

Evaluation of Seven Commercially Available Microcystins ELISAs for Applications Safeguarding Public Health



Introduction

Microcystins are a class of potent hepatotoxins produced by cyanobacteria during harmful algal blooms (HABs). They impact both drinking and recreational water safety, posing a threat to human and animal health. While microcystin-LR remains the most extensively studied and commonly monitored microcystin congener due to its prevalence and toxicity, research from North America, Europe, Asia and Australia demonstrates that other toxic congeners are also present in water bodies throughout the world (Harke et al., 2016). In fact, recent toxicological studies have shown that congener dominance can vary both seasonally and temporally and that multiple non-LR congeners, including microcystins-LA, LF, and LW, which exhibit toxicity up to 7 times greater than microcystin-LR, are often present (Chaffin et al., 2023). With approximately 300 known microcystin congeners (Meriluoto et al., 2017) varying in toxicity and structure, monitoring strategies aiming to protect public health must measure total microcystins, not just MC-LR or a small subset of microcystin congeners, to avoid underestimation of potential toxin exposure risk.

The US EPA has established two methods for the detection of microcystins in drinking water: the LC-MS/MS Method 544 and the ADDA-ELISA Method 546. Enzyme-linked immunosorbent assays (ELISAs) are widely used due to their speed, simplicity, and cost-effectiveness in detecting microcystin toxins and can offer significant advantages over LC-MS/MS in terms of their ability to assess total microcystins and the associated public health risks. As LC-MS/MS quantifies only those congeners for which reference materials are available (currently <20 congeners), ELISAs with high cross-reactivity to a broad range of toxic congeners, such as those with specificity to ADDA, the unique β -amino acid moiety, which is highly conserved across all toxic microcystins variants, ensure a more comprehensive assessment of total microcystins. The "Frequently Asked Questions" section of the US EPA's website addresses the advantages and limitations associated with the two methods, stating that **"while LC-MS/MS analysis is well suited for those six microcystins congeners [commercially available at the time the method was developed] (and possibly additional congeners if analytical standards are available), it does not identify and accurately quantify other microcystins that may be present in the sample; it may therefore produce lower values than measurement by ADDA-ELISA"**. The EPA goes on to say that "ADDA-ELISA is detecting any congener that contains the ADDA functional group (currently associated with more than 100 verified congeners), while Method 544 only detects six microcystin congeners and nodularin-R that were commercially available as analytical standards when Method 544 was developed. The microcystins addressed by Method 544 may or may not be the dominant congeners in the particular water sample." In short, use of ELISAs possessing broad congener cross-reactivity, specifically ADDA ELISAs referenced in the EPA method, can reduce the risk of underreporting the microcystin toxin concentrations and aid efforts to protect the public from exposure to harmful levels of microcystin toxins.

This study evaluates the congener cross-reactivity of seven commercially available microcystins ELISA kits to assess their effectiveness in detecting a broad spectrum of toxic microcystin congeners and therefore their utility in safeguarding public health from the potential threat of exposure to microcystin toxins.



Materials

1. ABRAXIS® Microcystins ADDA ELISA (Gold Standard Diagnostics Horsham, Part #520011)
2. ABRAXIS® Microcystins (ADDA)-DM ELISA (Gold Standard Diagnostics Horsham, Part #522015)
3. Attogene Congener Independent Microcystins ELISA (Attogene, Part #EL2024-05)
4. Beacon Analytical Systems, Inc.® Microcystin Plate ELISA (Beacon Analytical Systems, Inc.®, Part #20-0068)
5. Beacon Analytical Systems, Inc.® Microcystin BX Plate ELISA (Beacon Analytical Systems, Inc.®, Part #20-0300)
6. QuantiPlate™ Kit for Microcystins (Envirologix™, Part #EP 022)
7. QuantiPlate™ Kit for Microcystins High Sensitivity (Envirologix™, Part #EP 022 HS)
8. Microcystin-LR (Gold Standard Diagnostics Horsham, Part #300639)
9. Microcystin-LA (Gold Standard Diagnostics Horsham, Part #300628)
10. Microcystin-LF (Gold Standard Diagnostics Horsham, Part #300646)
11. Microcystin-LW (Gold Standard Diagnostics Horsham, Part #300650)
12. Microcystin-LY (Gold Standard Diagnostics Horsham, Part #300634)
13. Microcystin-RR (Gold Standard Diagnostics Horsham, Part #300636)
14. Microcystin-YR (Gold Standard Diagnostics Horsham, Part #300638)
15. Nodularins (Gold Standard Diagnostics Horsham, Part #300640)



