THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

TECHNOLOGY TYPE:	**MICROCYSTIN TEST KIT**	
APPLICATION:	**RECREATIONAL WATER MICROCYSTIN DETECTION**	
TECHNOLOGY NAME:	**Microcystin Plate Kit**	
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ETV Joint Verification Statement

The U.S. Environmental Protection Agency (EPA) has established the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies. Information and ETV documents are available at www.epa.gov/etv.

ETV works in partnership with recognized standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permitters), and with individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field and laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The Advanced Monitoring Systems (AMS) Center, one of six verification centers under ETV, is operated by Battelle in cooperation with EPA's National Risk Management Research Laboratory. The AMS Center evaluated the performance of microcystin test kits for water monitoring. This verification statement provides a summary of the test results for the Beacon Analytical Systems' Microcystins Plate Kit.

VERIFICATION TEST DESCRIPTION

This verification test of the Beacon Plate Kit was conducted from July 26 through August 12, 2010 at Battelle laboratories in Columbus, OH. Reference analyses by liquid chromatography tandem mass spectrometry

(LC-MS/MS) were performed the week of August 16th, 2010 by the University of Nebraska Water Sciences Laboratory.

The objective of this verification test was to evaluate the microcystin test kit performance in analyzing known concentrations of microcystin in ASTM International Type II deionized (DI) water and in natural recreational water samples. The technology was used to analyze a variety of water samples for the variants microcystin-LR, microcystin-LA, and microcystin-RR. Because the technology cannot distinguish individual congeners among the more than 80 microcystin variants, the samples prepared for this test were spiked with individual variants. The plate kit provided a quantitative determination of microcystins and was evaluated in terms of:

- Accuracy - comparison of test kit results (samples prepared in DI) to results from a reference method;
- Precision - repeatability of test kit results from three sample replicates analyzed in DI water, matrix interference, and recreational water samples;
- Linearity - determination of whether or not the test kit response increases in direct proportion to the known concentration of microcystin;
- Method detection limit - the lowest quantity of toxin that can be distinguished from the absence of that toxin (a blank value) at a 95% confidence level;
- Inter-kit lot reproducibility - determination of whether or not the test kit response is significantly different between two different lots of calibration standards within the kits;
- Matrix interference - evaluation of the effect of natural recreational water matrices and chlorophyll-*a* on the results of the test kits; and
- Operational and sustainability factors - such as general operation, data acquisition, set up, and consumables.

Each microcystin test kit was operated according to the vendor's instructions by a vendor-trained Battelle technician. The Battelle technician had previous experience with using ELISA test kits. Samples and calibration standards were analyzed in duplicate and positive and negative controls were analyzed at the vendor-specified frequency.

The ability of the plate kit to determine the concentration of microcystin was challenged using quality control (QC) samples, performance test (PT) samples and recreational water (RW) samples. QC, PT, and RW samples were prepared by Battelle technical staff the day before testing began. The test samples were prepared in glass volumetric flasks and stored in amber glass vials at 4 ^{o}C ± 3 ^{o}C. The reference samples that were aliquotted from the test samples were stored in amber glass bottles at < -10^{o}C. Replicate samples for the test kits were taken from the same sample bottle. The QC, PT, and RW samples were prepared blindly for the operator by coding the sample labels to ensure the results were not influenced by the operator's knowledge of the sample concentration and variant.

Unlike many contaminants, certified microcystin standards are not commercially available. In planning this verification test, multiple sources of standards were investigated. With agreement from all vendors and the EPA Project Officer, the standards used for this verification were purchased from reputable sources (Canadian National Research Council and Abraxis), based on a Performance Evaluation Audit, and used for both the testing solutions and the reference method calibration.

QA oversight of verification testing was provided by Battelle and EPA. Battelle QA staff conducted technical systems audits of the testing, and Battelle QA staff conducted a data quality audit of at least 10% of the test data. This verification statement, the full report on which it is based, and the test/QA plan for this verification test are available at www.epa.gov/etv/centers/center1.html.

TECHNOLOGY DESCRIPTION

The following is a description of the Beacon Plate Kit, based on information provided by the vendor. The information provided below was not verified in this test.

