September 2006

Environmental Technology Verification Report

BioVeris
BioVerify™ Botulinum Toxin A and Ricin Test Kits and M-SERIES® M1M Analyzer

Prepared by

Battelle
The Business of Innovation

Under a cooperative agreement with

U.S. Environmental Protection Agency

ETV ETV ETV

THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

ETV Joint Verification Statement

TECHNOLOGY TYPE:	**IMMUNOASSAY TEST KITS**
APPLICATION:	**DETECTING BOTULINUM TOXIN A AND RICIN**
TECHNOLOGY NAME:	**BioVerify™ Botulinum Toxin A and Ricin Test Kits and M-SERIES® M1M Analyzer**
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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 botulinum toxin A and ricin in water. This verification statement provides a summary of the test results for BioVerify™ Botulinum Toxin A and Ricin Test Kits using the M-SERIES® M1M analyzer.

VERIFICATION TEST DESCRIPTION

The verification test for the BioVerify™ Botulinum Toxin A and Ricin Test Kits using the M-SERIES® M1M analyzer was conducted at Battelle between December 2005 and August 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; lowest detectable concentration; field portability; ease of use; and sample throughput. The ability of the BioVerify™ Botulinum Toxin A and Ricin Test Kits to detect various concentrations of botulinum toxin A and ricin using the M-SERIES® M1M analyzer 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 BioVerify™ test kits to lipopolysaccharide, a potentially cross-reactive compound for botulinum toxin A, and lectin from soybean, a potentially cross-reactive compound for ricin. PT samples were analyzed in triplicate (with the exception of DI water fortified with target analytes at five times the vendor-stated LOD, for which ten 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 with and without the addition of botulinum toxin A and ricin at a concentration of 10 times the vendor-stated LOD. In addition to the PT and DW samples analyzed, method blank 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 the BioVerify™ Test Kits and M-SERIES® M1M analyzer was provided by the vendor and was not verified in this test.

BioVerify™ Test Kits detect biological agents such as bacteria, viruses, and toxins in various matrices, including food and environmental samples. The test kits use proprietary BioVeris Technology™ based on a process that uses labels designed to emit light when electrochemically stimulated. The tests use two antibodies specific for the antigen of interest in a single-tube lyophilized reagent format. One antibody is immobilized on paramagnetic microparticles, and the other is labeled with BioVeris' BV-TAG™ label. When the antigen of interest is present in the sample, both antibodies bind to the antigen, effectively linking the microparticle, the antigen, and the BV-TAG™ label. The electrode stimulates the BV-TAG™ labels bound (via the antibodies and antigen) to the microparticles, and the emitted light is measured. If the antigen of interest is not present in the sample, the microparticle and the label are not linked, and no signal is generated.

Sample analysis tubes are arranged in a 96-well format, and tests tubes containing reagents for specific target analytes are color coded for the operator's convenience. The analyzer provides real time data acquisition using preset test protocols and includes both audible and visual warnings in the event a positive sample is encountered. The system allows storage and retrieval of all plate, sample, and quality control data in Microsoft® Excel format. All reagent information is entered into the system through a bar code, and reagent usage is monitored electronically.

The analyzer includes an internal shaker and pipetting capability so that once a sample is added to a tube containing lyophilized reagents and loaded into the analyzer; no further user intervention is required. The analyzer is 38 centimeters (cm) (14.8 inches) wide, 30 cm high (11.7 inches), and 38 cm (14.8 inches) deep and weighs 16 kilograms (35 pounds). The analyzer and computer are contained within an instrument transport case with dimensions of 65 cm (25.5 inches) by 61 cm (24.1 inches) by 51 cm (20 inches). The total weight of the analyzer, computer, transport case, and accessories is 36.4 kilograms (80 pounds). The analyzer requires a power source or use of a battery backup.

