September 2006

Environmental Technology Verification Report

QTL BIOSYSTEMS LLC
QTL BIOSENSOR

Prepared by
Battelle

Battelle
The Business of Innovation

Under a cooperative agreement with

EPA **U.S. Environmental Protection Agency**

ETV ETV ETV

THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

Battelle
The Business of Innovation

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
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ETV Advanced Monitoring Systems Center

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QTL Biosensor

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Notice

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, has financially supported and collaborated in the extramural program described here. This document has been peer reviewed by the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technology across all media and to report this objective information to permitters, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of six environmental technology centers. Information about each of these centers can be found on the Internet at http://www.epa.gov/etv/.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. Under a cooperative agreement, Battelle has received EPA funding to plan, coordinate, and conduct such verification tests for "Advanced Monitoring Systems for Air, Water, and Soil" and report the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at http://www.epa.gov/etv/centers/center1.html.

Acknowledgments

The authors wish to acknowledge the support of all those who helped plan and conduct the verification test, analyze the data, and prepare this report. We sincerely appreciate the contribution of drinking water samples from the Metropolitan Water District of Southern California (Paul Rochelle and Melinda Stalvey), the New York Department of Environmental Protection (Virginia Murray), and Orange County Utilities, Orlando, Florida (Theresa Slifko and Liza Robles). Also, thank you to the Metropolitan Water District of Southern California for concentrating each drinking water sample. We would also like to thank Karen Bradham, U.S. EPA National Exposure Research Laboratory; Steve Allgeier, U.S. EPA Office of Water; Ricardo DeLeon, Metropolitan Water District of Southern California; and Stanley States, Pittsburgh Water and Sewer Authority, for their careful review of the test/QA plan and this verification report.

Contents

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List of Abbreviations

%FS	percent full scale
AMS	Advanced Monitoring Systems
ATEL	Aqua Tech Environmental Laboratories, Inc.
Ca	calcium
CDC	Centers for Disease Control and Prevention
cfu	colony-forming units
COA	certificate of analysis
DI	deionized
DW	drinking water
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
L	liter
LD	lethal dose
LOD	limit of detection
MB	method blank
MDL	method detection limit
Mg	magnesium
µL	microliter
mg/L	milligram per liter
mL	milliliter
mV	millivolt
PD	percent difference
PT	performance test
QA	quality assurance
QC	quality control
QMP	quality management plan
TSA	technical systems audit
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases

Chapter 1
Background

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative 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.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of 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 EPA's National Exposure Research Laboratory and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of the QTL Biosystems Biosensor immunoassay test cartridges and detector system. Immunoassay test kits were identified as a priority technology category for verification through the AMS Center stakeholder process.

Chapter 2
Technology Description

The objective of the ETV AMS Center is to verify the performance characteristics of environmental monitoring technologies for air, water, and soil. This verification report provides results for the verification testing of the QTL Biosensor. Following is a description of the QTL Biosensor, based on information provided by the vendor. The information provided below was not verified in this test.

The QTL Biosensor, shown in Figure 2-1, 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 the magnetic pellet comprised of the biological agent complexes is exposed to an excitation wavelength of light. The resulting fluorescence indicates the presence of the biological agent.

Figure 2-1. QTL Biosystems Biosensor

The QTL Biosensor contains both positive and negative controls to ensure the validity of results and proper functioning of the QTL Biosensor. The Negative Control Cartridge for each of its tests can be used to rule out false positives. This cartridge contains a sensor that is prepared with antibodies that are not directed towards an antigen that is found in human samples. These cartridges have two primary uses. First, they can be used to confirm the results of the test after a positive result is obtained with a test cartridge. If a negative control cartridge gives a positive result with the same sample matrix,

then the results from that test cartridge are ambiguous. However, if the negative control gives a negative result, it confirms the original positive result. The second use would be to determine whether or not the QTL test can be used reliably on a given sample matrix or with a particular interferent before any additional testing is performed. For example, if the QTL Negative Control Cartridge for the anthrax assay always gives positive results on water samples from a particular water system, then the assay cannot be used in that water system.

Liquid samples are sampled directly into the sampling syringe while a hydrated swab is provided to collect samples from surfaces. The swab sample is transferred to a sample buffer tube and drawn into a sample syringe. After a washing syringe is inserted into the QTL Biosensor cartridge, the sampling syringe is inserted. The cartridge is shaken by hand for one minute and inserted into the detector. After a three minute automatic pellet formation step, the washing syringe is pressed and the read button is pushed to obtain results. 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. The QTL Biosensor pricing starts at $11,500, and the price of each cartridge is $21.

Chapter 3
Test Design

The objective of this verification test was to evaluate the ability of the QTL Biosensor and test cartridges to detect specific biological toxins and agents in water samples and to determine whether the QTL Biosensor is susceptible to interferents in drinking water (DW).

During this verification test, the QTL Biosensor and test cartridges were subjected to various concentrations of anthrax and ricin in American Society for Testing and Materials Type II deionized (DI) water. Table 3-1 shows the contaminants, the vendor-stated limit of detection (LOD), the lethal dose (LD) concentrations, and the contaminant source. It should be recognized that there is a wide range of LD concentrations in the literature. In selecting an LD level for use in verification testing, literature oral LD50 values were reviewed and included in the test/QA plan and amendments.[1] In addition to reviewing the LD values in the literature, two factors were taken into consideration in selecting the final LD concentration for use in testing:

1) Consistency with the LD concentrations used in the first round of ETV immunoassay technology evaluations.
2) Applicability of the LD concentration level to the participating technologies' expected limits of detection.