The Beacon Plate Kit is an immunological laboratory test for the quantification of microcystins in water. The kit uses a polyclonal antibody that binds both microcystins and a microcystin-enzyme conjugate. Microcystins in the sample compete with the microcystin-enzyme conjugate for a limited number of antibody binding sites. The assay procedure included the following steps:

- Add microcystin-enzyme conjugate and a sample containing microcystins to the wells in the plate, followed by antibody solution. The conjugate competes with any microcystins in the sample for the same antibody binding sites. The test well is coated with anti-rabbit IgG to capture the rabbit anti-microcystin added.
- Wash away any unbound molecules, after a 20-minute incubation.
- Add clear substrate solution to each well in the plate. In the presence of bound microcystin-enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites that are available in every well, and each well receives the same number of microcystin-enzyme conjugate molecules, a sample containing a low concentration of microcystins allows the antibody to bind many microcystin-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of microcystins allows fewer microcystin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution. The color is analyzed using a colorimeter or spectrophotometer to obtain optical density (OD) values at 450 nanometers (nm). Reader software or a spreadsheet is used to generate a standard curve and interpolate the sample values off that curve.

VERIFICATION RESULTS

The verification of the Beacon Plate Kit is summarized by the parameters described in Table 1.

Table 1. Beacon Plate Test Kit Performance Summary[a]

Verification Parameters	LR	LA	RR
Accuracy (ppb, range of %D)			
0.10	34% to 81%		
0.50	16% to 72%	270% to 2900% The LR equivalent values were closer to the spiked values suggesting that the 2% CR for LA may differ from those provided by Beacon.	49% to 170%
1.0	26% to 47%		170% to 190%
2.0	21% to 38%		59% to 100%
4.0			
7.0			
Precision (range of %RSD)	1% to 15%	3% to 16%	4% to 18%
Precision (RW samples)	All RSDs < 9%, except one at 59%		
Linearity (y=)	1.2x + 0.052 r^2 = 0.99	2.9x + 9.8 r^2 = 0.76	1.6x + 0.29 r^2 = 0.91
Method Detection Limit (ppb)	0.15	0.043	0.20

[a] Cross reactivity values were used to quantify results for different variants based on the LR calibration. See the full report for more information.

Inter-kit lot reproducibility. Calibration standards from two different lots were measured and the RPD of the resulting optical densities were all less than 14%.

Matrix Interference. Matrix interference effects were assessed by using a t-test to compare the plate kit results generated from samples made by spiking undiluted and diluted interference matrices with the PT sample results at the same concentration. For chlorophyll-*a* and RW matrices, five comparisons resulted in statistically significant differences: 1) 4.0-ppb LA spike into DI water was significantly different from the 4.0-ppb LA spike into the tenfold diluted RW samples (p=0.006); 2) the RW matrix was between the RR spikes into undiluted and diluted RW (p=0.006); 3) 4.0-ppb LR spike into DI water was significantly different from the 4.0-ppb LR spike into the

10 mg/L (p=0.002); 4) the 1.0-mg/L (p=0.003) chlorophyll-*a*; 5) for LA, the 1.0-mg/L and 10-mg/L chlorophyll-*a* solutions average results were different by 0.01-ppb and when compared to the DI water results, and the 1.0-mg/L chlorophyll-*a* solution results were statistically different (p = 0.045).

Recreational Water (RW). Because the reference method did not measure all possible microcystin variants, no quantitative comparison was made between the plate kit and the reference method results. The reference data were converted into LR-equivalents according to the plate kit cross reactivity for the variants. In general, the samples that were determined to have higher total concentrations by the plate kit had higher total concentrations as determined by the reference method. All of the plate kit total microcystin results were greater than the reference method results, which was consistent with the likelihood that all of the microcystins were not being measured by the reference method, which only measured three variants. However, the results of the plate kit were usually within 25% of the reference method, indicating that the LR, LA, and RR variants make up a significant proportion of the microcystins that are measurable by the plate kit.

Operational Factors. The test kit operator reported that the plate kit was easy to use. Solution or sample preparation was minimal, mostly involving diluting the wash solution or the samples that were above the quantification range. The procedure included two incubation periods that were 30 minutes each. Previous knowledge or training on the use of micro-pipettes and or multi-channel pipettes with 96-well plates is recommended for consistent readings. A spectrophotometer plate reader was necessary for obtaining the spectrophotometric readings that were then analyzed using any commercial ELISA evaluation program (for example, 4-parameters, Logit/Log or alternatively point to point). According to the vendor, once the analysis was complete, the remaining solutions and tube contents could be flushed down the drain with no hazardous waste generated for disposal. Since waste disposal requirements vary from state-to-state, the reader is encouraged to consult with the appropriate state government agency for proper waste disposal requirements.

The listed price for the plate kit at the time of the verification test was $275 for a plate kit that will analyze 84 samples. According to the vendor, the kit has a 6-month shelf life as received and should be stored at 4 – 8 ^{o}C. Other consumables not included in the kit are pipettes, pipette tips, and distilled or DI water.

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