Method

As differences in the microcystin-LR stock material used for the calibration standards in the different ELISA kits (such as certified vs. non-certified stocks, differences in stock purity, etc.) can introduce bias in the cross-reactivity interpretations with the various antibodies, microcystin-LR standards prepared using ABRAXIS® Microcystin-LR part #300639 were used as calibration standards for all tested kits. Cross-reactivity standards for the various congeners were prepared at a range of concentrations and analyzed using each kit according to the manufacturer's instructions for use. Percent cross-reactivity was then calculated based on the EC-50 of the MC-LR calibration standards and the EC-50 of each of the cross-reactivity standards using the following formula:

$$\text{Percent Cross-Reactivity} = \frac{\text{EC-50 with MC-LR calibration standards}}{\text{EC-50 with cross-reactivity standards}} \times 100\%$$



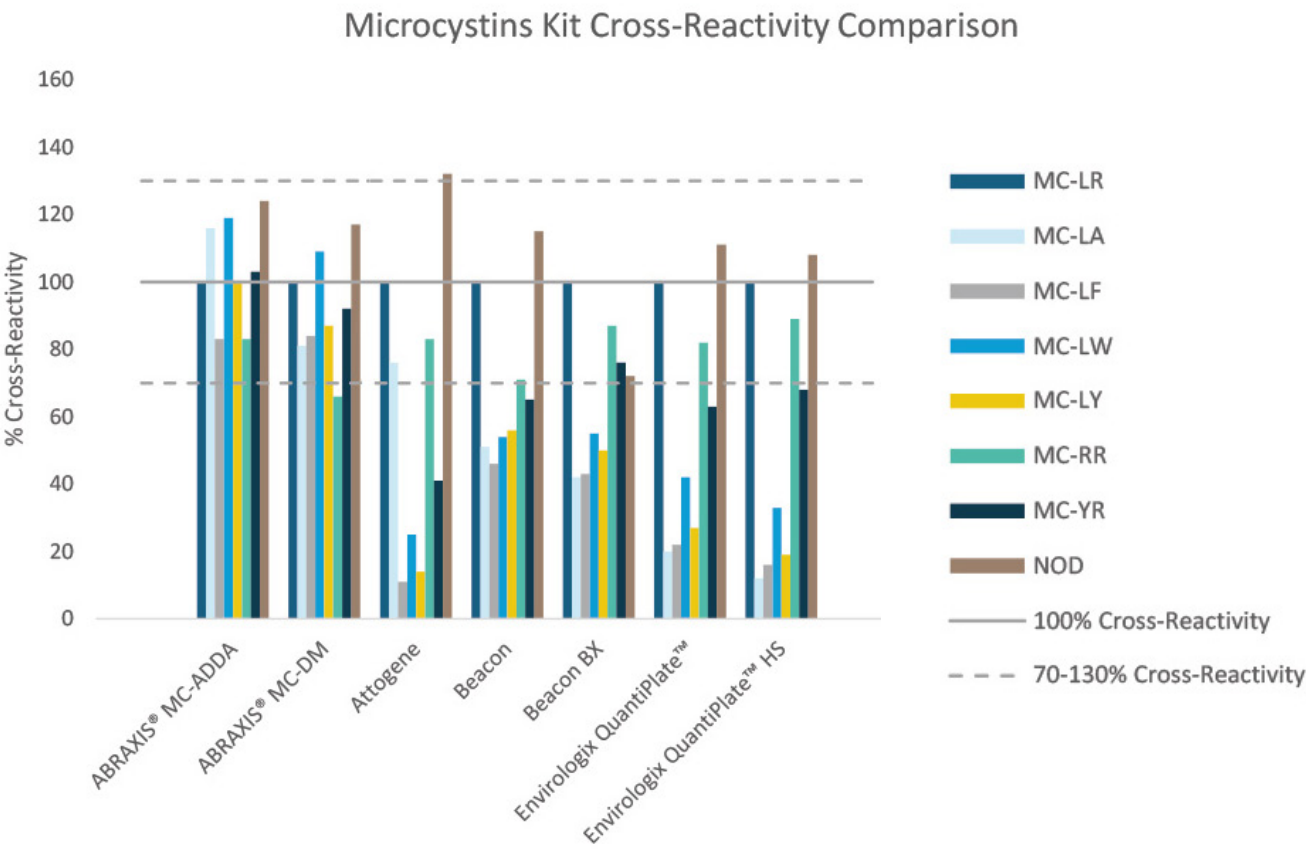
Results & Discussion

As indicated in the Table 1, the ABRAXIS® Microcystins ADDA ELISA demonstrated 83% to 124% cross-reactivity, and the ABRAXIS® Microcystins (ADDA)-DM ELISA demonstrated 66% to 117% cross-reactivity for all congeners tested, supporting the manufacturer's claims of ADDA specificity. In comparison, the Attogene Congener Independent Microcystins ELISA demonstrated cross-reactivity ranging from 11% for Microcystins-LF up to 132% for Nodularin, with <41% cross-reactivity for four of the congeners (MC-LF, MC-LW, MC-LY, and MC-YR). Although the manufacturer claims it is compatible for use with the US EPA Method 546, the lack of broad cross-reactivity suggests that the kit is not ADDA specific and therefore would not be suitable for use in Method 546 sample analysis. The Beacon Analytical Systems, Inc.® Microcystins ELISA showed ≤56% cross-reactivity for MC-LA, MC-LF, MC-LW, and MC-LY. The assay showed greater cross-reactivity for MC-RR (71%), MC-YR (65%), and NOD (115%). The Beacon Analytical Systems Inc.® Microcystins BX ELISA also showed lower cross-reactivities for MC-LA, MC-LF, MC-LW, and MC-LY (42% to 55%) and greater cross-reactivity with MC-RR, MC-YR, and NOD (72% to 87%). Both the Envirologix™ QuantiPlate™ and QuantiPlate™ High Sensitivity showed significantly lower cross-reactivities for MC-LA, MC-LF, MC-LW, and MC-LY (12% to 42%) and greater cross-reactivity with MC-RR, MC-YR, and NOD (63% to 111%).

The significant variability in cross-reactivity to the different microcystin congeners tested suggests that the antibodies used in the Attogene, Beacon, and Envirologix ELISA kits are not specific for the highly conserved ADDA region of the microcystin molecule (a requirement of EPA Method 546) and therefore lack true broad-spectrum detection and/or quantitation capabilities. Depending on the specific congeners present in a sample, these kits may produce significant under- or overreporting of total microcystin concentrations.