In some instances this resulted in an LD level being selected that was on the high end of the literature values reported. Given the range of LD concentrations that are available in the literature, it is recommended that all readers evaluate the LD concentrations used for verification testing with respect to their particular LD requirements. The lethal dose concentration was determined using a 250 mL ingestion volume.

The QTL Biosensor also was used to analyze contaminant-fortified DW samples that were collected from four water utilities that use a variety of treatment methods. 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. In addition, specificity was evaluated by exposing the QTL Biosensor to a potentially cross-reactive compound or spore for each target contaminant. *Bacillus thuringiensis* was used to test the anthrax cartridge and lectin was used to test the ricin cartridge.

Table 3-1. Lethal Dose and Source of Contaminants

Contaminant	Vendor-Stated LOD	Lethal Dose Concentration[a]	Source of Contaminant
Bacillus anthracis Ames Strain (anthrax)	1×10^5 spores/milliliter (mL)	200 spores/mL	Independent lot prepared at Battelle from U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) stock
Ricinus communis Agglutinin II (ricin)	0.05 milligrams/ liter (mg/L)	15 mg/L	Vector Laboratories, Inc. (Burlingame, California)

[a] The lethal dose of each contaminant was determined by calculating the concentration at which 250 mL of water would probably cause the death of a 154-pound person, based on human mortality data and as outlined in the Test/QA Plan for Verification of Immunoassay Test Kits Amendment Number 5 [1].

The verification test for the QTL Biosensor was conducted from November 2005 through March 2006, according to procedures specified in the *Test/QA Plan for Verification of Immunoassay Test Kits* including amendments 1-5.[1] This test was conducted at Battelle in West Jefferson, Ohio. Aqua Tech Environmental Laboratories, Inc. (ATEL) of Marion, Ohio, performed physicochemical characterization for each DW sample to determine the following parameters: turbidity; concentration of dissolved and total organic carbon; specific conductivity; alkalinity; concentration of Mg and Ca; pH; hardness; and concentration of total organic halides, trihalomethanes, and haloacetic acids. Battelle confirmed the presence of anthrax spores using plate enumeration. The QTL Biosensor was evaluated for the following parameters:

- Contaminant presence/absence
- False positive/false negative response
  - Interferents
  - DW matrix effects
  - Cross-reactivity
- Consistency
- Method detection limit
- Other performance factors
  - Field portability
  - Ease of use by technical and non-technical operators
  - Sample throughput.

3.1 Test Samples

Tables 3-2 and 3-3 summarize the samples analyzed for each contaminant. The ability of the QTL Biosensor to individually detect various concentrations of anthrax spores and ricin was evaluated by analyzing performance test (PT) and DW samples. PT samples included DI water fortified with the target contaminant, an interferent, both, or only a cross-reactive species. DW samples were analyzed using the QTL Biosensor with and without the addition of each target contaminant. Note that test cartridges specific to anthrax only were tested with anthrax and cartridges specific to ricin only were tested with ricin.

3.1.1 Performance Test Samples

The contaminant-only PT samples (shown in Table 3-2) were prepared in DI water using standards of anthrax and ricin. Anthrax concentrations were verified using a plate enumeration technique. Reference methods were not available for quantitative confirmation of ricin test solutions so certificates of analysis (COA) and QA oversight of solution preparation were used to determine their concentrations.

Table 3-2. Performance Test Samples

Type of PT Sample	Sample Characteristics	Approximate Concentrations
Contaminant	Anthrax	200 to 5 x 10^6 spores/mL
	Ricin	0.05 to 15 mg/L
Interferent	Contaminants in 50 mg/L Ca and 50 mg/L Mg	Anthrax – 1 x 10^6 spores/mL Ricin – 0.5 mg/L
	Contaminants in 250 mg/L Ca and 250 mg/L Mg	Anthrax – 1 x 10^6 spores/mL Ricin – 0.5 mg/L
	Contaminants in 0.5 mg/L humic acid and 0.5 mg/L fulvic acid	Anthrax – 1 x 10^6 spores/mL Ricin – 0.5 mg/L
	Contaminants in 2.5 mg/L humic acid and 2.5 mg/L fulvic acids	Anthrax – 1 x 10^6 spores/mL Ricin – 0.5 mg/L
Cross-reactive species	*Bacillus thuringiensis* (anthrax analogue)	1 x 10^6 spores/mL
	Lectin from soybean (ricin analogue)	0.5 mg/L

Table 3-3. Drinking Water Samples

Drinking Water Sample Description				Approximate Contaminant Concentrations	
Water Utility	**Water Treatment**	**Source Type**	**Conc. / Unconc.**	**Anthrax**	**Ricin**
Metropolitan Water District of Southern California (CA)	Filtered chloraminated	surface	both	unspiked and 1×10^6 spores/mL	unspiked and 0.5 mg/L
New York City, New York (NY)	Unfiltered chlorinated	surface	both		
Columbus, Ohio (OH)	Filtered chlorinated	surface	both		
Orlando, Florida (FL)	filtered chlorinated	ground	both		

The contaminant only PT samples were solutions of the contaminant in DI water at the vendor-stated LOD, the lethal dose, and approximately 5, 10, and 50 times the LOD. These solutions were used to evaluate contaminant presence/absence and method detection limit.

The interferent PT samples consisted of samples of humic and fulvic acids isolated from Elliott Soil (obtained from the International Humic Substances Society) and Ca and Mg (prepared from their chlorides with concentrations based on metals only), each spiked into DI water at two concentration levels. These solutions were analyzed both with and without the target contaminant. In addition, because the commercially available ricin contained a preservative (sodium azide), a preservative blank sample consisting of 0.16 mg/L sodium azide was prepared in DI water. This sodium azide solution represents the concentration of the preservative that would be found in the most concentrated contaminant solution (50 x LOD). This preservative blank was analyzed along with the contaminant solutions to ensure that the preservative did not have a significant effect on the performance of the QTL Biosensor during ricin testing. The interferent PT samples were used to evaluate whether interferences which are commonly found in water have the potential to cause false positive or negative results with the QTL Biosensor.

