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: BADD™ Test Strips

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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 ADVNT Biotechnologies Biowarfare Agent Detection Device (BADD™) test strips.

VERIFICATION TEST DESCRIPTION

The ability of the BADD™ test strips 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 BADD™ 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 BADD™ test strips were at detecting the presence of each contaminant at several concentration levels, the consistency of the responses, and the susceptibility of the BADD™ test strips 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 BADD™ test strip 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 BADD™ test strips. During the anthrax spore PT sample analysis, the lowest detectable concentration of the BADD™ test strips 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 BADD™ test strips. Solutions of vegetative anthrax cells also were analyzed to determine the sensitivity of the BADD™ test strips 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 BADD™ test strips was provided by the vendor and was not subjected to verification in this test.

BADD™ test strips are self-contained, qualitative assays for screening environmental samples for the presence of anthrax, botulinum toxin, and ricin. These test strips work on similar principles, but each is single use and can detect only one contaminant. The BADD™ test strips are stored in resealable packages, which include all the items necessary to analyze each sample. Each individually packaged test includes approximately 250 microliters (μL) of buffer in a small plastic screw-top vial, a sample collection swab, a bulb syringe, the test strip (within its own sealed package), and step-by-step instructions. This package is approximately 5 inches (12.7 centimeters) by 6 inches (15.2 centimeters) and weighs only a few ounces. The vendor suggests that the resealable package be used as a sealed waste receptacle for all testing materials. The testing procedure involves dipping the dry collection swab into a solution suspected of containing anthrax, botulinum toxin, or ricin, followed by eluting (extracting) the collected sample into a collection tube containing a sample diluent. After the sample is collected, it is transferred onto the BADD™ test strip where dye-labeled antibodies detect trace amounts of the contaminant collected by the swab, as indicated by the presence of two bands in the test result window. After 15 minutes, the results are read visually. BADD™ test strips are sold in boxes of 10 for approximately $250 per box.

VERIFICATION OF PERFORMANCE

The tables below summarize the performance of the BADD™ test strips 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	3/3
			4×10^7 spores/mL	2/3
			8×10^6 spores/mL	0/3
			8×10^5 spores/mL	0/3
		Vegetative cells	4×10^6 colony-forming units (cfu)/mL	1/1
			3×10^5 cfu/mL	2/3
			3×10^4 cfu/mL	0/1
		Dugway-prepared spores	8×10^7 spores/mL	3/3
			8×10^6 spores/mL	0/1
	Interferent PT samples	230 mg/L Calcium (Ca) 90 mg/L Magnesium (Mg)	1×10^8 spores/mL[b]	3/3
		2.5 mg/L humic acid 2.5 mg/L fulvic acid	1×10^8 spores/mL[b]	3/3
	DW samples	Concentrated CA	1×10^8 spores/mL[b]	3/3
		Concentrated NY	1×10^8 spores/mL[b]	2/3
		Unconcentrated DW	1×10^7 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		One false negative replicate resulted from the analysis of the interferent and DW samples spiked with detectable levels of anthrax spores (concentrated NY DW); the BADD™ test strips were not able to detect anthrax spores at the concentration levels claimed by the vendor, 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		90% 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		4×10^7 spores/mL - Battelle prep; 8×10^7 spores/mL - Dugway prep (vendor-stated limit of detection [LOD]: 1×10^6 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	1/3
			2	0/3
			5	3/3
			25	3/3
		Type B	0.3	0/3
			0.4	0/3
			2	1/3
			4	1/3
			20	0/3
			200	0/3
			1,000	0/3
	Interferent PT samples[(a)]	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)]	3/3
	DW samples[(a)]	Concentrated CA	5[(a)]	3/3
		Concentrated NY	5[(a)]	3/3
		Unconcentrated DW	4[(b)]	2/24
	Cross-reactivity	5 mg/L Lipopolysaccharide	unspiked	1/3
False positives		No false positives resulted from the analysis of the interferent or unspiked DW samples. There was one false positive replicate out of three when lipopolysaccharide was analyzed as a possible cross-reactive compound.		
False negatives		No false negatives resulted from the analysis of the interferent and DW samples spiked with detectable levels of Type A botulinum toxin; however, the BADD™ test strips were not able to reproducibly detect Type B botulinum toxin when spiked into DW or interferent samples at 4 mg/L or DI water up to 1,000 mg/L.		
Consistency		84% 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 (Type A), Type B was not reproducibly detectable. (vendor-stated LOD for botulinum toxin [non-specific]: 0.4 mg/L)		

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

Ricin Summary Table

Parameter		Ricin Concentration (mg/L)	Positive Results Out of Total Replicates
Qualitative contaminant positive results	Contaminant-only PT samples	0.4	0/3
		2	0/3
		5	0/3
		15	0/3
		20	3/3
		200	3/3
		2,000	3/3
	Interferent PT and DW Samples	5	0/36
	Cross-reactivity	4 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		Ricin was not detectable when spiked into DW and interferent samples at 5 mg/L. No expanded testing was done involving the interferent or DW samples.	
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		20 mg/L (vendor-stated LOD: 0.4 mg/L)	

Other Performance Factors for Anthrax, Botulinum Toxin, and Ricin Test Strips: All components for testing were provided in a resealable package weighing just a few ounces; strips used easily inside and outside a laboratory with trained operator; non-technical operator needed minor direction from a trained operator; indicator line color change for the anthrax test strips was very faint 44% of the time, 29% of the time for botulinum toxin, and 100% for ricin, increasing the likelihood of false negative results; and sample throughput was 20 to 30 samples per hour.

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