Got Blue-Green Algae? But, is it Toxic? – Evaluating Reliability and Accuracy of Rapid Test Kits for Detection of Cyanotoxins

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Harmful Algal Blooms

- Excessive growth of Cyanobacteria (often called blue-green algae) may result in dense blooms that can produce harmful toxins. These harmful blooms are called Harmful Algal Blooms (HABs).
- There is no way to visually determine if a bloom is toxic. So, to prevent toxin exposure, fast and reliable methods for toxin detection are needed.
- Rapid Test Kits provide waterbody owners/managers a rapid method for cyanotoxin assessment and make timely interventions to mitigate the risk and impact from cyanotoxins but their accuracy and reliability under field conditions is not well documented.

Research Objectives

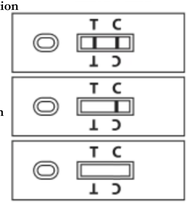
- Evaluate the application of 2 commercially available rapid test kits for microcystin detection: 5 Strands and Eurofins-Abraxis (now Gold Standard Diagnostics, GSD)
- Explore variability and reliability of rapid kit results between two test users (in field and lab settings).
- Assess accuracy of rapid test kit results through comparison to conventional cyanotoxin assessment (Enzyme Linked Immunosorbent Assay- ELISA) results.

Rapid Test Kits

5 Strands

BlueGreen Test[a]

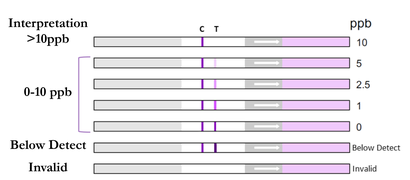
Interpretation



- 1 Test/Kit
- ~20-min. Test Time
- Presence/Absence

Eurofins-Abraxis

Microcystin Strip Test[b]



- 5 or 20 Tests/Kit
- ~35-min. Test Time
- Presence/Absence + toxin level color range

Figure 1. The 5 Strands BlueGreen Test[a] (left) and Eurofins-Abraxis Microcystin Strip Test[b] (right) were selected for evaluation. Each test has similar qualitative Presence/Absence of toxin result, but Eurofins also includes a semi-quantitative toxin level determined by color intensity of the test line(T).

Study 1: Reliability/Repeatability

- Six water samples were collected from livestock farm ponds or private waterbody suspected of having a HAB (ex: Figure 2).
- Each rapid kit was analyzed in the field (user 1) at the waterbody and then repeated in lab settings (user 2).
- Actual microcystin concentration confirmed with ELISA.
- Across field and lab application both rapid test kits matched on their respective Toxin or No Toxin assessment (Table 1).

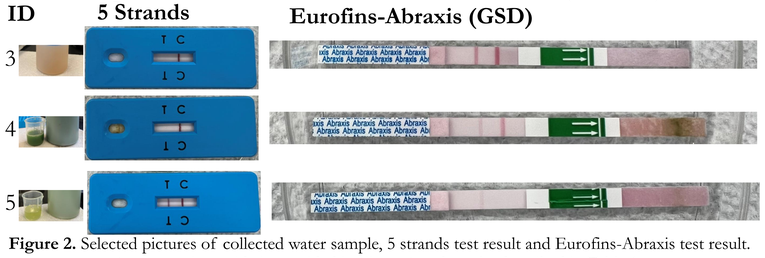


Figure 2. Selected pictures of collected water sample, 5 strands test result and Eurofins-Abraxis test result. Interpretation guide is provided in Figure 1 and results described in Table 1.

Table 1. Results of the 6 water samples analyzed with both rapid test kits and toxin concentration confirmed by ELISA analysis. Two mismatches between the field and lab tests were observed for semi-qualitative toxin level results from the Eurofins-Abraxis test (sample ID 1 and 6). Only 1 inaccurate result for Toxin or No Toxin occurred (Sample 4). ELISA results show the actual toxin levels ranged from 0.152 to >30ppb.

ID	5 Strands		Eurofins-Abraxis (GSD)		ELISA
	Field	Lab	Field	Lab	Microcystin(ppb)
1	Toxin	Toxin	Toxin >10ppb	Toxin 0-10ppb	6.788
2	Toxin	Toxin	Toxin >10ppb	Toxin >10ppb	>30
3	No Toxin	No Toxin	Below Detect	Below Detect	0.152
4	No Toxin	No Toxin	Toxin 0-10ppb	Toxin 0-10ppb	0.286
5	Toxin	Toxin	Toxin >10ppb	Toxin >10ppb	>30
6	Toxin	Toxin	Toxin 0-10ppb	Toxin >10ppb	1.504

Which Toxin Test is Right for You?