The last type of PT sample was a cross-reactivity check sample to determine whether the QTL Biosensor produced false positive results in response to similar analytes. *Bacillus thuringiensis* (for anthrax) and lectin from soybean (for ricin) are biologically or chemically (respectively) similar to the specified targets. Solutions of these were prepared in DI water at concentrations ten times greater than the vendor-stated LOD for the specified targets and analyzed to evaluate cross-reactivity interference.

Three replicates of each PT sample were analyzed except for the method detection limit sample for which seven replicates were analyzed. The results provided information about how well the QTL Biosensor detected the presence of each contaminant at several concentration levels, the consistency of its responses, and its susceptibility to interferents.

3.1.2 Drinking Water Samples

The DW samples were collected from four geographically distributed municipal sources (Table 3-3). These samples were unique in terms of their source, treatment, and disinfection process. All collected samples were finished DW either ready for the distribution system or from within the distribution system.

Approximately 175 liters (L) of each of the DW samples were collected in pre-cleaned low-density polyethylene containers. One hundred twenty-five liters of each DW sample were shipped to the Metropolitan Water District of Southern California and dechlorinated with sodium thiosulfate. Out of this, 100L was concentrated using ultra-filtration techniques to a final volume of 250 mL. This concentration factor was selected because it is the goal of an EPA on-site ultra-filtration sample concentration method that is being developed to increase the concentration of insoluble microbiological species in a water sample so they may be detected by available detection technologies. Concentrated water samples were included in the test/QA plan due to stakeholder interest in this technique and because the large concentration factor could affect the amount of potential interferences in various types of water compared to testing only with unconcentrated water. Twenty-five liters of each water sample was shipped to ATEL for water

quality analysis. The remaining 25L of each sample was shipped to Battelle where the sample was dechlorinated with sodium thiosulfate. Each DW sample (unconcentrated and concentrated) was analyzed without adding any contaminant, as well as after fortification with individual contaminants at a single concentration level.

3.1.3 Quality Control Samples

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. A positive control cartridge provided by QTL Biosystems LLC was analyzed to ensure that the QTL Biosensor was operating properly. Analysis frequencies for the MB and positive control samples are discussed in Section 4.2.

3.2 Test Procedures

3.2.1 Laboratory Testing

Each day, fresh samples were prepared from standards or stock solutions in either DI water, an interferent matrix, or a DW matrix. Each sample was prepared in its own container and labeled with a sample identification number that was recorded on a data sheet. To test a liquid sample for the presence of anthrax or ricin, the procedure described below was used.

The QTL Biosensor case was opened, plugged into a power source, and turned on. Test cartridges (specific to either anthrax or ricin) were provided in individually sealed foil bags that contained one test cartridge, a small sample syringe, a large wash syringe, and a wet sampling tube. The QTL Biosensor was set for analyzing samples following the Biosensor's Sample Wizard, which prompts the operator through the sample analysis using on-screen instructions, by pressing the button labeled "SMPL". The foil bag was opened and the test cartridge, which must be used immediately after opening the bag, was removed. For each individual sample analyzed, approximately 300 µL of sample were drawn into the small sample syringe and then added to the wet sampling tube which was then capped and manually shaken for 15 seconds. After shaking, approximately 150 µL of this solution were withdrawn from the wet sampling tube back into the small sampling syringe. Then the large wash syringe and the small sample syringe were seated firmly into the test cartridge without pressing either plunger. While holding the test cartridge at eye-level, the small sample syringe plunger was pressed slowly and consistently until liquid filled the cartridge detection chamber, being careful not to overfill the chamber. The "NEXT" button then the "START" button were pressed. This initiated a 60 second countdown timer. During this 60 seconds, the test cartridge with syringes in place was shaken up and down. After the 60 second shaking period, the "NEXT" button was pressed and the test cartridge was placed into its port in the Biosensor. The "NEXT" button was pressed again starting a three minute countdown during which the cartridge was left undisturbed in the Biosensor. After the three minute period, the large wash syringe plunger was pressed completely in a slow uniform manner. The "NEXT" button on the Biosensor was pressed to activate the detection process. Results were displayed as a mV and %FS reading. For this verification test, mV values were manually recorded and used for data interpretation which is described in Section 5.1. The Biosensor also has red/green warning lights that could be set to a pre-determined mV

threshold reading to indicate a positive/negative response; however, this feature was not used in verification testing.

3.2.2 Non-Laboratory Testing

Because of the toxic nature of ricin and anthrax, only MB samples and the vendor-provided positive control cartridges were analyzed at a non-laboratory location in order to evaluate field portability. The non-laboratory location was a well-lit shipping/receiving area where there was a power source. The temperature and relative humidity were ambient (20 +/- 2 ºC and 40-50%, respectively). Both a trained technician and a non-technical, untrained first-time user performed analyses at the non-laboratory location.