Required reagents as well as waste are contained in a second transport case to segregate liquids and electronics during transport. The transport case dimensions are 48 cm (19 inches) by 38 cm (14.9 inches) by 35 cm (13.7 inches). The transport case, including reagents and liquid waste, weighs 9.5 kilograms (20.9 pounds). A BioVerify™ test kit containing 96 tests and the controls to run them is $1,440. The M-SERIES® M1M analyzer is $69,500. Additional materials that may be purchased include BV-GLO™ Plus ($148 per bottle), BV-CLEAN™ Plus ($148 per bottle), BV-STORE™ ($100 per bottle), BV-DILUENT™ ($100 per bottle), and BV-SANITIZE™ ($690 for eight single-use bottles for decontaminating the instrument system).

VERIFICATION OF PERFORMANCE

The tables below summarize the performance of the BioVerify™ test kits using the M-SERIES® M1M analyzer in detecting botulinum toxin A and ricin, respectively.

Botulinum Toxin A Summary Table

Parameter	Sample Information	Botulinum Toxin A Concentration (mg/L)		No. of Positive Results[(a)]	
Contaminant-only PT samples	DI water	0.00005 (vendor-stated limit of detection)		0	
		0.00025		0	
		0.0005		3	
		0.0025		3	
		0.3 (lethal dose)		3	
Interferent PT samples	0.5 milligrams per liter (mg/L) humic and fulvic	unspiked	0.0005	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	0
DW samples	Unconcentrated CA	unspiked	0.0005	0	3
	Concentrated CA			0	3
	Unconcentrated FL			0	0
	Concentrated FL			0	3
	Unconcentrated NY			0	3
	Concentrated NY			0	3
	Unconcentrated OH			0	3
	Concentrated OH			0	3
Cross-reactivity	0.5 mg/L lipopolysaccharide	unspiked		0	
False positives	There were no false positive results.				
False negatives	False negatives were observed in the presence of 250 mg/L Ca and Mg and in the unconcentrated FL drinking water samples.				
Consistency	Results were consistent (i.e., produced positive or negative results without variation among replicates) in 29 out of 29 sets of replicates or 100%.				
Lowest detectable concentration	The lowest concentration where at least two-thirds of the replicates generated a positive response was 0.0005 mg/L.				
Other performance factors	Test kits require storage at 2-8º C. Analyzer software requires training. The M-SERIES® M1M analyzer uses electricity or battery backup and includes a rugged carrying case. Analyzer console weighs approximately 80 pounds. Test kits and analyzer were used inside and outside a laboratory by a trained operator; one 96 tube sample set can be processed in approximately two hours, provided the analyzer is primed and system diagnostics have already been performed.				

[(a)] Number of positive results out of three replicates, except for the 0.00025 mg/L contaminant-only PT sample which is out of 10 replicates.

Shading indicates results for unspiked sample.

Ricin Summary Table

Parameter	Sample Information	Ricin Concentration (mg/L)		No. of Positive Results[(a)]	
Contaminant-only PT samples	DI water	0.00005 (vendor-stated limit of detection)		0	
		0.00025		6	
		0.0005		3	
		0.0025		3	
		15 (lethal dose)		3	
Interferent PT samples	0.5 mg/L humic and fulvic	unspiked	0.0005	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	0
DW samples	Unconcentrated CA	unspiked	0.0005	0	3
	Concentrated CA			0	3
	Unconcentrated FL			0	3
	Concentrated FL			0	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	There were no false positive results.				
False negatives	False negatives were observed only in the 250 mg/L Ca and Mg sample.				
Consistency	Results were consistent (i.e., produced positive or negative results without variation among replicates) in 28 out of 29 sets of replicates or 97%.				
Lowest detectable concentration	The lowest concentration where at least two-thirds of the replicates generated a positive response was 0.0005 mg/L, although the 0.00025 mg/L concentration was detected in 6 out of 10 replicates.				
Other performance factors	Test kits require storage at 2-8º C. Analyzer software requires training. The M-SERIES® M1M analyzer uses electricity or battery backup and includes a rugged carrying case. Analyzer console weighs approximately 80 pounds. Test kits and analyzer were used inside and outside a laboratory by a trained operator; one 96 tube sample set can be processed in approximately two hours, provided the analyzer is primed and system diagnostics have already been performed.				

