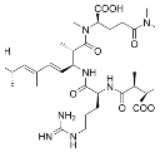
# Use of rapid lateral flow test to screen lab strains of cyanobacteria strains for Microcystin expression

## Introduction:

Microcystins are hepatoxic cyclic heptapeptides that are often produced by freshwater blue-green algae (*Microcystis aeruginosa* and other members of *Planktothrix*, *Anabaena*, *Oscillatoria* and *Nostoc* genera by non-ribosomal peptide synthesis. These toxins can covalently bind and inhibit protein phosphatase 1 and 2A which are important in regulating many critical cellular functions. Microcystins are responsible for the deaths of fish, birds, wild animals, and agricultural livestock in many countries where freshwaters contain toxic cyanobacterial blooms, and adverse effects of the toxins on human health have been recognized.

A tiered notification system that takes different actions based on specific numeric thresholds for Microcystin-LR concentrations in recreational waters has been developed. This guidance allows states to take various actions—such as posting information about harmful algal blooms (HABs), issuing a recreational public health advisory, or temporarily closing recreational waters through a no contact advisory—depending on the severity of the bloom event.

Recommended Actions for Specific Microcystin-LR Concentrations
Informational sign postings about HABs at recreational waters: < 6 µg/L
Recreational public health advisory: 6 µg/L
Elevated recreational public health advisory (e.g., no contact): 20 µg/L



To improve and expand microcystin monitoring, Attogene has developed a rapid, easy to use lateral flow assay (architecture shown in Figure 1) to detect these algal toxins at or above the key 5µg/L threshold. The assay is based on a competitive inhibition (the higher the concentration of microcystin in the sample, the fainter the test line on the device). As an example of the utility of this rapid test, we implemented the screening of microcystin from pure algae cultures.

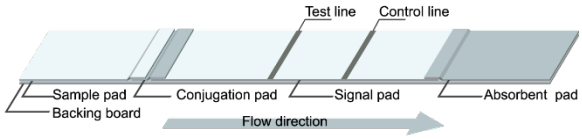



**Figure 1**. Architecture of a lateral flow device that includes a backing card with laminated sample pad, conjugate pad, nitrocellulose, and absorbent pad. On the nitrocellulose pad

## Results and Discussion:

Attogene leveraged its rapid microcystin lateral flow test (catalog number AU2024C - https://www.attogene.com/shop/microcystin-lateral-flow-kit-for-labs/) to Identify cultures that are producing appreciable levels of microcystins. Our Attogene microcystin lateral flow assay has the key benefit of being field deployable and capable of detecting samples in the field. We also make a laboratory version of this kit which does not include sample collection bottles like the field-based version. In these experiments, we removed 200ul of media directly from tubes containing suspensions of the algae listed in **Table 1**. These cultures were centrifuged, and the supernatant was moved to a new tube. We then added 20ul of running buffer included with our microcystin kit and applied the sample directly into the port of the lateral flow cassette to detect extracellular levels of microcystins.