3.2.3 Drinking Water Characterization

An aliquot of each DW sample, collected as described in Section 3.1.2, was sent to ATEL to characterize the water samples based on the water quality parameters shown in Table 3-4. The table lists the methods used as well as the characterization data for the four water samples collected as part of this verification test. Water quality parameters were characterized upon sampling in June 2005, while the QTL Biosensor was tested with DW in November and December 2005. The time delay between collection and testing was due to the fact that the water samples were collected for use during a separate ETV test conducted prior to this one. Because of this, an aliquot of each DW was tested by ATEL again in January 2006 to verify some of the parameters with the most potential to change over time. Note that dissolved organic carbon was not retested as this result was verified by the total organic carbon results, additionally the total organic halides and calcium and magnesium were not verified as there was no reason to expect a change in these parameters. The concentrations of most water quality parameters were similar; however, there was a decrease in levels of volatile compounds such as trihalomethanes and haloacetic acids over this time-period.

Table 3-4. Water Quality Characterization of Drinking Water Samples

Parameter	Method	Columbus, Ohio		Metropolitan Water District of Southern California		New York City, New York		Orlando, Florida	
		2005	2006	2005	2006	2005	2006	2005	2006
Alkalinity (mg/L)	SM 2320 B[2]	40	44	71	97	14	12	142	125
Specific conductivity (µmho)	SM 2510 B[2]	572	602	807	812	84	78	322	325
Hardness (mg/L)	EPA 130.2[3]	118	107	192	182	20	26	143	130
pH	EPA 150.1[3]	7.6	7.4	8.0	7.9	6.9	6.8	8.5	7.6
Total haloacetic acids (µg/L)	EPA 552.2[5]	32.8	<6.0	17.4	<6.0	39.0	<6.0	34.6	<6.0
Total organic carbon (mg/L)	SM 5310 B[2]	2.1	2.3	2.5	2.7	1.6	4.1	1.7	2.1
Dissolved organic carbon (mg/L)	SM 5310 B[2]	2.1	NA	2.9	NA	1.1	NA	1.6	NA
Total organic halides (µg/L)	SM 5320B[2]	220	NA	170	NA	82	NA	300	NA
Total trihalomethanes (µg/L)	EPA 524.2[4]	74.9	16.6	39.2	24.1	39.0	23.1	56.4	41.8
Turbidity (NTU)	SM 2130 B [7]	0.1	0.6	0.1	0.2	1.1	1.3	0.5	0.1
Calcium (mg/L)	EPA 200.7 [6]	33	NA	45	NA	5.6	NA	8.8	NA
Magnesium (mg/L)	EPA 200.7 [6]	7.7	NA	20	NA	1.3	NA	43	NA

NTU = nephelometric turbidity unit
NA = not retested

Chapter 4
Quality Assurance Quality Control

Quality assurance/quality control (QC) procedures were performed in accordance with the quality management plan (QMP) for the AMS Center[8] and the test/QA plan[1] for this verification test.

4.1 Quality Control of Stock Solution Confirmation Methods

4.1.1 Anthrax testing solutions

Solutions of Ames strain *Bacillus anthracis* prepared at Battelle from a USAMRIID stock was used for testing. The concentrations of the anthrax testing solutions were confirmed by a plate enumeration method (Battelle Standard Operating Procedure MREF X-054, *Enumeration of BL-2 and BL-3 Bacteria Samples Via the Spread Plate Technique*) within 24 hours of any solution being analyzed. As an example, for a 10^9 spores/mL sample to be enumerated, the method requires that the sample be diluted to at least 10^3 spores/mL so 100 microliters (µL) of sample would provide a countable number of spores on a culture plate. The number of counted colony forming units multiplied by the dilution factor results in the enumeration confirmation concentration. It should be noted that while all units for anthrax are listed as spores/mL in this verification report, colony forming units are counted during plate enumeration. Because of colony clumping, this could cause the reference results (in spores/mL) to be slightly overestimated.

$$PD(\%) = \frac{|E - A|}{E} \times 100$$

Table 4-1 provides the results of all plate enumerations performed throughout the verification test on anthrax solutions prepared in DI water. The expected concentration, the actual concentration, and the percent difference (PD) between the two are given in the table. PD is determined using the following equation, where *E* is the expected concentration and *A* is the actual concentration as determined by the enumeration.

With PD values all less than 24%, the enumeration data confirm that the PT samples containing the Battelle-prepared spores from USAMRIID stock were prepared accurately at various concentration levels.

Table 4-1. Anthrax Enumeration Data for PT Samples

	Date	Spore Solution Description	Expected Concentration (spores/mL)	Actual Concentration (spores/mL) [(a)]	PD (%)
Battelle-prepared solution	11/7/05	stock	1×10^9	0.95×10^9	5
	11/8/05	50 x LOD	5×10^6	5.2×10^6	3
	11/8/05	10 x LOD	1×10^6	0.82×10^6	18
	11/8/05	5 x LOD	5×10^5	5.2×10^5	4
	11/8/05	LOD	1×10^5	0.87×10^5	13
	11/8/05	LD	200	247	24
	12/5/05	10 x LOD	1×10^6	0.83×10^6	17

LOD= vendor-stated limit of detection.
LD = lethal dose concentration.
[(a)] The value reported is the average of three plates developed for each enumeration. The uncertainty about the average is approximately 8%.

Table 4-2 gives the enumeration data for all of the interferent PT and DW samples that were spiked with anthrax spores. Interferent and DW samples had similar percent differences between expected and actual concentration as compared to the contaminant PT samples, with the exception of the 250 mg/L calcium and magnesium solution (PD = 59%). In the Chapter 6 tables, only the expected concentration of the test samples is given along with the results from the QTL Biosensor. No correction for the enumeration data was performed since the QTL Biosensor test is qualitative and not quantitative.

The stock solution containing *Bacillus thuringiensis* (analogue of anthrax) was confirmed by the same enumeration method used for anthrax. The expected concentration was 1×10^7 spores/mL and the actual concentration was 4.93×10^7 spores/mL. Based on this, the stock solution was diluted to create a 1×10^7 spores/mL solution, which was then diluted by a factor of ten to make the testing solution of 1×10^6 spores/mL.

