THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

Battelle
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ETV Joint Verification Statement

TECHNOLOGY TYPE:	**IMMUNOASSAY TEST KITS**		
APPLICATION:	**DETECTING ANTHRAX AND RICIN**		
TECHNOLOGY NAME:	**QTL Biosensor**		
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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 technology areas under ETV, is operated by Battelle in cooperation with EPA's National Exposure Research Laboratory. The AMS Center evaluated the performance of immunoassay test kits used to detect anthrax and ricin in water. This verification statement provides a summary of the test results for the QTL Biosystems Biosensor.

VERIFICATION TEST DESCRIPTION

The verification test for the QTL Biosensor was conducted at Battelle between November 2005 and March 2006 according to procedures specified in the *Test/QA Plan for Verification of Immunoassay Test Kits* for the following parameters: contaminant presence/absence; false positive/false negative response to interferents, drinking water (DW) matrix effects, and cross-reactivity; consistency; method detection limit; field portability; ease of use; and sample throughput. The ability of the QTL Biosensor to detect various concentrations of anthrax and ricin was evaluated by analyzing performance test (PT) and DW samples. PT samples included American Society for Testing and Materials Type II deionized (DI) water fortified with the target contaminant, an interferent, both, or only a cross-reactive species. Target analytes were added to DI water at lethal dose concentrations as well as at several concentrations selected based on the vendor-stated limit of detection (LOD). The effect of interferents was evaluated by analyzing two types of interferent solutions. The first type contained both humic and fulvic acids in DI water, and the second type contained magnesium (Mg) and calcium (Ca) in DI water. Both types of interferent solutions were prepared with and without the addition of the contaminants at a single concentration level (10 times the vendor-stated LOD). In addition, specificity was evaluated by exposing the QTL Biosensor to *Bacillus thuringiensis*, a potentially cross-reactive compound for anthrax, and lectin from soybean, a potentially cross-reactive compound for ricin. PT samples were analyzed in triplicate (with the exception of samples used to determine the method detection limit for which seven replicates were analyzed). DW samples were collected from four water utilities that use a variety of treatment methods. DW samples, both unconcentrated and concentrated by a factor of 400, were analyzed in triplicate both with and without the addition of anthrax and ricin at a concentration of 10 times the vendor-stated LOD. In addition to the PT and DW samples analyzed, method blank (MB) samples consisting of DI water were analyzed to confirm negative responses in the absence of any contaminant and to ensure that no sources of contamination were introduced during the analysis procedures.

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 QTL Biosensor was provided by the vendor and was not verified in this test.

The QTL Biosensor is a handheld device that is similar to standard immunomagnetic sandwich assays and is capable of detecting anthrax spores and ricin toxins in samples. The sample is added to the QTL Biosensor cartridge which contains sensing reagents. The sensing reagents are composed of two materials: a magnetic component and a fluorescent component. Receptors for the biological agent(s) of interest are contained in both sensing reagents. Upon mixing the sample with the reagents, the magnetic and fluorescent components form a complex with the biological agent(s) for which they are specific. A magnetic field is then applied. This separates all magnetic materials (including any complexes containing the biological agent) from the solution, which contains excess fluorophore. A wash is performed to remove all excess reagents materials from the sample chamber. Then, an excitation wavelength of light is exposed to the magnetic pellet comprised of the biological agent complexes, and the resulting fluorescence indicates the presence of the biological agent.

The QTL Biosensor contains both positive and negative controls to ensure the validity of results and proper functioning of the QTL Biosensor. Both liquid and solid samples can be analyzed using the QTL Biosensor. Results are displayed as a millivolt (mV) and percent of full scale (%FS) reading. The QTL Biosensor also has red/green warning lights that can be set to a pre-determined mV threshold reading to indicate a positive/negative response where a green light indicates no toxin below the mV threshold, and a red light indicates the presence of a toxin above the mV threshold.

The QTL Biosensor includes an integrated bar code reader (use is optional), and a starter kit comes with 20 test cartridges. Test cartridges are shipped with a sample collector, bio-hazard bag, and a bar code label. The cartridge is single-use, self contained, self sealing, and includes all necessary reagents. It is 1.9 inches wide by 1.7 inches

high by 0.5 inch deep. The QTL Biosensor is 11 inches long by 10 inches wide by 5 inches high and weighs 6 pounds. Its battery life is 16 hours of continuous operation, and it has a recharge cycle of 2 to 4 hours.