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## Table 1: Reader Results

| Name | Control Line | Test Line | Ratio | Result |
|---|---|---|---|---|
| **0ppb Microcystin LR** | **408358** | **759404** | **1.86** | |
| **5ppb Microcystin LR** | **665219** | **221010** | **0.33** | |
| **10ppb Microcystin LR** | **911955** | **167065** | **0.18** | |
| **20ppb Microcystin LR** | **762449** | **107272** | **0.14** | |
| Microcystis aeruginosa UTEX 2386 | 212229 | 402511 | 1.9 | Clear Negative |
| Microcystis aeruginosa UTEX 2385 | 521117 | 46393 | 0.09 | > 20ppb |
| Microcystis UTEX aeruginosa 2063 | 230626 | 321265 | 1.39 | 0-5ppb |
| Microcystis aeruginosa UTEX 2061 | 247383 | 513119 | 2.07 | Clear Negative |
| Microcystis flos-aquae UTEX 2673 | 271612 | 598352 | 2.2 | Clear Negative |
| Microcystis aeruginosa UTEX 2665 | 272204 | 536171 | 1.97 | Clear Negative |
| Microcystis aeruginosa UTEX 2664 | 355138 | 292848 | 0.82 | 5-20ppb |
| Microcystis aeruginosa UTEX 2388 | 460496 | 40978 | 0.09 | > 20ppb |
| Microcystis aeruginosa UTEX 3037 | 322704 | 28721 | 0.09 | > 20ppb |
| Microcystis aeruginosa UTEX B2670 | 202282 | 467633 | 2.31 | Clear Negative |
| Microcystis aeruginosa UTEX B2663 | 431271 | 241764 | 0.56 | 5-20ppb |
| Microcystis aeruginosa UTEX B2676 | 229101 | 504363 | 2.2 | Clear Negative |
| Oscillatoria tenuis UTEX B428 | 412132 | 294990 | 0.72 | 5-20ppb |
| Anabaena catenula utex B 375 | 238980 | 348847 | 1.46 | 0-5ppb |
| Microcystis aeruginosa B 2662 | 465041 | 233601 | 0.5 | 5-20ppb |
| Anabaena spiroides utex B 1552 | 340337 | 364980 | 1.07 | 0-5ppb |
| Oscillatoria prolifera B 1270 | 384372 | 298063 | 0.78 | 5-20ppb |
| Nostoc muscorum utex 1038 | 182016 | 237959 | 1.31 | 0-5ppb |
| Oscillatoria lutea utex B 1814 | 346805 | 310665 | 0.9 | 5-20ppb |
| Anabaena cylindrica utex 1611 | 253309 | 445867 | 1.76 | Clear Negative |
| Phormidium autumnale utex 1580 | 366365 | 535266 | 1.46 | 0-5ppb |
| Nostoc muscorum utex 2209 | 320006 | 392927 | 1.23 | 0-5ppb |
| Nostoc muscorum utex 1832 | 302039 | 386426 | 1.28 | 0-5ppb |
| Anabaena raddhawae utex 1823 | 233194 | 513027 | 2.2 | Clear Negative |
| Phormidium luridum var. olivace utex B426 | 246426 | 478781 | 1.94 | Clear Negative |
| Nostoc sp. Utex B2211 | 368591 | 478059 | 1.3 | 0-5ppb |
| Plectonema boryanun utex 485 | 199290 | 395629 | 1.99 | Clear Negative |

**ppb = parts per billion = µg/L**

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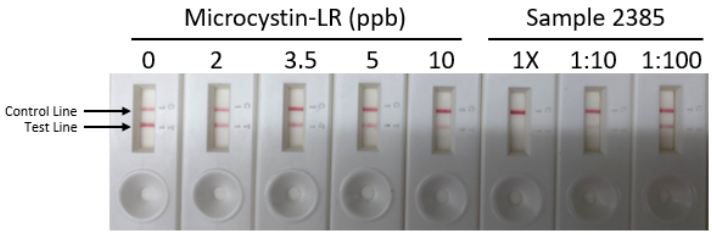
## Discussion:

This proof of principle study represents an excellent example of how the Attogene rapid lateral flow test can be used and leveraged to rapidly detect microcystin in in a qualitative fashion. It may also be used in a semi-quantitative or quantitative fashion with the use of a reader (Figure 2).

The limit of detectability of this lateral flow test can readily be made less than 5ppb with implementation of a lateral flow reader (e.g., this is how the results shown in Table 1 were generated). However, for rapid reviewing of the results by eye, a cut off value of 5ppb or greater is recommended. Several types of lateral flow readers are on the market and can be used for performing very accurate and quantitative analysis. Images may also be analyzed from photos in image J or similar processing programs.




| Sample | Name | Control | Test | Ratio |
|---|---|---|---|---|
| 0ppb | Standard | 427570 | 758377 | 1.77 |
| 2ppb | Standard | 433258 | 481339 | 1.11 |
| 3.5ppb | Standard | 692773 | 500093 | 0.72 |
| 5ppb | Standard | 640760 | 412207 | 0.64 |
| 10ppb | Standard | 801629 | 283004 | 0.35 |
| 2385 1X | Microcystis aeruginosa UTEX 2385 | 920084 | 64750 | 0.07 |
| 2385 1:10 | Microcystis aeruginosa UTEX 2385 | 661701 | 132165 | 0.2 |
| 2385 1:100 | Microcystis aeruginosa UTEX 2385 | 688647 | 378776 | 0.55 |

**Figure 2.** Lateral Flow running buffer spiked with 0, 2, 3.5, 5 or 10ppb microcystin-LR were added into sample ports. Highly positive sample UTEX2385 was added to sample ports at 1X, 10X or 100X. Samples were run for 15 minutes. A picture was taken of the cassettes and results were read in a lateral flow reader.

Interestingly, while analyzing the extracellular levels of microcystin, we also have found that the intercellular levels of microcystins in algae are often several fold higher than that found in the media or in contaminated water samples. Thus, extracting intercellular microcystin can be performed using a probe sonication followed by filtration or centrifugation and direct addition to the sample port if understanding the levels of microcystin within the cells is desired.

Finally, while we did not normalize our results with the number of algae cells in each culture, it is fully possible to keep close track of cell population and directly relate the number of cells with amount of microcystins. Calculations can then be readily done to determine the amount of microcystin being expressed per cell.