4.1.2 Ricin testing solutions

The COA for ricin was provided by the supplier. Because standard reference methods for the determination of ricin do not exist, the concentration of ricin was not independently confirmed. The COA stated that the ricin standard (Vector Laboratories, Burlingame, CA) had a concentration of 5.0 mg/mL. Test samples containing these contaminants were prepared by diluting aliquots of this stock solution with DI water. All records pertaining to stock solution dilutions were reviewed as part of the technical systems audit (TSA).

Table 4-2. Anthrax Enumeration Results for Fortified Interferent and Drinking Water Samples

Test Solution	Sample Description	Date	Expected Concentration (spores/mL)	Actual Concentration[(a)] (spores/mL)	PD (%)
Interferent	0.5 mg/L each humic/fulvic acids	11/11/05	1×10^6	0.85×10^6	15
	2.5 mg/L each humic/fulvic acids	11/11/05	1×10^6	0.71×10^6	29
	50 mg/L each Ca/Mg	11/11/05	1×10^6	0.69×10^6	31
	250 mg/L each Ca/Mg	11/11/05	1×10^6	0.41×10^6	59
DW	Conc. CA DW	11/16/05	1×10^6	0.78×10^6	22
	Unconc. CA DW	11/16/05	1×10^6	0.71×10^6	29
	Conc. OH DW	11/16/05	1×10^6	0.76×10^6	24
	Unconc. OH DW	11/16/05	1×10^6	0.71×10^6	29
	Conc. NY DW	11/16/05	1×10^6	0.83×10^6	17
	Unconc. NY DW	11/16/05	1×10^6	0.91×10^6	9
	Conc. FL DW	11/16/05	1×10^6	0.93×10^6	7
	Unconc. FL DW	11/16/05	1×10^6	0.92×10^6	8

[(a)] The value reported is the average of three plates developed for each enumeration. The uncertainty about the average is approximately 8%.

4.1.3 Interferent solutions

For the interferent samples, the concentration of calcium and magnesium was confirmed by EPA Method 200.7.[(6)]

4.2 Quality Control of Drinking Water Samples

A method blank sample consisting of DI water was analyzed once for approximately every 12 water samples analyzed for anthrax and once for approximately every 11 samples analyzed for ricin for a frequency of approximately 9% for both anthrax and ricin. A positive control sample was also analyzed once for approximately every 12 water samples for anthrax and once for approximately every 16 samples for ricin for frequencies of approximately 9% for anthrax and 6% for ricin. While performance limits were not placed on the results of the positive control sample, the vendor informed Battelle that, if the positive control samples did not cause a significantly higher millivolt (mV) reading than the method blank, it would indicate to the operator that the system was not functioning properly.

4.3 Technical Systems Audit

The Battelle Quality Manager conducted a TSA to ensure that the verification test was performed in accordance with the test/QA plan[1] and the AMS Center QMP.[8] As part of the audit, the Battelle Quality Manager reviewed the standards and methods used, compared actual test procedures with those specified in the test/QA plan,[1] and reviewed data acquisition and handling procedures. Observations and findings from this audit were documented and submitted to the Battelle Verification Test Coordinator for response. No findings were documented that required any significant action. The records concerning the TSA are permanently stored with the Battelle Quality Manager.

4.4 Audit of Data Quality

At least 10% of the data acquired during the verification test was audited. Battelle's Quality Manager or designee traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting, to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

4.5 QA/QC Reporting

Each internal assessment and audit was documented in accordance with Sections 3.3.4 and 3.3.5 of the QMP for the ETV AMS Center.[8] Once the assessment report was prepared, the Battelle Verification Test Coordinator responded to each potential problem and implemented any necessary follow-up corrective action. The Battelle Quality Manager ensured that follow-up corrective action was taken. The results of the TSA were sent to the EPA.

4.6 Data Review

Records generated in the verification test were reviewed before they were used to calculate, evaluate, or report verification results. Table 4-3 summarizes the types of data recorded. The review was performed by a technical staff member involved in the verification test, but not the staff member who originally generated the record. The person performing the review added his/her initials and the date to a hard copy of the record being reviewed.

Table 4-3. Summary of Data Recording Process

Data to Be Recorded	Responsible Party	Where Recorded	How Often Recorded	Disposition of Data
Dates and times of test events	Battelle	ETV data sheets	Start/end of test, and at each change of a test parameter	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Sample collection and preparation information, including chain-of-custody	Battelle and Water Utilities providing DW samples	ETV data sheets, laboratory record books and/or chain-of-custody forms	At time of sample collection and preparation	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Biosensor procedures and sample results	Battelle	ETV data sheets	Throughout test duration	Manually incorporated in data spreadsheets
Anthrax enumeration data	Battelle	Enumeration data forms	With every enumeration	Manually incorporated in data spreadsheets
Reference method procedures and sample results	ATEL	Data acquisition system, as appropriate	Throughout sample analysis process	Transferred to spreadsheets and reported to Battelle

Chapter 5
Statistical Methods and Reported Parameters

The methods presented in this chapter were used to verify the performance parameters listed in Chapter 3. The QTL Biosensor produces qualitative results; i.e., the Biosensor indicates only the presence or absence of the contaminant and does not measure the concentration present. Therefore, the data evaluation methods were applied in that context.