[(a)] Number of positive results out of three replicates, except for the 0.00025 mg/L contaminant-only PT sample which is out of 10 replicates.

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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Vice President	Director
Energy, Transportation, and Environment Division	National Homeland Security Research Center
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ETV Advanced Monitoring Systems Center

BioVeris
BioVerify™ Botulinum Toxin A and RicinTest Kits
and
M-SERIES® M1M Analyzer

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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.

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List of Abbreviations

AMS	Advanced Monitoring Systems
ATEL	Aqua Tech Environmental Laboratories, Inc.
Ca	calcium
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
Mg	magnesium
mg/L	milligram per liter
µL	microliter
mL	milliliter
PT	performance test
QA	quality assurance
QC	quality control
QMP	quality management plan
TSA	technical systems audit

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 BioVeris BioVerify™ Botulinum Toxin A and Ricin Test Kits using the BioVeris M-SERIES® M1M analyzer. 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 BioVerify™ Botulinum Toxin A and Ricin Test Kits using the M-SERIES® M1M analyzer. The M-SERIES® M1M analyzer is shown in Figure 2-1. Following is a description of the system, based on information provided by the vendor. The information provided below was not subjected to verification in this test.

BioVerify™ Test Kits detect biological agents such as bacteria, viruses, and toxins in various matrices, including food and environmental samples. The test kits use proprietary BioVeris Technology™ based on a process that uses labels designed to emit light when electrochemically stimulated. The tests use two antibodies specific for the antigen of interest in a single-tube lyophilized reagent format. One antibody is immobilized on paramagnetic microparticles, and the other is labeled with BioVeris' BV-TAG™ label. When the antigen of interest is present in the sample, both antibodies bind to the antigen, effectively linking the microparticle, the antigen, and the BV-TAG™ label. The electrode stimulates the BV-TAG™ labels bound (via the antibodies and antigen) to the microparticles, and the emitted light is measured. If the antigen of interest is not present in the sample, the microparticle and the label are not linked, and no signal is generated.

Figure 2-1 BioVeris M-SERIES® M1M Analyzer

Sample analysis tubes are arranged in a 96-well format, and tests tubes containing reagents for specific target analytes are color coded for the operator's convenience. The analyzer provides real time data acquisition using preset test protocols and includes both audible and visual warnings in the event a positive sample is encountered. The system allows storage and retrieval of all plate, sample, and quality control data in Microsoft® Excel format. All reagent information is entered into the system through a bar code, and reagent usage is monitored electronically.

The analyzer includes an internal shaker and pipetting capability so that once a sample is added to a tube containing lyophilized reagents and loaded into the analyzer; no further user intervention is required. The analyzer is 38 centimeters (cm) (14.8 inches) wide, 30 cm high (11.7 inches), and 38 cm (14.8 inches) deep and weighs 16 kilograms (35 pounds). The analyzer and computer are contained within an instrument transport case with dimensions of 65 cm (25.5 inches) by 61 cm (24.1 inches) by 51 cm (20 inches). The total weight of the analyzer, computer, transport case, and accessories is 36.4 kilograms (80 pounds). The analyzer requires a power source or use of a battery backup.

Required reagents as well as waste are contained in a second transport case to segregate liquids and electronics during transport. The transport case dimensions are 48 cm (19 inches) by 38 cm (14.9 inches) by 35 cm (13.7 inches). The transport case, including reagents and liquid waste, weighs 9.5 kilograms (20.9 pounds). A BioVerify™ test kit containing 96 tests and the controls to run them is $1,440. The M-SERIES® M1M analyzer is $69,500. Additional materials that may be purchased include BV-GLO™ Plus ($148 per bottle), BV-CLEAN™ Plus ($148 per bottle), BV-STORE™ ($100 per bottle), BV-DILUENT™ ($100 per bottle), and BV-SANITIZE™ ($690 for eight single-use bottles for decontaminating the instrument system).

