CDIA™ Anatoxin-a Colloidal Gold Test Strip (Drinking and Recreational Waters)

Cat. No.: DTSJYJ056
Pkg. Size: 25T

Intended use

The CDIA™ Anatoxin-a Colloidal Gold Test Strip (Drinking and Recreational Waters) is an immunochromatographic test for the detection of Anatoxin-a in drinking and recreational waters.

General Description

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.

The CDIA™ Anatoxin-a Colloidal Gold Test Strip (Drinking and Recreational Waters) is a rapid immunochromatographic test, designed solely for use in the qualitative screening of Anatoxin-a in fresh water. The CDIA™ Anatoxin-a Colloidal Gold Test Strip (Drinking and Recreational Waters) provides only preliminary qualitative test results. If necessary, positive samples can be confirmed by ELISA, HPLC or other conventional methods.

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Principle of the Test

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. Semiquantitative 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 can be used to prepare Anatoxin-a controls.

Reagents and Materials Provides

1. Anatoxin-a test strips in a desiccated container
2. Sample preservation vials
3. 3 mL graduated disposable pipettes
4. Conical test vials
5. Disposable transfer pipettes
6. One instruction

Materials Required but Not Provided

1. Timer
2. (+) Anatoxin-a standard, for the preparation of control solutions which can be analyzed with samples to obtain semi-quantitative sample results.

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Sample Collection and Handling

1. Fresh water samples must be preserved at the time of collection to prevent degradation of Anatoxin-a, which will produce inaccurate (biased low) sample results. The use of the Anatoxin-a test strips with samples which have not been treated with the reagents contained in the Sample Preservation Vials will produce inaccurate results.
2. 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 Anatoxina due to exposure to natural or artificial light and/or the lack of preservation reagents, producing inaccurate (falsely low) results.
3. Chlorinated water samples must be treated (quenched) with ascorbic acid at 0.1 mg/L 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 E, 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.
4. Preserved samples can be stored refrigerated (4-8ºC) for up to 28 days. Samples which must be held for greater than 28 days should be stored frozen.
5. Samples should be protected from exposure to natural and artificial light, as light exposure will cause degradation of Anatoxin-a.

Sample Collection/Preservation Procedure

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 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.

Assay operation

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

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1. Using a new disposable transfer pipette for each sample, transfer 7 drops (approximately 200 µL) of the preserved water sample (from section “Sample Collection/Preservation Procedure”, above) to the appropriately labeled conical test vial.
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.

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 strip with a test line which is lighter than the control line indicates a result which is ≥ 0.4 ppb. Test strip 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.

Control Line	Test Line	Interpretation
No control line present	No test line present	Invalid result
Control line present	No test line present	>2.5 ng/mL (ppb)
Control line present	Moderate to equal intensity test line present	Between 0 and 2.5 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 0-2.5 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.

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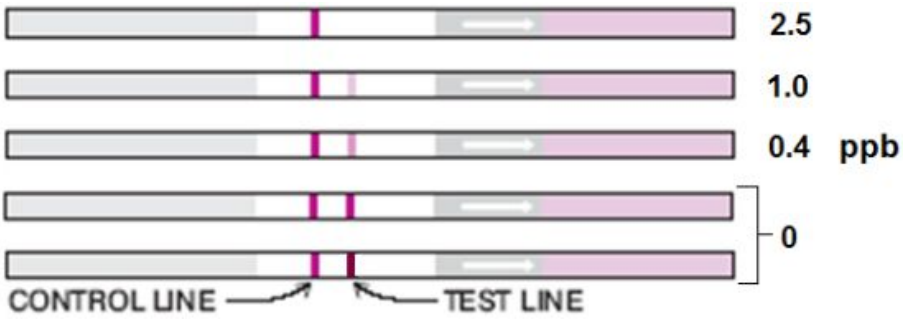

Alternately, test strips can also be interpreted using the Strip Reader, which provides objective determination of line intensities for consistent interpretation of results as well as a digital photographic record of all test strips. For sample screening requiring with a higher concentration range (greater 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 and mixed thoroughly), then transferred into the Sample Preservation Vial and prepared as described in section “Sample Collection/Preservation Procedure”. The diluted sample would then be analyzed according to the procedure described in section “Assay operation”. Analysis of the diluted sample would then provide results in the range of 1.6 ppb to 10 ppb.

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.

Limitation

1. 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.
2. 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

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performance (lower than 10ºC or higher than 30ºC), use of the test with water samples which have not been preserved properly.

3. The test is designed for use with fresh water. The 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.

Performance

Test sensitivity: The CDIA™ Anatoxin-a Colloidal Gold Test Strip (Drinking and Recreational Waters) for fresh water will detect Anatoxin-a at 0.4 ng/mL or higher. At this level, the test line exhibits moderate intensity. At levels greater than 2.5 ng/mL 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 CDIA™ Anatoxin-a Colloidal Gold Test Strip (Drinking and Recreational Waters) and ELISA methods showed a good correlation.

Storage

The Kit should be stored between 4-30ºC. The test strips, test vials, and water samples to be analyzed should be at room temperature before use.

Warnings and Precautions

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

2. Water samples must be preserved using the Sample Preservation Vials included in the kit before analyzing with the Anatoxina strip test. Use of the Anatoxin-a test strips with samples which have not been treated with the reagents contained in the Sample Preservation Vials will produce inaccurate results (see section “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 instruction may also produce inaccurate results.

3. 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. If desired, preserved water samples (see section “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.

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