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TECHBRIEF
Environmental Technology Verification Program

ETV

Immunoassay Test Kits for Microcystins

Some cyanobacteria, also known as blue-green algae, can produce toxic compounds called microcystins, that may pose a significant threat to human and animal health. In 2010 and 2011, the U.S. EPA Environmental Technology Verification (ETV) Program's Advanced Monitoring Systems (AMS) Center, operated by Battelle under a cooperative agreement with EPA, evaluated the performance of six test kits for microcystins in recreational waters. The Nebraska Department of Environmental Quality (NDEQ) and the Suffolk County Department of Health Services (SCDHS) in New York collaborated in this verification effort.

Technology Description and Verification Testing

In the summer of 2009, Battelle collected recreational water samples from a total of nine lakes in Nebraska and New York. Technicians evaluated the test kits against known microcystin concentrations in deionized (DI) water as well as unknown concentrations in recreational water samples, and then compared to the reference method by calculating the percent difference (%D). Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis for the determination of algal toxins was the proven reference method of choice. Five of the six kits provided quantitative results and one kit provided semi-quantitative results; technology descriptions are provided in Table 1. The kits were evaluated for concentrations of microcystin-LR, -LA, and -RR. The results were expressed as microcystin-LR equivalent concentrations.

ETV evaluated the test kits for accuracy, precision, linearity, method detection limit, inter-kit lot reproducibility, matrix interference, and operational and sustainability factors. Battelle completed the recreational water analysis at their laboratory in Columbus, Ohio, while the reference method analysis was performed at the University of Nebraska Water Center in Lincoln, Nebraska. Table 2 provides selected performance results for the six verified kits. Additional information is available in the verification reports and statements on ETV's website at http://www.epa.gov/nrmrl/std/etv/vt-ams.html#itkm.

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Environmental, Health, and Regulatory Background of Microcystins at a Glance

Cyanobacteria, also known as blue-green algae, are a family of single-celled organisms that proliferate in warm, nutrient-rich fresh waters and estuaries. Many cyanobacteria species produce toxic compounds called microcystins. Microcystins are nonribosomal peptides that are naturally produced within the cell wall of living cyanobacteria. When a cell dies or ruptures through a process called lysis, microcystins are released into the water. Human and animal exposures to microcystins have the potential to result in skin rashes, eye irritations, respiratory symptoms, and liver damage.

Current research has identified over 80 structural variants or congeners of toxic microcystins with microcystin-LR (Leucine-Arginine) being the most frequently detected and extensively studied. The World Health Organization (WHO) has set a provisional drinking water guideline concentration of 1 microgram/liter (µg/L) and a recreational water standard of 20 µg/L for microcystin-LR (free and cell-bound). In addition to microcystin-LR, microcystin-LA (Leucine-Alanine) is highly toxic, and microcystin-RR (Arginine-Arginine) is highly prevalent but less toxic.

Under the Safe Drinking Water Act (SDWA), EPA is responsible for publishing a list of contaminants, called the Contaminant Candidate List (CCL), that are not subject to any current regulation but may require future regulation under the SDWA. In 2005, EPA published a second CCL (CCL2), including cyanobacteria and their toxins. In June 2011, EPA published a draft CCL3 which included three cyanotoxins: Anatoxin-a, Microcystin-LR, and Cylindrospermopsin. The final CCL3 will be out for public comment in mid-2012 and the final rule is expected in August 2013.

A verified immunoassay technology.

[1]The ETV Program operates largely as a public-private partnership through competitive cooperative agreements with non-profit research institutes. The program provides objective quality-assured data on the performance of commercial-ready technologies. Verification does not imply product approval or effectiveness. ETV does not endorse the purchase or sale of any products and services mentioned in this document.