Chapter 3
Test Design

The objective of this verification test was to evaluate the ability of the BioVerify™ Botulinum Toxin A and Ricin Test Kits using the M-SERIES® M1M analyzer to detect specific biological toxins and agents in water samples and to determine whether the test kits are susceptible to interferents in drinking water (DW).

During this verification test, the BioVerify™ Botulinum Toxin A and Ricin Test Kits were subjected to various concentrations of their respective target analyte in American Society for Testing and Materials Type II deionized (DI) water and analyzed with the M-SERIES® M1M analyzer. 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 BioVerify™ Botulinum Toxin A and Ricin Test Kits also were 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 various solutions of interferences. One type of interference solution 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 BioVerify™ Botulinum Toxin A and Ricin Test Kits to a potentially cross-reactive compound for each target contaminant.

Table 3-1. Lethal Dose and Source of Contaminants

Contaminant	Vendor-Stated LOD	Lethal Dose Concentration[a]	Source of Contaminant
Botulinum toxin A	0.00005 milligrams/liter (mg/L)	0.3 mg/L	Metabiologics, Inc. (Madison, Wisconsin)
Ricinus communis Agglutinin II (ricin)	0.00005 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 BioVerify™ Botulinum Toxin A and Ricin Test Kits was conducted from December 2005 through August 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.

The BioVerify™ Botulinum Toxin A and Ricin Test Kits were evaluated for the following parameters:

- Contaminant presence/absence
- False positive/false negative response
  - Interferents
  - DW matrix effects
  - Cross-reactivity
- Consistency
- Lowest detectable concentration
- Other performance factors
  - Field portability
  - Ease of use by 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 BioVerify™ Botulinum Toxin A and Ricin Test Kits to individually detect various concentrations of botulinum toxin A and ricin was evaluated by analyzing performance test (PT) and DW samples. PT samples included DI water fortified with either the target contaminant, an interferent, both, or only a cross-reactive species. DW samples were analyzed using the BioVerify™ Botulinum Toxin A and Ricin Test Kits with and without the addition of each target contaminant.

Table 3-2. Performance Test Samples

Type of PT Sample	Sample Characteristics	Approximate Concentrations
Contaminant	Botulinum toxin A	0.00005 to 0.3 mg/L
	Ricin	0.00005 to 15 mg/L
Interferent	Contaminants in 50 mg/L Ca and 50 mg/L Mg	Botulinum toxin A- 0.0005 mg/L Ricin - 0.0005 mg/L
	Contaminants in 250 mg/L Ca and 250 mg/L Mg	Botulinum toxin A- 0.0005 mg/L Ricin - 0.0005 mg/L
	Contaminants in 0.5 mg/L humic acid and 0.5 mg/L fulvic acid	Botulinum toxin A- 0.0005 mg/L Ricin - 0.0005 mg/L
	Contaminants in 2.5 mg/L humic acid and 2.5 mg/L fulvic acids	Botulinum toxin A- 0.0005 mg/L Ricin - 0.0005 mg/L
Cross-reactive species	Lipopolysaccharide (botulinum toxin analogue)	0.5 mg/L
	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.**	**Botulinum Toxin A (mg/L)**	**Ricin (mg/L)**
Metropolitan Water District of Southern California (CA)	Filtered chloraminated	surface	both	unspiked and 0.0005	unspiked and 0.0005
New York City, New York (NY)	Unfiltered chlorinated	surface	both		
Columbus, Ohio (OH)	Filtered chlorinated	surface	both		
Orlando, Florida (FL)	Filtered chlorinated	ground	both		

3.1.1 Performance Test Samples

The contaminant-only PT samples (shown in Table 3-2) were prepared in DI water using certified standards of ricin or botulinum toxin A. Reference methods were not available for quantitative confirmation of botulinum toxin A or ricin test solutions so certificates of analysis

(COA) and QA oversight of solution preparation were used to determine their concentrations. All test samples were prepared from the standards or stock solutions on the day of analysis.

