THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

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ETV Joint Verification Statement

TECHNOLOGY TYPE: IMMUNOASSAY TEST KITS

APPLICATION: DETECTING ANTHRAX, BOTULINUM TOXIN, AND RICIN

TECHNOLOGY NAME: RAMP® Test Cartridges

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The U.S. Environmental Protection Agency (EPA) supports 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 or 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 Exposure Research Laboratory. The AMS Center has recently evaluated the performance of immunoassay test kits used to detect anthrax, botulinum toxin, and ricin. This verification statement provides a summary of the test results for the Response Biomedical Corp. Rapid Analyte Measurement Platform (RAMP®) test cartridges.

VERIFICATION TEST DESCRIPTION

The ability of the RAMP® test cartridges to individually detect various concentrations of anthrax spores, botulinum toxin, and ricin was evaluated between January 14 and April 23, 2004, by analyzing performance test (PT) and drinking water (DW) samples. PT samples included deionized (DI) water fortified with either the target contaminant, an interferent, both, or only a cross-reactive species. In addition to the PT and DW samples analyzed, method blank (MB) samples consisting of DI water also were analyzed to confirm negative responses in the absence of contaminants and to ensure that no sources of contamination were introduced during the analysis procedures. MB samples were analyzed by both a trained technician and a non-technical/untrained, first-time user at a non-laboratory location to evaluate the RAMP® performance and ease of use outside of the laboratory. The test strips generated either positive or negative qualitative results. Verification test results showed how effective the RAMP® test cartridges were at detecting the presence of each contaminant at several concentration levels, the consistency of the responses, and the susceptibility of the RAMP® test cartridges to selected interferents and cross-reactive species. In most cases, three replicates of each PT and DW sample were analyzed to evaluate the reproducibility of the RAMP® test cartridge results. Approximately 120 liters (L) of four DW samples were collected from geographically distributed municipal sources located in Florida (FL), New York (NY), Ohio (OH), and California (CA). These samples were dechlorinated with sodium thiosulfate, and then 100 L of each sample were concentrated using an ultra-filtration technique to a final volume of 250 milliliters (mL). Each DW sample (non-concentrated and concentrated) was analyzed without adding any contaminant, as well as after fortification with individual contaminants at a single concentration level to evaluate the effect of the DW matrix on the performance of the RAMP® test cartridges. During the anthrax spore PT sample analysis, the lowest detectable concentration of the RAMP® test cartridges was shown to be much higher than claimed by the vendor. Therefore, three preparations of spores were analyzed to further investigate these results. The three preparations included spores prepared at Battelle and preserved in a solution of water and phenol, spores prepared at Battelle and not preserved in phenol, and spores prepared at Dugway Proving Ground and stored in spent culture media. Most of the samples analyzed were made from the Battelle-prepared, phenol-preserved spores. The other two preparations were used to determine if the phenol preservation or the preparation technique was negatively affecting the sensitivity of the RAMP® test cartridges. Solutions of vegetative anthrax cells also were analyzed to determine the sensitivity of the RAMP® test cartridges to vegetative anthrax cells.

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

TECHNOLOGY DESCRIPTION

The following description of RAMP® test cartridges was provided by the vendor and was not subjected to verification in this test.

RAMP® is a rapid immunochromatographic system for screening environmental samples. The RAMP® system comprises a portable fluorescence reader and RAMP® test cartridges specific for detecting anthrax, botulinum toxin, and ricin. Test cartridges specific for detecting smallpox are also available, but were not tested. The RAMP® reader is a scanning fluorometer and data analysis system used to measure fluorescence from RAMP® test cartridges. The reader can be operated on built-in battery power or using an alternating current adapter. RAMP® uses an immunochromatographic strip, housed in the disposable test cartridges. Each test cartridge is single-use, disposable, and analyte-specific and is used to detect whether an analyte (e.g., anthrax spores) is present in an aqueous sample. Twenty-five individually packaged RAMP® test cartridges are provided in a small box. In addition to the test cartridges, the box contains 25 small plastic screw-top vials containing approximately 250 microliters (µL) of buffer, a box of sample collection swabs, a 70-µL micropipette, a lot card for insertion into the reader, a marking pen, and step-by-step instructions. To perform a test on a liquid sample, a small amount (10 µL) of sample is added to the provided buffer, and that solution is mixed and 70 µL of sample is pipetted onto the RAMP® test cartridge. The cartridge is then read using the reader, and a positive or negative result is generated on the reader's display. Each result, along with the time, date, and sample identification is printed using a printer provided by Response Biomedical Corp. The reader is also capable of downloading the results to a computer. The

dimensions of the RAMP® are 10.5 inches wide by 10 inches deep by 6 inches high (27 centimeters wide by 25 centimeters deep by 5 centimeters high), and it weighs 4.6 pounds (2.1 kilograms). A RAMP® system including 25 test cartridges, a reader, a printer, and a carrying case costs approximately $10,000. Regardless of whether the test strips are specific to anthrax, botulinum toxin, ricin, or smallpox, each additional box of 25 test cartridges costs approximately $500.

VERIFICATION OF PERFORMANCE

The tables below summarize the performance of the RAMP® test cartridges in detecting anthrax, botulinum toxin, and ricin.