Table: Cross-reactivity profiles of seven commercially available Microcystins ELISA kits

Congener	ABRAXIS® Microcystins ADDA ELISA (520011)	ABRAXIS® Microcystins (ADDA)- DM ELISA (522015)	Attogene Congener Independent Microcystins ELISA (EL2024-05)	Beacon Analytical Systems, Inc.® Microcystins Plate ELISA (20-0068)	Beacon Analytical Systems, Inc.® Microcystins BX Plate ELISA (20 0300)	Envirologix™ QuantiPlate™ for Microcystins (EP 022)	Envirologix™ QuantiPlate™ for Microcystins High Sensitivity (EP 022 HS)
MC-LR	100%	100%	100%	100%	100%	100%	100%
MC-LA	116%	81%	76%	51%	42%	20%	12%
MC-LF	83%	84%	11%	46%	43%	22%	16%
MC-LW	119%	109%	25%	54%	55%	42%	33%
MC-LY	100%	87%	14%	56%	50%	27%	19%
MC-RR	83%	66%	83%	71%	87%	82%	89%
MC-YR	103%	92%	41%	65%	76%	63%	68%
NOD	124%	117%	132%	115%	72%	111%	108%





Conclusion

This study's evaluation of cross-reactivity for the ABRAXIS® Microcystins ADDA, ABRAXIS® Microcystins (ADDA)-DM, Attogene Congener Independent Microcystins, Beacon Analytical Systems, Inc.® Microcystins and Microcystins BX, and Envirologix™ QuantiPlate™ and QuantiPlate™ High Sensitivity ELISAs shows significant differences in the cross-reactivity profiles of the kits. **The ADDA-specific ABRAXIS® Microcystins ELISAs exhibit strong specificity for the ADDA moiety, a highly conserved structural feature associated with toxic microcystin congeners, which ensures broad cross-reactivity across both known and unknown microcystin congeners. This allows for more accurate quantitation of total microcystin toxins.** In contrast, the Attogene Congener Independent Microcystins, Beacon Analytical Systems, Inc.® Microcystins, and Microcystins BX, and Envirologix™ QuantiPlate™ and QuantiPlate™ High Sensitivity ELISAs all showed generally lower cross-reactivities for MC-LF, MC-LY, and MC-LW, with some kits also showing lower cross-reactivities with MC-LA, MC-RR, MC YR, and/or NOD.

Although the cross-reactivity profiles of some of the evaluated ELISA kits do allow for varying degrees of detection for certain microcystin congeners, the generally lower, less uniform cross-reactivity may result in an underestimation of public health risk, depending on the dominant toxic congeners present in a particular sample. As such, **ELISA kits with ADDA specificity, as specified in US EPA Method 546, such as the ABRAXIS® Microcystins ADDA and ABRAXIS® Microcystins (ADDA)-DM ELISAs, offer more comprehensive and reliable assessment of total microcystins contamination, enhancing the ability of water utilities, researchers, and regulatory agencies to monitor harmful algal blooms, minimize public exposure and reduce public health impacts associated with these potent cyanotoxins.**



References

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