The QTL Biosensor has a liquid crystal display screen with a four-button user interface. The bar code reader captures the cartridge type and serial number. Date, time, location, user identification, and serial numbers (Biosensor and cartridge) are stored with each result. There is on-board data storage for the last 100 samples and results can be uploaded electronically to a laptop computer (not included with the QTL Biosensor). The QTL Biosensor pricing starts at $11,500, and the price of each cartridge is $21.

VERIFICATION OF PERFORMANCE

The tables that follow summarize the performance of the QTL Biosensor in detecting anthrax and ricin, respectively.

Anthrax Summary Table

Parameter	Sample Information	Anthrax Concentration (spore/mL)		Positive Results out of 3 Replicates	
Contaminant-only PT samples	DI water	200 (lethal dose)		0	
		1×10^5 (vendor-stated limit of detection)		1	
		5×10^5		3	
		1×10^6		3	
		5×10^6		3	
Interferent PT samples	0.5 mg/L humic and fulvic	unspiked	1×10^6	0	3
	2.5 mg/L humic and fulvic			0	3
	50 mg/L Ca and Mg			3	3
	250 mg/L Ca and Mg			3	3
DW samples	Unconcentrated CA	unspiked	1×10^6	3	2
	Concentrated CA			3	3
	Unconcentrated FL			2	2
	Concentrated FL			3	3
	Unconcentrated NY			1	3
	Concentrated NY			2	3
	Unconcentrated OH			0	2
	Concentrated OH			2	3
Cross-reactivity	1×10^6 spores/mL *Bacillus thuringiensis*	unspiked		0	
False positives	False positive results occurred in Ca and Mg interferent samples as well as the unconcentrated water from CA, FL, and NY and all concentrated drinking water samples.				
False negatives	False negative results occurred only in the unconcentrated CA, FL, and OH drinking water samples.				
Consistency	Results were consistent (i.e., produced positive or negative results without variation among replicates) in 21 out of 29 sets of replicates or 72%.				
Method Detection Limit	The method detection limit was determined to be the concentration generating a 65 mV response. It was between $1x10^5$ spores/mL (vendor-stated limit of detection) and $5x10^5$ spores/mL.				
Other Performance Factors	Long term storage of the test cartridges should be at 2-8 ºC, but cartridges may be kept at room temperature for up to six months. Analysis software was user-friendly. The QTL Biosensor uses electricity or rechargeable batteries and includes a rugged carrying case. Test cartridges and detector were used inside and outside a laboratory by trained operator as well as non-technically trained operator; sample throughput was 12 samples per hour.				

Shading indicates results for unspiked sample.

Ricin Summary Table

Parameter	Sample Information	Ricin Concentration (mg/L)		Positive Results out of 3 Replicates	
Contaminant-only PT samples	DI water	0.05 (vendor-stated limit of detection)		0	
		0.25		3	
		0.5		3	
		2.5		3	
		15 (lethal dose)		3	
Interferent PT samples	0.5 mg/L humic and fulvic	unspiked	0.5	0	3
	2.5 mg/L humic and fulvic			0	3
	50 mg/L Ca and Mg			0	3
	250 mg/L Ca and Mg			0	1
DW samples	Unconcentrated CA	unspiked	0.5	0	3
	Concentrated CA			0	3
	Unconcentrated FL			1	3
	Concentrated FL			1	3
	Unconcentrated NY			0	3
	Concentrated NY			0	3
	Unconcentrated OH			0	3
	Concentrated OH			0	3
Cross-reactivity	0.5 mg/L Lectin from soybean	unspiked		0	
False positives	False positive results occurred in the unconcentrated and concentrated FL drinking water samples.				
False negatives	False negative results occurred only in the 250 mg/L Ca and Mg interferent sample.				
Consistency	Results were consistent (i.e., produced positive or negative results without variation among replicates) in 26 out of 29 sets of replicates or 90%.				
Method Detection Limit	The method detection limit was determined to be the concentration generating a 76 mV response. It was between 0.05 mg/L (vendor stated limit of detection) and 0.25 mg/L.				
Other Performance Factors	Long term storage of the test cartridges should be at 2-8 ºC, but cartridges may be kept at room temperature for up to six months. Analysis software was user-friendly. The QTL Biosensor uses electricity or rechargeable batteries and includes a rugged carrying case. Test cartridges and detector were used inside and outside a laboratory by trained operator as well as non-technically trained operator; sample throughput was 12 samples per hour.				

Shading indicates results for unspiked sample.

Original signed by Gregory A. Mack 10/26/2006	Original signed by Jonathan G. Herrmann 11/12/2006
Gregory A. Mack Date	Jonathan G. Herrmann Date
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