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 samples were analyzed both with and without the target contaminant and were used to evaluate whether interferences which are commonly found in water have the potential to cause false positive or negative results with the BioVerify™ Botulinum Toxin A and Ricin Test Kits. In addition, because the commercially available ricin contained sodium azide as a preservative, a preservative blank sample consisting of 0.16 mg/L sodium azide was prepared in DI water and processed with the ricin samples. This solution was prepared as for a concentrated stock solution, actual samples would contain lower concentrations because of the dilution of the stock to testing concentrations. However, if there was to be an interference effect, it would be more likely to occur at higher concentrations. 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 BioVerify™ Ricin Test Kits during testing.

The last type of PT sample was a cross-reactivity check sample to determine whether the test kits produced false positive results in response to similar analytes. Lectin from soybean (for ricin) and lipopolysaccharide (for botulinum toxin) are chemically or biologically similar to the specified targets. Solutions of these were prepared in DI water.

Three replicates of each PT sample were analyzed, except for the sample concentration five times greater than the vendor-stated LOD (0.00025 mg/L), for which a total of ten replicates were analyzed. The results provided information about how well the BioVerify™ Botulinum Toxin A and Ricin Test Kits 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, 100 L 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. Twenty-five liters of each water sample was shipped to ATEL for water quality analysis. The remaining 25 L 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. Positive controls included in the BioVerify™ Botulinum Toxin A and Ricin Test Kits were analyzed to verify that the M-SERIES® M1M analyzer was operating properly.

3.2 Test Procedure

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 botulinum toxin A or ricin the procedure described below was used. Test kits were specific to either botulinum toxin A or ricin.

Before starting, the M-SERIES® M1M Analyzer was put through system checks and diagnostics to ensure that sufficient volume of reagents were present in the analyzer, that the proper tubing connections were in place, and that the dispensing and shaker functions were performing properly. For each sample and control, test tubes, color coded based on the target analyte, were removed from the kit and placed in a 96-well tube holder. The first four test tubes in the tube holder of each analysis set are used as calibrators and are always reserved for two negative control samples followed by two positive control samples. For this study, the negative controls consisted of DI water. This was selected at the suggestion of the vendor. However, note that it is possible that analysis of other sources of water could cause matrix effects requiring that a different negative control (such as the water matrix being analyzed) be used as the negative control. The positive control is provided in the test kit and is prepared by adding 125 µL of DI water to the positive control tube included in the kit. The kit includes multiple positive control tubes as one is needed for each positive control sample to be analyzed (possibly several per sample set). DI water (100 µL) was added to the first two tubes in the tube holder (slated for negative controls) and to any other tubes intended for method blank samples. The reconsitituted positive control solution (100 µL) was added to the third and fourth tube in the tube holder (slated for positive controls) and to any other tubes intended for additional positive control samples. Finally 100 µL of each test sample to be processed with the set were placed individually into the remaining test tubes. A description of each sample was entered into the analysis software. The 96-well tube holder was then loaded in the M-SERIES® M1M Analyzer and the run sequence was started. Once the 96-well tube holder was placed into the analyzer, the sample analyses, including reagent additions and electrochemiluminescence measurements, were performed automatically without additional operator intervention. The M-SERIES® M1M Analyzer calculates a raw data threshold value based on the response of the first two negative control samples analyzed (referred to as the negative calibrator by BioVeris). Other samples were then automatically designated as positive or negative based on whether their resulting raw data value was above the raw data threshold (positive) or below the raw data threshold (negative). An audible alarm was also automatically triggered each time the analyzer detected a

sample with a positive result. Note that the raw data threshold value is recalculated with each set of samples analyzed based on the results of the two negative controls and two positive controls in the first four tubes of the plate. Upon assay completion, the data were transferred via a removable drive to a personal computer and imported into Microsoft Excel.