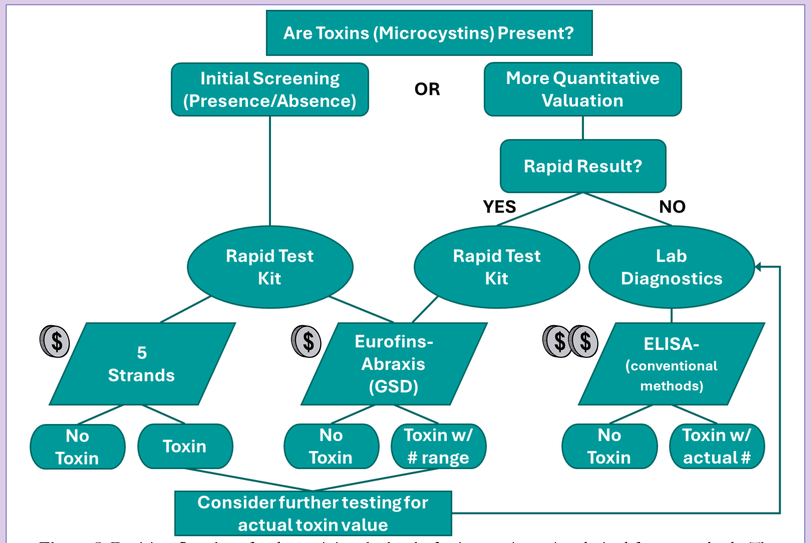


Figure 5. Decision flowchart for determining the level of microcystin testing desired for a waterbody. The flowchart guides users through selecting between qualitative (presence/absence) and quantitative testing options based on data needs, analytical complexity, and budget considerations.

Study 2: 5 Strands Accuracy

- Additional sampling with 5 Strands Rapid Tests and ELISA analysis (total samples 28, ex: Figure 3).
- Confusion Matrix analysis used to examine the 5 Strand performance as compared to ELISA results under different Microcystin threshold levels.
- The Microcystin Threshold of 0.3, had the strongest scores for Accuracy, Kappa, and MCC metrics among the tested thresholds. Suggesting 5 Strands has a sensitive Microcystin toxin detection.

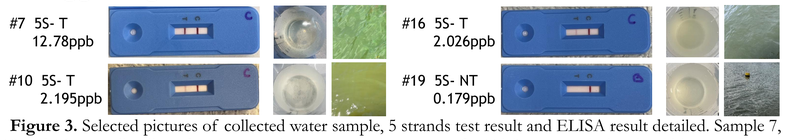


Figure 3. Selected pictures of collected water sample, 5 strands test result and ELISA result detailed. Sample 7, 10 and 16 were all collected at the same location over the course of a HAB event with the visual bloom indication from surface (7) to mixed (10) to reduced (16). Sample 16 shows reduced visual bloom but still has toxin.

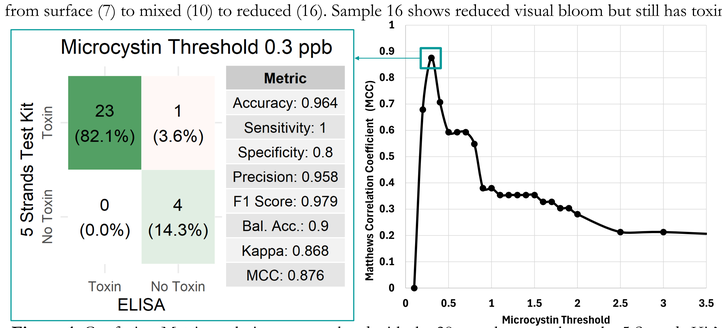


Figure 4. Confusion Matrix analysis was completed with the 28 samples to evaluate the 5 Strands Kit's performance and level of detection. The 5 Strand Toxin/No Toxin result was compared to ELISA actual toxin levels by defining Microcystin Thresholds. As the threshold increases above 0.3ppb, the number of samples of "falsely" ID'd 5 Strand Toxin result increases as the measured actual Microcystin value is lower than the defined Threshold, this results in a decrease in MCC value as the threshold increases. MCC is a balanced measure of correlation between the test prediction and actual value, with 1 being a perfect prediction.

Conclusion & Key Findings

- **Study 1 Result:** Across Field and Lab application both 5 Strands and Eurofins correctly classify Toxin or No Toxin. However, user interpretation of line color for toxin level in Eurofins test differed for 2 Toxin samples (Table 1).
- **Study 2 Result:** The confusion matrix analysis revealed the 5 Strands test has a relatively sensitive Microcystin toxin detection level, 0.3ppb (Figure 4). This value may be overly sensitive for some applications but does provide beneficial information that Microcystin is present at some capacity and further testing would need to be considered (Figure 5).

➢ **Rapid test kits provide rapid test and result, but result interpretation influenced by experience of sampler and conditions of water sample (scum/turbidity may interfere)**

➢ **Rapid test kits provide qualitative to semi-quantitative result. Depending on waterbody use rapid test kit results may require confirmation by conventional testing methods.**

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Rapid Kit Reference: a)5 Strands- https://tinyurl.com/5SBGAtest ; **b)Eurofins-Abraxis GSD-** https://tinyurl.com/GSDMCteststrip