5.1 Qualitative Contaminant Presence/Absence

Contaminant presence/absence was assessed by comparing the mV reading for the contaminant-only PT solutions with the mV threshold determined during the Method Detection Limit (MDL) evaluation described in Section 5.4. Note that both the contaminant-only samples and MDL samples were analyzed at the same time and all data were interpreted at the conclusion of all analyses. Samples generating results above the MDL mV threshold level (determined to be 65 mV for anthrax and 76 mV for ricin) were considered positive. The QTL Biosensor features a red/green light that is set at a factory determined mV level to switch from green (no contaminant present) to red (contaminant present). The red/green light feature was not evaluated during verification testing and instead the MDL determined mV threshold was used.

5.2 False Positive/Negative Responses

A result was considered a false positive when a DI water or DW sample was spiked with a potential interferent, a cross-reactive compound, or not spiked at all and a response above the MDL mV threshold level was obtained. A result was considered a false negative when any DW or interferent sample was spiked with a contaminant at 10 times the vendor stated limit of detection and produced a response below the MDL mV threshold level. Interferent PT samples, cross-reactivity PT samples, and DW samples were included in the analysis. The number of false positive and negative results is reported.

5.3 Consistency

The reproducibility of the results was assessed by calculating the percentage of individual test samples within a set (i.e., within a single concentration level or type of interferent) that produced positive or negative results without variation among replicates.

5.4 Method Detection Limit

The MDL for each contaminant was determined according to procedures described in 40CFR136 Appendix B[9] and was assessed from seven replicate analyses of a fortified sample with the target contaminant concentration approximately five times the vendor-stated LOD. The MDL was calculated using the following equation:

$$MDL = t \times S$$

Where t is the Student's value of 3.143 for a 99% confidence level when the degrees of freedom (N-1) equals six, and S is the standard deviation of the replicate samples.

5.5 Other Performance Factors

Aspects of the QTL Biosensor performance such as ease of use, field portability, and sample throughput are discussed in Section 6. Also addressed are qualitative observations of the verification staff pertaining to the performance of the QTL Biosensor.

Chapter 6
Test Results

6.1 Qualitative Contaminant Presence/Absence

The responses for the QTL Biosensor using the contaminant-only PT samples containing anthrax and ricin are shown in Tables 6-1a and 6-1b and are discussed in the following sections.

6.1.1 Anthrax

The results obtained for the PT samples containing anthrax are given in Table 6-1a. The QTL Biosensor generated positive results (i.e., had a mV reading greater than the MDL mV threshold of 65mV) for concentrations of anthrax in all three replicates of solutions containing five times the vendor-stated LOD (5×10^5 spores/mL) and higher. At the vendor-stated LOD (1×10^5 spores/mL) only one of three replicates was considered positive. There were no positive results at the LD concentration of 200 spores/mL, which was not unexpected given that the LD concentration is considerably lower than the vendor-stated LOD.

6.1.2 Ricin

The results obtained for the PT samples containing ricin are given in Table 6-1b. The QTL Biosensor generated positive results (i.e., had a mV reading greater than the MDL mV threshold of 76 mV) for concentrations of ricin in all three replicates of solutions containing five times the vendor-stated LOD (0.25 mg/L) and higher. These positive results included the LD level for ricin of 15 mg/L. At the vendor-stated LOD (0.05 mg/L) none of the three replicates generated a response greater than the 76 mV MDL threshold, although two readings approached that level at 64 mV and 66 mV.

6.2 False Positive/Negative Responses

Three types of samples were analyzed to evaluate the susceptibility of the QTL Biosensor to false positive and negative results. These included interferent PT samples, consisting of DI water fortified with Ca and Mg or with humic and fulvic acids, both with and without the addition of target contaminants; cross-reactivity PT samples consisting of DI water fortified with a contaminant similar biologically or chemically with the target contaminant; and DW samples both concentrated and unconcentrated and both with and without the addition of target contaminants. In addition, a preservative blank containing sodium azide, which is used as a preservative in commercially available ricin, was analyzed with the ricin test cartridges to evaluate the potential for interference from the preservative. A false positive result was defined

as a result above the MDL threshold mV reading (determined to be 65 mV for anthrax and 76 mV for ricin) in the absence of the target contaminant and a false negative result was defined as a result below the MDL threshold mV reading from a DW or interferent sample containing the target contaminant at levels ten times the vendor-stated LOD.

Table 6-1a. Anthrax Contaminant-Only PT Sample Results-Contaminant Presence/Absence Evaluation

Testing Level	Concentration (spores/mL)	Result (mV)	No. of Positive Results[(a)]
LD	200	23	0
		18	
		17	
LOD	1×10^5	54	1
		45	
		67	
5 x LOD	5×10^5	124	3
		209	
		205	
10 x LOD	1×10^6	299	3
		380	
		540	
50 x LOD	5×10^6	1546	3
		1594	
		1512	

LD = Lethal dose concentration.
LOD = Vendor-stated limit of detection.
[(a)] Number of positive results (as indicated by a mV reading >65) out of three replicates.
Shaded areas indicate positive results.

Table 6-1b. Ricin Contaminant-Only PT Sample Results-Contaminant Presence/Absence Evaluation

Testing Level	Concentration (mg/L)	Result (mV)	No. of Positive Results[(a)]
LOD	0.05	64	0
		66	
		33	
5 x LOD	0.25	125	3
		175	
		245	
10 x LOD	0.5	199	3
		207	
		205	
50 x LOD	2.5	396	3
		682	
		466	
LD	15	954	3
		945	
		827	

LD = Lethal dose concentration.
LOD= Vendor-stated limit of detection.
[(a)] Number of positive results (as indicated by a mV reading >76) out of three replicates.
Shaded areas indicate positive results.