3.2.2 Non-Laboratory Testing

Because of the toxic nature of the contaminants, only positive and negative control samples were analyzed at a non-laboratory location. 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). Because the M-SERIES ® M1M Analyzer requires training to operate, only trained technical staff performed the non-laboratory testing.

3.2.3 Drinking Water Characterization

An aliquot of each DW sample, collected as described in Section 3.1.2, was sent to ATEL. Table 3-4 lists the methods used to characterize the DW samples, as well as the characterization data for the four water samples collected as part of this verification test. Water quality parameters were measured by ATEL in June, 2005. Test kits were analyzed with DW from December 2005 through August 2006. 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 with the following exception.

The cross-reactivity reagents for ricin (lectin from soybean) and botulinum toxin A (lipopolysaccharide) were inadvertently prepared at 0.5 mg/L (a concentration 10,000 times the vendor stated LOD) rather than at the 0.0005 mg/L concentration (ten times the vendor stated LOD) specified in Section 2.3.1 of the test/QA plan.[1] Because no interference effects were noted at the higher solution concentration, where they would be more likely to occur than at lower concentrations, results are reported from the 0.5 mg/L solutions. The cross reactivity results are presented in Section 6.2.3.

4.1 Quality Control of Stock Solutions

The COAs for botulinum toxin A and ricin were provided by the suppliers. Because standard reference methods do not exist, the concentrations of botulinum toxin A and ricin were not independently confirmed. The botulinum toxin A COA (Metabiologics, Inc., Madison, Wisconsin) indicated that the standard had a concentration of 1000 mg/L and was prepared in phosphate buffer saline at a pH of 6.2 and had passed Metabiologics' tests for activity, identity and purity. The ricin COA (Vector Laboratories, Inc.; Burlingame, California) indicated that the ricin standard had a concentration of 5.0 mg/mL and was prepared in a 0.08% sodium azide buffer at pH 7.8. Test samples containing these contaminants were prepared by diluting aliquots of these stock solutions. All records pertaining to stock solution dilutions were reviewed as part of the TSA review. For the interferent samples, the concentration of calcium and magnesium was confirmed by EPA Method 200.7.[6] Subsequent preparations of calcium and magnesium at the same stock concentrations were made following the same preparation procedure.

4.2 Technical Systems Audit

The Battelle Quality Manager conducted a technical systems audit (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.

4.3 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.

4.4 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.

4.5 Data Review

Records generated in the verification test were reviewed before they were used to calculate, evaluate, or report verification results. Table 4-1 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 4-1. 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
M-SERIES® M1M analyzer and BioVerify™ Botulinum Toxin A and Ricin Test Kit procedures and sample results	Battelle	ETV data sheets	Throughout test duration	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 BioVerify™ Botulinum Toxin A and Ricin Test Kits and M-SERIES® M1M analyzer produced qualitative results; i.e., they indicated only the presence or absence of the contaminant and did 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 reporting the number of positive results out of the total number of contaminant-only PT samples tested for botulinum toxin A and ricin. A positive result was determined automatically by the M-SERIES® M1M analyzer through an evaluation of the background signal generated by the negative control analyzed as the first two samples on each 96 tube assay.

5.2 False Positive/Negative Responses

A false positive response was defined as a positive response when the DI water or DW sample was spiked with a potential interferent, a cross-reactive compound, or not spiked at all. A false negative response was defined as a negative response when any sample was spiked with ten times the vendor-stated LOD or more for each analyte. 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 that produced positive or negative results without variation within replicates.

5.4 Lowest Detectable Concentration

The lowest detectable concentration for each contaminant was determined to be the concentration level at which at least two-thirds of the replicates generated positive responses. These concentration levels are determined for each target contaminant in solutions of DI water.

5.5 Other Performance Factors

Aspects of the BioVerify™ Botulinum Toxin A and Ricin Test Kits and M-SERIES® M1M analyzer 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 BioVerify™ Botulinum Toxin A and Ricin Test Kits and M-SERIES® M1M analyzer.

