Detection of microcystin in drinking water using Attogene's highly sensitive microcystin testing solutions

Introduction:

Microcystins are hepatoxic cyclic heptapeptides that are often produced by freshwater blue-green algae by non-ribosomal peptide synthesis. The toxin can covalently bind and inhibit protein phosphatase 1 and 2A which are important in regulating many critical cellular functions. Microcystins are responsible for the sickening and deaths of fish, birds, wild animals, and agricultural livestock in many countries where freshwaters contain toxic cyanobacterial blooms. Importantly, adverse effects of the toxins on human health have also been recognized. Humans and animals can be exposed directly to microcystins by ingesting contaminated drinking water or through dermal contact, and indirectly through routes such as eating vegetables that were watered during their growth from surface waters contaminated from microcystins and from eating vegetables that were washed with microcystins contaminated water. There is also data documenting the accumulation of cyanotoxins in plants and crops irrigated with water containing cyanobacterial blooms - including evidence that microcystins can accumulate in some food crops. Additionally, microcystins have been found to remain stable in lettuce, carrots, and green beans when cooked, as boiling, frying, or steaming does not degrade toxins prior to consumption.

To protect people from adverse health effects caused by microcystins exposure in drinking water, the World Health Organization (WHO) has proposed a provisional upper limit for Microcystin-LR of 1.0 ppb (µg/L) in drinking water and the U.S. Environmental Protection Agency (EPA) has established health advisories for microcystins in drinking water that includes limits of 0.3 µg/L (ppb) for children below school age and 1.6 µg/L (ppb) for all other age groups as a level not to be exceeded based on exposure for 10 days. This guidance allows states to take actions—such as and up to a do-not-drink advisory. Health Advisories are non-regulatory values that serve as informal technical guidance to assist federal, state, and local officials, and managers of public or community water systems to protect public health from contaminants.

To improve and expand microcystin monitoring and provide additional tools to drinking water assessment programs, Attogene has developed rapid, easy to use assays to detect microcystins at or above the key health advisory limits set for drinking water. We offer both rapid lateral flow, catalog number AU2024-02 and quantitative enzyme linked immunosorbent assay (ELISA), catalog number EL2024-02, which are competitive inhibition (the higher the concentration of microcystin in the sample, the fainter the signal) test kits that can readily detect less than 0.3 ppb. The ELISA test may be effectively utilized following the EPA 546 guideline to monitor water systems. As an example of the utility of these tests for detecting advisory levels for drinking water, we screened for microcystin from tap and milli Q purified water samples. During these tests, we show that both the ELISA and lateral flow strip tests for drinking water can easily detect the lowest concentration of a regulatory advisory for children of 0.3ppb for drinking water. These tests can be effectively integrated into routine drinking water screening programs for testing water directed for consumption.

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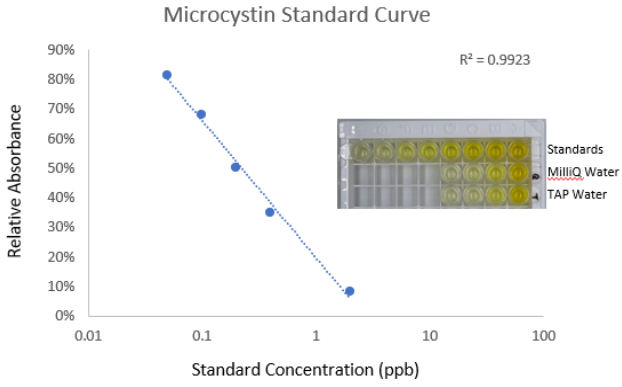
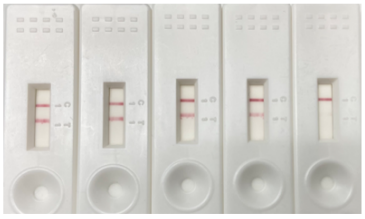
Results:

We obtained water samples directly from the tap or water purified using a milli Q water purification system. These water samples were spiked with microcystin LR at 0.3, 1 and 1.6 parts per billion levels. These samples were tested using Attogene's EL2024 microcystin ELISA. Briefly, we added 50ul of each of the samples or standards to the wells of the 96-well plate that is coated with the microcystin protein conjugate, microcystin antibody was added, incubated for 30 minutes, washed, incubated for 30 minutes with a secondary antibody conjugated with HRP, washed and an HRP substrate added. After 15 minutes, the wells were read using a Tecan M200 Plex plate reader at OD 450 (**Figure 1**). The OD readings were used to calculate the B/B0 (B = the average absorbance of the sample and B0 = the average absorbance of the background value). Although the kit is capable of screening 42 samples in duplicate, it also has the flexibility of breakable wells so less samples can be analyzed in one run. Importantly, this kit is both less labor intensive, faster, and less expensive than using analytical instrumentation such as LC-MS/MS to screen for microcystin. This enables users to screen more samples in less time at a lower cost which has an obvious impact on improving surveillance of water systems because more samples can be screened more regularly to understand the nature of microcystin levels.



Standard	B/B0	Sample	B/B0	Sample	B/B0
0ppb	-	Milli Q	-	Tap Water	-
0.3ppb	30%	0.3ppb	34%	0.3ppb	31%
1ppb	15%	1ppb	14%	1ppb	14%
2ppb	8%	1.6ppb	11%	1.6ppb	12%

Figure 1. Data showing the standard curve generated using the set of standards applied in the ELISA kit EL2024. The advisory limits of 0.3, 1 and 1.6ppb can be clearly differentiated by easily comparing the B/B0 readouts with the controls.



Microcystin LR concentration	Control Line	Test Line	Ratio
Tap water	782271	721455	0.92
0.1ppb	610033	419979	0.69
0.5ppb	755253	539328	0.71
1ppb	835028	417598	0.5
5ppb	847280	78523	0.09

Figure 1. Tap water spiked with the indicated concentration of microcystin LR were used in the AU2024-02 kit. After following the kit's instructions, the cassettes were read using a lateral flow reader. Numerical readings of the control line, test line, and line intensity ratios are provided in the lower panel.

Next, Attogene tested drinking water samples for microcystin using our rapid lateral flow test kit for drinking water assessment in a laboratory setting (AU2024-02). This kit comes with 10 lateral flow cassettes and sample dilutant buffer. In **Figure 2** we deployed this kit to detect microcystin in tap water. Tap water was spiked with Microcystin LR at four concentrations (0.1, 0.5, 1.0 and 5.0 ppb). The visual and reader results are depicted in **Figure 2**. The data demonstrated as little as 0.1ppb of microcystin LR can be differentiated from tap water sample lacking any spiked microcystin LR. Although the change in line intensity can be readily seen by eye, the use of the lateral flow reader (bottom half of **Fig. 2**) provides a

greater level of confidence, particularly at the lower end of the concentration. At 5ppb the test line reaches a level where only a slight shadow line remains at best.

Discussion:

Attogene offers several products that are helpful to federal, state, and local municipalities to perform screening of microcystin in both recreational and drinking water systems. These kits represent rapid and efficient screening tools as the tests can be performed in either the field or in a laboratory setting. Due to the ease of use and low cost for these tools, increasing the frequency and sample number of microcystin screening in water sources would be reasonable to undertake. As a lesson learned from the 2014 microcystin water crisis in Ohio, microcystin levels can rise quickly and close attention/monitoring are required to ensure that a spike in microcystin levels is readily detected in a timely fashion. Daily - and even hourly - monitoring are not out of the question when Attogene's sensitive and low-cost tools are integrated into the workflow for hazard analysis and critical control point (HACCP) plans.