Conical test vials stored with indicating desiccant which has turned from blue to pink (indicating excessive exposure to moisture) should not be used for testing and should be discarded.

-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 the date of use is prior to the expiration date on the label.

-Avoid cross-contamination of water samples by using a new sample vial and disposable pipette for each sample.

-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

-Freshwater samples **must** be preserved at the time of collection (see Water Sample Collection/Preservation Procedure, section D below) to prevent degradation of Anatoxin-a, which will produce inaccurate (biased low) sample results. The use of the ABRAXIS® Anatoxin-a test strips with samples which have not been treated with the reagents contained in the Sample Preservation Vials **will produce inaccurate results**. Please see the ABRAXIS® Benthic Mat Extraction Kit for Anatoxin-a Strip Test Instructions for use for information on sample preparation for benthic mat samples.

-The Sample Preservation Vials are amber glass containers which contain dried sample preservation reagents. The use of other types of containers may result in adsorptive loss of Anatoxin-a to the sample container and/or degradation of Anatoxin-a due to exposure to natural or artificial light and/or the lack of preservation reagents, producing inaccurate (falsely low) results.

-Studies comparing the quantities of extracellular and intracellular Anatoxin-a in water samples have found significant differences in the amounts of Anatoxin-a located inside and outside of algal cells depending on the species of cyanobacteria present, cellular age, and nutrient content of the water source (see References section). If desired, preserved water samples (see section D, Water Sample Collection/Preservation Procedure) may be subjected to freeze/thaw sample preparation (sample is frozen then allowed to thaw for a total of three cycles) before analysis.

-Chlorinated water samples must be treated (quenched) with **ascorbic acid** at 0.1 mg/mL at the time of collection to remove residual chlorine. Each chlorinated water sample should be collected in a clean amber glass container, then immediately treated with ascorbic acid to neutralize chlorine. Immediately after quenching, the sample should be transferred to a Sample Preservation Vial following the procedure described in section D, Water Sample Collection/Preservation Procedure, below.

Do not use sodium thiosulfate to quench residual chlorine in water samples to be tested for Anatoxin-a. Sodium thiosulfate will degrade Anatoxin-a and produce biased low sample results.

-Preserved samples can be stored refrigerated (2-8°C) for up to 28 days. Samples which must be held for greater than 28 days should be stored frozen.

-Samples should be protected from exposure to natural and artificial light, as light exposure will cause degradation of Anatoxin-a.

A. Materials Provided

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|-----------------------------------------------------------------------------------|------------------------------|
| 1. Anatoxin-a test strips in a desiccated container | 4. Sample preservation vials |
| 2. 3 mL graduated disposable pipettes | 5. Conical test vials |
| 3. Disposable exact volume transfer pipettes (see package for usage instructions) | 6. Instructions for use |

B. Additional Materials (not provided with the test)

1. Timer
2. ABRAXIS® (+) Anatoxin-a standard (PN 300620), for the preparation of control solutions which can be analyzed with samples to obtain semi-quantitative sample results (see section C, Controls, below)
3. ABRAXIS® Benthic Mat Extraction Kit for Anatoxin-a Strip Test (PN 529918)

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 Anatoxin-a (positive and negative controls) should be analyzed with each lot of test strips to provide a reference for line intensity to be expected. Control samples should be preserved in the same manner as samples, using the Sample Preservation Vials, in order to produce accurate results. Analysis of control samples which have not been treated with the reagents in the Sample Preservation Vials will produce inaccurate positive and negative control sample results.

D. Water Sample Collection/Preservation Procedure

Water samples must be treated with sample preservation reagents at the time of collection to prevent loss of Anatoxin-a:

1. Using a new graduated disposable pipette for each water sample, draw the sample to the 3 mL line (graduation mark slightly below the bulb) and add to an appropriately labeled amber glass Sample Preservation Vial.
2. Tightly cap the vial and shake thoroughly for 30 seconds to mix. Allow the vial to sit at room temperature for 5 minutes. Shake thoroughly to mix. Sample is now ready for testing or storage at 4°C for later testing.

E. Test Preparation

-Allow the reagents and preserved water sample to reach room temperature before use.

-Remove the number of conical test vials required from the package. The remaining conical test vials are stored in the tightly closed container with desiccant.

F. Procedure

Water samples must be preserved at the time of collection using the Sample Preservation Vials (provided in the kit) according to the procedure described in section D, Water Sample Collection/Preservation Procedure, before proceeding to the analysis procedure listed below. Water samples which are not preserved appropriately will produce inaccurate results.

1. Using a new disposable exact volume transfer pipette for each sample, transfer 200 µL of the preserved water sample (from section D, above) to the appropriately labeled conical test vial (see pipette package for usage instructions).
2. 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).
3. Incubate the conical test vial at room temperature for 10 minutes.
4. Insert the test strip (arrows down) into the conical vial.
5. Allow the test to develop for 10 minutes.
6. Remove the test strip. Lay the strip flat and allow to continue developing for 5 minutes.
7. Read the results visually, as explained below in section G, Interpretation of Results.

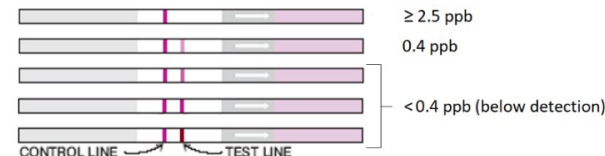
G. Interpretation of Results

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 ≥ 0.4 ppb. Test strips with no test line visible (only the control line is visible) indicates a result which is ≥ 2.5 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.

<u>Control Line</u>	<u>Test Line</u>	<u>Interpretation</u>
No control line present	No test line present	Invalid result
Control line present	No test line present	≥ 2.5 ppb
Control line present	Test line present	Between 0 and < 2.5 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 test kit range of 0-2.5 ppb, solutions of known Anatoxin-a 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.

For sample screening requiring a higher concentration range (greater than 0.4 – 2.5 ppb) or for samples which exceed the detection range (≥ 2.5 ppb) for which a more definitive qualitative screening result is necessary, samples can be diluted in deionized or distilled water prior to addition to the Sample Preservation Vials. The detection range of the strip test kit is then determined by multiplying the screening levels by the dilution factor of the samples. For example, a sample which is being screened for Anatoxin-a content up to 10 ppb would be diluted 1:4 in deionized or distilled water (1 mL of sample into 3 mL of deionized or distilled water in a clean vial, capped, and mixed thoroughly), then transferred into the Sample Preservation Vial and prepared as described in section D, Water Sample Collection/Preservation Procedure. The diluted sample would then be analyzed according to the procedure described in section F. Analysis of the diluted sample would then provide results in the range of 1.6 ppb to 10 ppb.