Importance of Cylindrospermopsin Determination

Most of the world’s population relies on surface freshwaters as its primary source for drinking water. The drinking water industry is consequently 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. Cylindrospermopsin is a toxin produced by several different strains of cyanobacteria (blue-green algae) and has been found in fresh water throughout the world. Certain strains of Cylindrospermopsis raciborskii (found in Australia, Hungary, and the United States), Umezakia natans (found in Japan), and Anabaenopsis ovalisporum (found in Australia and Israel) have been found to produce Cylindrospermopsin. The production of Cylindrospermopsin seems to be strain specific rather than species specific.

Acute poisoning of humans and animals constitutes the most obvious problem from toxic cyanobacterial blooms and, in several cases, has led to death. Human exposure to Cylindrospermopsin can occur through ingestion of contaminated food or fish (fish) or during recreational activities in which water is swallowed. Dermal contact with Cylindrospermopsin may occur during showering or bathing, or during recreational activities such as swimming or boating. These toxins mediate their toxicity by inhibiting liver function and are potent inhibitors of protein synthesis and glutathione, leading to cell death.

To protect against adverse health effects, the U.S. Environmental Protection Agency (EPA) has established guidelines for Cylindrospermopsin in drinking water:

-For children pre-school age and younger (less than six years old), 0.7 μL (ppb)
-For school-age children and adults, 3.0 μL (ppb)

### Performance Data

**Test sensitivity:**
The detection limit for this assay is 0.040 ppb (μg/L).

**Test reproducibility:**
Coefficients of variation (CVs) for standards: <10%; for samples: <15%.

**Specificity:**
This ELISA recognizes Cylindrospermopsin and related compounds with varying degrees:
- Cylindrospermopsin 100%
- Deoxy-Cylindrospermopsin 112%
- 7-Epi-Cylindrospermopsin 157%

### Standard Curve:

![Standard Curve](image)

### Samples:

A sample correlation between the ELISA and HPLC methods showed a good correlation.

### Recovery

<table>
<thead>
<tr>
<th>Spike Level (ppb)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>91.01</td>
</tr>
<tr>
<td>0.25</td>
<td>92.69</td>
</tr>
<tr>
<td>0.5</td>
<td>94.14</td>
</tr>
<tr>
<td>1.0</td>
<td>95.82</td>
</tr>
</tbody>
</table>

### Precision

<table>
<thead>
<tr>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicates</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Days</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mean</td>
<td>0.198</td>
<td>0.501</td>
<td>1.01</td>
</tr>
<tr>
<td>% CV (within assay)</td>
<td>2.1</td>
<td>4.3</td>
<td>9.24</td>
</tr>
<tr>
<td>% CV (between assay)</td>
<td>8.3</td>
<td>5.3</td>
<td>4.9</td>
</tr>
</tbody>
</table>

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This product is for research only
A. Materials Provided
1. Microtiter plate (12 X 8 strips) coated with a second antibody (goat anti-rabbit)
2. Standards (7): 0, 0.05, 0.10, 0.25, 0.50, 1.0, 2.0 ppb, 1 mL each
3. Control: 0.75 ± 0.15 ppb, 1 mL, prepared from a secondary source, for use as a Quality Control Standard (QCS)
4. Sample Diluent, 25 mL, for use as a Laboratory Reagent Blank (LRB) and for dilution of samples above the range of the standard curve
5. Cylindrospermopsin-HRP Conjugate Solution (Vortex before use.), 6 mL
6. Antibody Solution (rabbit anti-Cylindrospermopsis), 6 mL
7. Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section E)
8. Substrate (Color) Solution (TM), 12 mL
9. Stop Solution, 12 mL

B. Additional Materials (not delivered with the test kit)
1. Micro-pipettes with disposable plastic tips (20-200 μL)
2. Multi-channel pipette (10-300 μL), stepper pipette (10-300 μL), or electronic repeating pipette with disposable plastic tips
3. Deionized or distilled water
4. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section E)
5. Graduated cylinder
6. Paper towels or equivalent absorbent material
7. Timer
8. Tape or paraffin
9. Microtiter plate reader (wavelength 450)
10. Microtiter plate washer (optional)

C. Sample Collection and Handling
Water samples should be collected in glass, polyethylene terephthalate glycol (PETG), high density polyethylene (HDPE), polycarbonate (PC), polypropylene (PP), or polystyrene (PS) containers. Samples can be stored refrigerated for up to 3 days. If samples must be held for greater than 5 days, samples should be stored frozen.

Finished (treated) drinking water samples do not require additional reagents at the time of collection.

D. Notes and Precautions
Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary.

The use of a multi-channel pipette, steppe pipette, or electronic repeating pipette is recommended for the addition of the antibody, enzyme conjugate, substrate, and stop solutions in order to equalize the incubation periods on the entire microtiter plate.

To avoid drift and obtain accurate results, the addition of the antibody, conjugate, color, and stop solutions should be performed in less than 2 minutes for each reagent. If additions to the entire microtiter plate cannot be completed in less than 2 minutes, run size should be decreased to the number of rows which can be pipetted in less than 2 minutes.

Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

E. Test Preparation
1. Allow the reagents and samples to reach ambient temperature before use.
2. Remove the number of microtiter plate strips required from the resealable pouch. The remaining strips are stored in the pouch with the desiccant (tightly closed).
3. The standards, control, sample diluent (LRB), antibody, enzyme conjugate, substrate, and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the Wash Buffer (5X) Concentrate at a ratio of 1:5 with deionized or distilled water. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
5. The stop solution must be handled with care as it contains diluted H₂SO₄.

F. Working Scheme
The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

<table>
<thead>
<tr>
<th>Std0-Std6: Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contr.: Control (QCS)</td>
</tr>
<tr>
<td>LRB: Laboratory Reagent Blank</td>
</tr>
<tr>
<td>Samp1, Samp2, etc.: Samples</td>
</tr>
</tbody>
</table>

G. Assay Procedure
1. Add 50 μL of the standards, control (QCS), LRB, or samples into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Vortex the enzyme conjugate solution. Add 50 μL of the enzyme conjugate solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
3. Add 50 μL of the antibody solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with paraffin or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 45 minutes at room temperature.
4. Remove the covering, decant the contents of the wells into a sink, and blot the invereted plate on a stack of paper towels.
5. Add 100 μL of substrate (color) solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with paraffin or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 30-45 minutes at room temperature. Protect the strips from sunlight.
6. Add 100 μL of stop solution to the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping, or electronic repeating pipette.
7. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

H. Evaluation
The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter (preferred) or Logit/Log. For a manual evaluation, calculate the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B₀ for each standard on the vertical linear (y) axis versus the corresponding Cylindrospermopsis concentration on the horizontal logarithmic (x) axis on graph paper. %B/B₀ for the control (QCS), LRB, and samples will then yield levels in ppb of Cylindrospermopsis by interpolation using the standard curve. Results can also be determined by using a spreadsheet macro available from Abraxis upon request.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing a lower concentration of Cylindrospermopsis than standard 1 (0.05 ppb) should be reported as containing < 0.05 ppb of Cylindrospermopsis. Samples showing a higher concentration than standard 6 (2.0 ppb) must be diluted to obtain accurate results. The concentration of the positive control (QCS) provided should be 0.75 ± 0.15 ppb.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards. Samples which have lower absorbances than a standard will have concentrations of Cylindrospermopsis less than that standard. Samples which have higher absorbances than a standard will have concentrations of Cylindrospermopsis greater than that standard.

As with any analytical technique (GC, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

I. References