Anthrax Summary Table

Parameter		Sample Information	Actual Fortified Anthrax Concentration[a]	Positive Results Out of Total Replicates
Qualitative contaminant results	Contaminant-only PT samples	Battelle-prepared, phenol-preserved spores	8×10^8 spores/mL	3/3
			8×10^7 spores/mL	0/3
			8×10^6 spores/mL	0/3
			3×10^5 spores/mL	0/3
		Vegetative cells	3×10^5 colony-forming units (cfu)/mL	2/3
			3×10^4 cfu/mL	0/1
		Dugway-prepared spores	7×10^8 spores/mL	3/3
			8×10^7 spores/mL	0/1
	Interferent PT samples	230 mg/L Calcium (Ca) 90 mg/L Magnesium (Mg)	1×10^9 spores/mL[b]	3/3
		2.5 mg/L humic acid 2.5 mg/L fulvic acid	1×10^9 spores/mL[b]	3/3
	DW samples	Concentrated CA	5×10^8 spores/mL[b]	3/3
		Concentrated NY	5×10^8 spores/mL[b]	3/3
		Unconcentrated DW	4×10^6 spores/mL[b]	0/24
	Cross-reactivity	5×10^5 spores/mL *Bacillus thuringiensis*	unspiked	0/3
False positives		No false positives resulted from the analysis of the interferent, DW, or cross-reactivity samples. *Bacillus thuringiensis* was prepared at concentrations much lower than the lowest detectable concentration of *Bacillus anthracis.* Therefore, negative results with these samples do not necessarily indicate a lack of cross-reactivity.		
False negatives		No false negative results were generated from the analysis of the interferent and DW samples spiked with detectable levels of anthrax spores; the RAMP® test cartridges were not able to detect anthrax spores at the vendor-stated limit of detection (LOD), but they were able to detect much higher concentration levels. All of the unconcentrated DW samples were spiked at concentrations less than detectable by the test strips and, therefore, were, as expected, negative.		
Consistency		96% of the results were obtained in replicate sets in which all the individual replicates had the same result, whether positive or negative.		
Lowest detectable concentration		8×10^8 spores/mL - Battelle prep ; 7×10^8 spores/mL - Dugway prep (vendor-stated LOD: 4×10^5 spores/mL); 3×10^5 cfu/mL - vegetative anthrax (no vendor-stated LOD)		

[a] The uncertainty of the enumeration technique is approximately 15%.
[b] Battelle-prepared, phenol-preserved spores.

Botulinum Toxin Summary Table

Parameter		Sample Information	Botulinum Toxin Concentration (mg/L)	Positive Results Out of Total Replicates
Qualitative contaminant positive results	Contaminant-only PT samples	Type A	0.5	0/3
			2	2/3
			5	2/3
			25	3/3
		Type B	0.3	0/3
			0.5	0/3
			2.5	0/3
			5	0/3
			200	0/3
			1,000	0/3
	Interferent PT samples	230 mg/L Ca 90 mg/L Mg	5[(a)]	3/3
		2.5 mg/L humic acid 2.5 mg/L fulvic acid	5[(a)]	2/3
	DW samples	Concentrated CA	5[(a)]	3/3
		Concentrated NY	5[(a)]	3/3
		Unconcentrated DW	5[(b)]	0/24
	Cross-reactivity	5 mg/L Lipopolysaccharide	unspiked	0/3
False positives		No false positives resulted from the analysis of the interferent, DW, or cross-reactivity samples.		
False negatives		One false negative replicate resulted from the analysis of the 2.5 mg/L humic and fulvic acid interferent samples spiked with a detectable level of Type A botulinum toxin; in addition, the RAMP® test cartridges were not able to detect Type B botulinum toxin spiked into DW at 5 mg/L or in DI water at concentrations up to 1,000 mg/L.		
Consistency		95% of the results were obtained in replicate sets in which all the individual replicates had the same result, whether positive or negative.		
Lowest detectable concentration		2 mg/L (Type A), Type B was not detectable up to concentrations of 1,000 mg/L. (vendor-stated LOD for botulinum toxin [non-specific]: 0.5 mg/L)		

(a) Type A botulinum toxin.
(b) Type B botulinum toxin.

Ricin Summary Table

Parameter		Sample Information	Ricin Concentration (mg/L)	Positive Results Out of Total Replicates
Qualitative contaminant positive results	Contaminant-only PT Samples	Ricin in DI water	1	0/3
			5	3/3
			15	3/3
			20	3/3
			50	3/3
	Interferent PT Samples	Ca and Mg	10	6/6
		Fulvic and humic acid	10	6/6
	DW Samples	Concentrated DW	10	12/12
		Unconcentrated DW	10	12/12
	Cross-reactivity	10 mg/L Lectin from soybean	unspiked	0/3
False positives		No false positives resulted from the analysis of the interferent, DW, or cross-reactivity samples.		
False negatives		No false negative results were generated by analyzing DW and interferent samples spiked with detectable levels of ricin.		
Consistency		100% of the results were obtained in replicate sets in which all the individual replicates had the same result, whether positive or negative.		
Lowest detectable concentration		5 mg/L (vendor-stated LOD: 1 mg/L)		

Other Performance Factorfor Anthrax, Botulinum Toxin, and Ricin Test Strips: All components for testing were provided in a box of 25 test cartridges; the required cartridge reader was operated using electricity or batteries, was easy to operate, and was contained in a rugged carrying case; test cartridges used easily inside and outside a laboratory with trained operator; non-technical operator needed minor direction from a trained operator; "low signal" resulted from highly concentrated anthrax solutions; and sample throughput was 4 samples per hour.

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