Chapter 6
Test Results

6.1 Qualitative Contaminant Presence/Absence

The responses for the BioVerify™ Botulinum Toxin A and Ricin Test Kits using the contaminant-only PT samples containing botulinum toxin A and ricin are discussed in the following sections. Three replicates of each PT sample were analyzed except for the sample concentration five times greater than the vendor-stated LOD (0.00025 mg/L) for which a total of ten replicates were analyzed. A total of ten replicates were analyzed at this concentration level because three replicates were contaminant-only PT samples and seven were included in the test/QA plan as a method detection limit study. Because of the qualitative nature of the BioVerify™ Botulinum Toxin A and Ricin Test Kits, the results of all ten analyses are reported as additional contaminant-only PT replicates because a method detection limit cannot be calculated for a technology that reports a presence/absence result. For the M-SERIES® M1M analyzer used in this study, results were considered positive if a sample's raw data value exceeded a threshold raw data value automatically calculated as approximately two times the average background signal generated by the negative control samples (for this ETV test, DI water) analyzed at the start of each set of samples. Note that these raw data threshold values will be unique for every set of samples analyzed and every type of analyte-specific test kit used since it is based on the negative control sample responses.

6.1.1 Botulinum Toxin A

The results obtained for the PT samples containing botulinum toxin are given in Table 6-1a. The instrument raw data threshold for a positive result generated during testing of these samples was a raw data value of 945 for all concentration levels except the lethal dose (due to its analysis on a different day), for which the positive result threshold was 1172. Based on these threshold levels, positive results were obtained in all solutions at 0.0005 mg/L (10 x LOD) and higher.

6.1.2 Ricin

The results obtained for the PT samples containing ricin are given in Table 6-1b. The instrument raw data threshold for a positive result generated during testing of these samples was a raw data value of 676 for all concentration levels except the lethal dose (due to its analysis on a different day), for which the positive result threshold was 898. Based on these threshold levels, positive results were obtained in all solutions at 0.0005 mg/L (10 x LOD) and higher and in six out of ten solutions at 0.00025 mg/L (5 x LOD).

Table 6-1a. Botulinum Toxin A Contaminant-Only PT Sample Results-Contaminant Presence/Absence Evaluation

Testing Level	Concentration (mg/L)	Raw Data Result	No. of Positive Results[(a)]
LOD	0.00005	559	0
		495	
		495	
5 x LOD	0.00025	746	0
		776	
		774	
		838	
		808	
		844	
		793	
		758	
		781	
		784	
10 x LOD	0.0005	1198	3
		1058	
		1168	
50 x LOD	0.0025	4220	3
		4233	
		4305	
LD	0.3	235643	3
		237258	
		218150	

LD = Lethal dose concentration.
LOD = Vendor-stated limit of detection.
[(a)] Number of positive results in each concentration level. Due to analysis on different days, the positive result raw data threshold was 1172 for LD concentration and 945 for all other concentrations.
Shaded areas indicate positive results.

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

Testing Level	Concentration (mg/L)	Raw Data Result	No. of Positive Results[(a)]
LOD	0.00005	391	0
		416	
		408	
5 x LOD	0.00025	661	6
		669	
		777	
		638	
		627	
		722	
		704	
		777	
		772	
		703	
10 x LOD	0.0005	1100	3
		1224	
		1269	
50 x LOD	0.0025	4892	3
		4096	
		4754	
LD	15	102884	3
		111269	
		113522	

LD = Lethal dose concentration.
LOD = Vendor-stated limit of detection.
[(a)] Number of positive results in each concentration level. Due to analysis on different days, the positive result raw data threshold was 898 for LD concentration and 676 for all other concentrations.
Shaded areas indicate positive results.