6.2.1 Interferent PT Samples

The results from the interferent PT samples are given in Table 6-2. The QTL Biosensor anthrax-specific cartridges generated false positive results from the PT samples containing calcium and magnesium, but no contaminant. The ricin-specific cartridges generated no false positive results in the presence of possible interferences, but no contaminant. For the anthrax–specific cartridges, there were no false negative results when contaminant was present in solutions containing possible interferences; however, for the ricin-specific cartridges there were false negative results for two out of three replicates in the presence of 250 mg/L each of calcium and magnesium. A preservative blank (0.16 mg/L sodium azide) was also analyzed with the ricin-specific test cartridge to assess possible interference from the preservative in the commercially available ricin. The preservative did not cause any false positive responses (average of three readings equaled 22 ± 7 mV).

6.2.2 DW Samples

Table 6-3 shows the results of testing with drinking water obtained from four different geographic locations, both unconcentrated and concentrated and both with and without contaminant.

For the unspiked, unconcentrated water samples, the QTL Biosensor anthrax-specific cartridge generated false positive results in at least one of three replicates in water from all locations except Ohio, for which there were no false positive results. With the anthrax-specific cartridge, false positive results were generated in at least two of three replicates in unspiked, concentrated water samples from each of the four locations. In the presence of anthrax, for the unconcentrated water samples from each location, there was a false negative result in one of three replicates in waters from California, Florida, and Ohio, but no false negative results in water from New York. For concentrated waters in the presence of contaminant there were no false negative results using the anthrax-specific cartridge.

The QTL Biosensor ricin-specific cartridge generated false positive results in one of three replicates for both the unspiked, unconcentrated and unspiked, concentrated water samples from Florida. There were no other false positive results in either the unconcentrated or concentrated waters from the other locations. There were no false negative results in the presence of ricin for any of the waters, both concentrated and unconcentrated from all locations.

Because of the false positive results obtained in the presence of calcium and magnesium and in various types of DW samples, the vendor has subsequently undertaken an evaluation of the effect of calcium and magnesium on their technology performance. The vendor has determined that calcium interferes with the reagents used in their technology more than magnesium and is working to incorporate a high affinity binder of cations (such as calcium and magnesium) into their sample buffer lyophilates to mitigate this problem. Any improvement based on the addition of a high affinity binder has not been verified through the ETV program.

Table 6-2. Interferent PT Sample Results- False Positive/Negative Evaluation

	Anthrax			Ricin		
Interferent	**Anthrax (spores/mL)**	**Result (mV)**	**No. of Positive Results[(a)]**	**Ricin (mg/L)**	**Result (mV)**	**No. of Positive Results[(a)]**
0.5 mg/L each humic and fulvic acids	none	20	0	none	25	0
		22			18	
		19			20	
	1×10^6	394	3	0.5	474	3
		398			294	
		250			305	
2.5 mg/L each humic and fulvic acids	none	35	0	none	35	0
		18			47	
		19			27	
	1×10^6	365	3	0.5	424	3
		299			586	
		270			365	
50 mg/L each Ca and Mg	none	394	3	none	27	0
		268			22	
		368			23	
	1×10^6	255	3	0.5	246	3
		278			406	
		446			515	
250 mg/L each Ca and Mg	none	932	3	none	40	0
		639			40	
		280			20	
	1×10^6	697	3	0.5	35	1
		609			59	
		882			88	

[(a)] Number of positive results out of three replicates as indicated by a mV reading >65 for anthrax and >76 for ricin. Shaded areas indicate positive results.

Table 6-3. DW Sample Results-False Positive/Negative Evaluation

	Anthrax			Ricin		
DW Sample	Anthrax (spores/mL)	Result (mV)	No. of Positive Results[(a)]	Ricin (mg/L)	Result (mV)	No. of Positive Results[(a)]
California	none	644	3	none	16	0
		541			16	
		131			15	
	1×10^6	807	2	0.5	317	3
		62			229	
		1186			271	
California-Concentrated	none	620	3	none	21	0
		1126			20	
		850			14	
	1×10^6	627	3	0.5	313	3
		558			253	
		272			196	
Florida	none	48	2	none	117	1
		68			53	
		1088			25	
	1×10^6	62	2	0.5	384	3
		350			281	
		924			174	
Florida-Concentrated	none	269	3	none	86	1
		227			18	
		397			19	
	1×10^6	1394	3	0.5	406	3
		588			218	
		432			404	

[(a)] Number of positive results out of three replicates as indicated by a mV reading >65 for anthrax and >76 for ricin. Shaded areas indicate positive results.

Table 6-3. DW Sample Results- False Positive/Negative Evaluation (continued)

	Anthrax			Ricin		
DW Sample	Anthrax (spores/mL)	Result (mV)	No. of Positive Results[(a)]	Ricin (mg/L)	Result (mV)	No. of Positive Results[(a)]
New York	none	38	1	none	27	0
		123			26	
		31			24	
	1×10^6	114	3	0.5	261	3
		93			163	
		149			194	
New York-Concentrated	none	40	2	none	24	0
		76			20	
		157			30	
	1×10^6	78	3	0.5	162	3
		110			216	
		143			242	
Ohio	none	28	0	none	17	0
		41			20	
		48			17	
	1×10^6	56	2	0.5	218	3
		164			223	
		79			285	
Ohio-Concentrated	none	41	2	none	18	0
		141			22	
		341			25	
	1×10^6	140	3	0.5	232	3
		302			291	
		249			309	

[(a)] Number of positive results out of three replicates as indicated by a mV reading >65 for anthrax and >76 for ricin. Shaded areas indicate positive results.

