THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

ETV Joint Verification Statement

TECHNOLOGY TYPE:	**IMMUNOASSAY TEST KITS**		
APPLICATION:	**DETECTING ANTHRAX, BOTULINUM TOXIN, AND RICIN**		
TECHNOLOGY NAME:	**BioThreat Alert® 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 Tetracore, Inc., BioThreat Alert® test strips.

VERIFICATION TEST DESCRIPTION

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

The BioThreat Alert® test strip from Tetracore, Inc., is a lateral flow immunochromatographic device that uses two antibodies in combination to specifically detect target antigen in solution. One of the specific antibodies is labeled with a colloidal gold derivative. Samples applied to the BioThreat Alert® test strips mix with the colloidal gold-labeled antibody and move along the strip membrane by capillary action. The second specific antibody captures the colloidal gold-labeled antibody and bound target. When a sufficient amount of target antigen is present, the colloidal gold label accumulates in the sample ("S") window on the test strip, forming a visible reddish-brown colored line. As an internal control, a second band in the control ("C") window indicates that the test strip functioned properly. Two bands or colored lines (in the "S" and "C" windows) are required for a positive result determination. Twenty-five individually packaged BioThreat Alert® test strips (including a disposable pipette) are provided in a small box. In addition to the test strips, the box contains several small plastic vials, 25 mL of sample buffer, and step-by-step instructions. To complete a test on a liquid sample, the sample is mixed with the provided buffer, and five or six drops are added to the sample well of the BioThreat Alert® test strip. A positive result is indicated by the appearance of a colored line in the test window of the test strip and can be read

visually or with a reader. During this verification test, a reader was used to make the determination of a positive or negative result. One kit of 25 strips including sample buffer, instruction brochure, and vials needed for sampling costs approximately $625. The Alexeter strip reader used during this verification test costs approximately $4,000.

VERIFICATION OF PERFORMANCE

The tables below summarize the performance of the BioThreat Alert® 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
			8×10^6 spores/mL	0/3
			8×10^5 spores/mL	0/1
		Vegetative cells	4×10^6 colony-forming units (cfu)/mL	1/1
			3×10^5 cfu/mL	1/1
			3×10^4 cfu/mL	2/3
			3×10^3 cfu/mL	0/1
		Dugway-prepared spores	7×10^8 spores/mL	2/2
			8×10^7 spores/mL	0/1
	Interferent PT samples	230 mg/L Calcium (Ca) and 90 mg/L Magnesium (Mg)	1×10^8 spores/mL[b]	0/3
		2.5 mg/L humic acid and 2.5 mg/L fulvic acid	1×10^8 spores/mL[b]	3/3
	DW samples	Concentrated CA	1×10^8 spores/mL[b]	2/3
		Concentrated NY	1×10^8 spores/mL[b]	1/3
		Unconcentrated DW	1×10^6 spores/mL	0/24
	Cross-reactivity	1×10^5 spores/mL *Bacillus thuringiensis*	unspiked	0/3
False positives		Two false positives resulted from the analysis of the DW samples. One out of three replicates for each of the FL DW and concentrated NY DW falsely generated positive results. *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		None of three results was positive for the 230-mg/L Ca and 90-mg/L Mg spiked with a detectable concentration of anthrax. In addition, one and two false negative results were generated for the concentrated CA and concentrated NY DW samples, respectively. BioThreat Alert® test strips were not able to detect anthrax spores at the vendor-stated limit of detection (LOD). 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% (25 of 26 replicates) of the contaminant and interferent PT sample results were obtained in replicate sets in which all the individual replicates had the same result, whether positive or negative. This was the case for 78% of the DW samples.		
Lowest detectable concentration		8×10^7 spores/mL - Battelle prep; 7×10^8 spores/mL - Dugway prep (vendor-stated LOD: 1×10^5 spores/mL); 3×10^4 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	0.01	3/3
			0.05	3/3
			0.1	3/3
			0.5	3/3
		Type B	0.01	1/3
			0.05	3/3
			0.1	3/3
			0.3	3/3
			0.5	3/3
	Interferent PT samples	Ca and Mg	0.1	3/3 Type A 6/6 Type B
		Humic acid and fulvic acid	0.1	3/3 Type A 6/6 Type B
	DW samples	Concentrated DW	0.1	6/6 Type A 12/12 Type B
		Unconcentrated DW	0.1	12/12 Type B
	Cross-reactivity	0.1 mg/L Lipopolysaccharide	unspiked	1/3
False positives		There was one false positive replicate out of three for the unspiked 2.5-mg/L humic and fulvic acid interferent PT sample; the unspiked concentrated OH DW sample and the lipopolysaccharide each generated one false positive result out of three replicates.		
False negatives		No false negatives resulted from the analysis of the interferent and DW samples spiked with detectable levels of Types A and B botulinum toxin.		
Consistency		92% 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		0.01 mg/L (Type A); 0.05 mg/L (Type B) (vendor-stated LOD for both Types A and B: 0.01 mg/L)		

Ricin Summary Table

Parameter			Ricin Concentration (mg/L)	Positive Results Out of Total Replicates
Qualitative contaminant positive results	Contaminant-only PT samples		0.035	3/3
			0.2	3/3
			0.4	3/3
			2	3/3
			15	3/3
	Interferent PT samples	Ca and Mg	0.4	6/6
		Humic acid and fulvic acid	0.4	6/6
	DW samples	Concentrated DW	0.4	12/12
		Unconcentrated DW	0.4	11/12
	Cross-reactivity	0.4 mg/L Lectin from soybean	unspiked	0/3
False positives		The unspiked concentrated FL DW generated two false positives out of three. All other DW and cross-reactivity samples resulted in correctly negative responses.		
False negatives		There was one false negative out of three for the NY DW sample spiked with a detectable concentration of ricin. The other spiked interferent and DW samples were correctly determined to be positive.		
Consistency		100% of the contaminant and interferent PT results were obtained in replicate sets in which all the individual replicates had the same result, whether positive or negative. That was the case 88% (14 out of 16) of the time for the DW samples.		
Lowest detectable concentration		0.035 mg/L (Vendor-stated LOD: 0.035 mg/L)		

Other Performance Factors for Anthrax, Botulinum Toxin, and Ricin Test Strips: All components for testing were provided in a box of 25 test strips; the strip reader used during the verification test was powered using electricity or batteries, was easy to operate, and was contained in a rugged carrying case; test strips and reader were used easily inside and outside a laboratory with trained operator; non-technical operator performed tests as well as a trained operator; and sample throughput was 20 samples per hour.

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