Table 1. Description of Microcystin Test Kit Technologies		
Vendor & Technology	**Type of Result**	**Technology Description**
Abraxis, LLC *Microcystin ADDA Plate Kit*	Quantitative	Enzyme-linked immunosorbent assay (ELISA) utilizing polyclonal antibodies raised against the ADDA moiety of the molecule, allowing recognition of microcystins, nodularins and their variants. Employs a 96-well assay plate that accommodates a large number of samples.
Abraxis, LLC *Microcystin DM Plate Kit*	Quantitative	ELISA utilizing monoclonal antibodies raised against the ADDA moiety of the molecule, allowing recognition of microcystins, nodularins and their variants. Employs a 96-well assay plate that accommodates a large number of samples.
Abraxis, LLC *Microcystin Strip Test Kit*	Semi-quantitative	Rapid immunochromatographic test utilizing rapid cell lysis step (QuikLyse™) prior to testing. Strip kit allows single sample analysis.
Beacon Analytical Systems *Microcystin Plate Kit*	Quantitative	Immunological laboratory test utilizing polyclonal antibodies that bind microcystins and microcystin-enzyme conjugates that compete for a limited number of antibody binding sites. Employs a 96-well assay plate that accommodates a large number of samples.
Beacon Analytical Systems *Microcystin Tube Kit*	Quantitative	Utilizes the same principles as the plate kit. Tube kit allows single sample analysis.
Zeu-Immunotec, S.L *MicroCystest Plate Kit*	Quantitative	Utilizes protein phosphatase inhibition assay (PPIA) to detect toxicity of microcystins associated with inhibition of protein phosphatases (PP) 1 and 2A in liver cells. Employs a 96-well assay plate that accommodates a large number of samples.

Selected Potential Outcomes of Verified Microcystin Immunoassay Technologies

Immunoassay technologies are faster and less costly than traditional methods of microcystin detection. The cost of analyzing a sample for one analyte using immunoassay techniques can range from $17 to $25, depending on the number of replicates run, while GC-MS analysis can cost between $500 to $900 per sample. Therefore, test kits can provide a cost-effective method to screen for microcystins while GC-MS and LC-MS methods provide true quantitative confirmation. Also, the analysis time for immunoassay techniques is typically less than one day, as compared to GC-MS, which can be two to three weeks. Since all verified test kits were reported by the operators as easy to use, these kits can likely be used by non-technical operators.

Table 2. Selected Performance Results (Microcystin-LR equivalents)

Technology	Accuracy[1] (%D)	Precision[2] (%RSD)	Linearity (R^2)	Method Detection Limit[3] (MDL, ppb)
Microcystin ADDA Plate	-45% to 58%	5% to 45%	0.91	0.14
Microcystin DM Plate	24% to 94%	2% to 11%	0.99	0.11
Microcystin Strip[4]	N/A	N/A	N/A	N/A
Microcystin Plate	16% to 81%	1% to 15%	0.99	0.15
Microcystin Tube	-76% to 21%	3% to 10%	0.98	0.18
MicroCystest Plate	20% to 280%	1% to 13%	0.95	0.24

1. The accuracy %D range covers 0.1, 0.5, 1.0, 2.0, and 4.0 ppb of microcystin LR-equivalents.
2. Precision includes known DI water spikes of the microcystin-LR variant and recreational water samples with unknown variants.
3. MDL was calculated with seven replicate analyses with a toxin concentration of five times the vendor's estimated detection limit.
4. Due to the semi-quantitative test design, parameters reported in this table cannot be calculated.

References:

Butler, N., et al., *Microcystins: A brief overview of their toxicity and effects, with special reference to fish, wildlife, and livestock*. January, 2009, Office of Environmental Health Hazard Assessment.

Guidelines for Safe Recreational Water Environments, Volume 1, Coastal and Fresh Waters. 2003: World Health Organization.

Hollrah, M., *Standard Operating Procedure (SOP) Determination of algaltoxin residues in water extracts by liquid chromatography (LC)-atmospheric pressure electrospray ionization tandem mass spectrometry (MS/MS)*. December, 2005, Water Sciences Laboratory, University of Nebraska.

U.S. EPA Office of Water, http://water.epa.gov/scitech/drinkingwater/dws/ccl/

U.S. EPA ETV Program, http://www.epa.gov/etv

EPA/600/S/12/511
April 2012