6.2.3 Cross-Reactivity PT Samples

The results from the cross-reactivity PT samples are given in Table 6-4. A PT sample fortified with a spore or chemical similar to each target contaminant was analyzed in the absence of any of the target contaminant. The mV readings obtained for each cross reactivity compound are given for each sample. No false positive results were obtained with the potentially cross-reactive compounds tested.

Table 6-4. Potentially Cross-Reactive PT Sample Results

Cross-Reactivity Compound	Result (mV)	No. of Positive Results[(a)]
Anthrax: *Bacillus thuringiensis* (1×10^6 spores/mL)	22	0
	24	
	20	
Ricin: Lectin from soybean (0.5 mg/L)	23	0
	26	
	23	

[(a)] Number of positive results out of three replicates as indicated by a mV reading >65 for anthrax and >76 for ricin.

6.3 Consistency

Using the QTL Biosensor and the anthrax-specific cartridge, results were consistent (i.e., produced positive or negative results without variation among replicates) in 21 out of 29 sets of replicates or 72%. Using the ricin-specific cartridge, results were consistent in 26 out of 29 sets of replicates or 90%. Replicates included in the consistency calculation are the contaminant-only PT samples, the interferent PT samples and the DW samples.

6.4 Method Detection Limit

The MDL for each contaminant was determined as described in Section 5.4. Note that the contaminant, interferent, DW and MDL samples were analyzed at the same time and MDL threshold values were determined afterwards, then applied to the sample data to interpret results. Results are presented in Table 6-5. A mV reading of 65 was determined as the MDL threshold value for anthrax and a mV reading of 76 was determined as the MDL threshold value for ricin. Based on the data presented in tables 6-1a and 6-1b, these mV MDL levels correspond to contaminant concentrations in between the LOD and 5 x LOD concentrations tested.

Table 6-5. Method Detection Limit

Analyte	Concentration	Testing Level	Result (mV)	Average Result (mV)	Standard Deviation (mV)	MDL (mV)
Anthrax	5×10^5 spores/mL	5 x LOD	168	169	21	65
			192			
			127			
			181			
			166			
			167			
			181			
Ricin	0.25 mg/L	5 x LOD	125	143	24	76
			178			
			175			
			135			
			143			
			117			
			129			

LOD = vendor-stated limit of detection

6.5 Other Performance Factors

6.5.1 Ease of Use

The QTL Biosensor came with clearly written and informative instructions as well as a demonstration video disc. Contents of the test cartridge kits were clearly labeled and storage requirements were readily available. Overall, all packaging was easy to open, even when the verification testing staff were outfitted with double-gloves.

The test cartridges come in packages containing everything needed to prepare a sample for analysis with the QTL Biosensor. The cartridges were required to be stored between 2-8º C for long term storage, but could be held at room temperature for up to six months. The test cartridges came ready-to-use and required no preparation. The QTL Biosensor software was easy to use and included a software feature called the "Sample Wizard" which prompted the operator through the sample analyses through on-screen instructions. Use of the Sample Wizard is optional and samples could be processed without the display prompts. The surfaces of the QTL Biosensor were easily wiped clean. Two syringes, a sample prep tube, and a test cartridge were generated as waste with each analysis.

As tested in this study, mV readings were obtained and manually recorded; however, the QTL Biosensor can be set with a mV threshold that trips an indicator light from green, which indicates

a response below the set mV threshold, to red, indicating a result exceeding the mV threshold. The indicator light feature was not evaluated during this study.

No formal scientific education would be required for using the QTL Biosensor and cartridges, but general good laboratory skills are helpful. Because the kit is intended for evaluating biological and chemical agents, users should know and understand the procedures for safely working with or near these agents before using this product. The verification testing staff were able to conduct tests with the kit after a two hour training session. Additionally, a non-technical operator who had not received training from the vendor, and who relied solely on the instruction manual and training disc was able to successfully operate the QTL Biosensor. QTL technical support contact information, including phone, e-mail, fax and website addresses are included in the instruction manual for easy access to the vendor's contact information.

6.5.2 Field Portability

Field portability testing took place in a non-laboratory location with analyses performed by both a trained operator and a non-technical, untrained first-time user. Field portability testing was accomplished by transporting the QTL Biosensor to a well lit shipping/receiving area. The temperature and relative humidity were ambient (20 +/- 2 ºC and 40-50%, respectively). The equipment was light-weight and easy for one person to transport. A cooler was used to transport the test cartridges to keep them between 2-8ºC prior to use. Once at the field testing location, the equipment was set up within minutes. All reagents are pre-packaged and ready-to-use as provided in the kit allowing a sample to be prepared and analyzed within five minutes of set-up. In addition to the cooler used to transport test cartridges, testing staff needed to provide something on which to record data and a waste container; otherwise, all other supplies were provided with the QTL Biosensor and the test cartridge kit.

At this non-laboratory location, the QTL Biosensor was operated using its self-contained rechargeable nickel-metal hybrid battery pack. For long-term field deployment, a power source would be needed to keep the QTL Biosensor battery charged. Additionally, for short-term use a cooler is sufficient for transporting the test cartridges to keep them between 2-8ºC; however, for long-term field deployment a refrigerator would be useful to keep the test cartridges cold.

The technology was field tested with a method blank and the vendor-provided positive control cartridge. Results in the field were similar to results obtained in the laboratory with the method blank result well below and the positive control result well above the MDL mV threshold.

6.5.3 Throughput

Approximately 12 sample analyses were completed per hour. Each test cartridge is single-use.

Chapter 7
Performance Summary

Table 7-1. 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.				

Shading indicates results for unspiked sample.

Table 7-1. Anthrax Summary Table (Continued)

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 test cartridges should be at 2-8º C, 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.

Table 7-2. 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 test cartridges should be at 2-8º C 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.

Chapter 8
References

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