6.2 False Positive/Negative Responses

Three types of samples were analyzed to evaluate the susceptibility of the BioVerify™ Botulinum Toxin A and Ricin Test Kits to false positive and negative results. These included interferent PT samples, made up 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 made up of DI water fortified with a contaminant similar biologically or chemically with each specific 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 kit to evaluate the potential for interference from the preservative. A false positive result was defined as a positive result (i.e., above the instrument generated threshold for positive values) in the absence of the target contaminant and a false negative result was defined as a result below the instrument generated threshold for positive values from a DW or interferent sample containing the target contaminant at levels ten times the vendor-stated LOD.

6.2.1 Interferent PT Samples

The results from the interferent PT samples are given in Table 6-2. There were no false positive results generated by any of the intereferent solutions for either the BioVerify™ Botulinum Toxin A or Ricin Test Kit. There were, however, false negative results for both the BioVerify™ Botulinum Toxin A and Ricin Test Kits for all replicates containing the target contaminant 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 Test Kit to assess possible interference from the preservative in the commercially available ricin. The preservative did not cause any false positive responses (average raw data result based on three replicates equaled 489 ± 67).

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

	Botulinum Toxin A			Ricin		
Interferent	**Botulinum Toxin A (mg/L)**	**Raw Data Result**	**No. of Positive Results[a]**	**Ricin (mg/L)**	**Raw Data Result**	**No. of Positive Results[a]**
0.5 mg/L each humic and fulvic acids	none	468	0	none	439	0
		404			490	
		465			489	
	0.0005	1489	3	0.0005	1847	3
		1466			2133	
		1447			2331	
2.5 mg/L each humic and fulvic acids	none	457	0	None	479	0
		447			492	
		449			491	
	0.0005	1516	3	0.0005	2084	3
		1449			2339	
		1365			2372	
50 mg/L each Ca and Mg	none	486	0	None	475	0
		473			484	
		429			491	
	0.0005	1155	3	0.0005	839	3
		1094			956	
		1025			1041	
250 mg/L each Ca and Mg	none	486	0	None	525	0
		418			485	
		436			492	
	0.0005	715	0	0.0005	408	0
		543			414	
		518			409	

[a] Number of positive results in each concentration level. Due to analysis on different days, the botulinum toxin A positive result raw data threshold was 892 for unspiked samples and 945 for all other samples. Similarly, the ricin positive result raw data threshold was 925 for unspiked samples and 676 for all other samples.

Shaded areas indicate positive results.

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. There were no false positive results generated by any of the drinking waters in the absence of contaminant for either the BioVerify™ Botulinum Toxin A or Ricin Test Kit. There were also no false negative results in the presence of ricin for any of the waters, both concentrated and unconcentrated, from all locations with the BioVerify™ Ricin Test Kit. For the BioVerify™ Botulinum Toxin A Test Kit there were false negative results only with the unconcentrated water from Florida.

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

	Botulinum Toxin A			Ricin		
DW Sample	Botulinum Toxin A (mg/L)	Raw Data Result	No. of Positive Results[a]	Ricin (mg/L)	Raw Data Result	No. of Positive Results[a]
California	none	537	0	none	480	0
		508			511	
		448			466	
	0.0005	1107	3	0.0005	1448	3
		1108			1396	
		1133			1391	
California-Concentrated	none	627	0	none	485	0
		626			480	
		585			468	
	0.0005	1082	3	0.0005	1826	3
		966			1949	
		1063			1838	
Florida	none	540	0	none	493	0
		516			496	
		506			476	
	0.0005	826	0	0.0005	1345	3
		772			1391	
		769			1381	
Florida-Concentrated	none	563	0	none	494	0
		530			477	
		466			472	
	0.0005	1117	3	0.0005	1382	3
		1004			1411	
		1050			1370	

[a] Number of positive results in each concentration level. Due to analysis on different days, the botulinum toxin A positive result raw data threshold was 1106 for unspiked samples and 945 for all other samples. Similarly, the ricin positive result raw data threshold was 893 for unspiked samples and 676 for all other samples.

Shaded areas indicate positive results.

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

	Botulinum Toxin A			Ricin		
DW Sample	Botulinum Toxin A (mg/L)	Raw Data Result	No. of Positive Results[a]	Botulinum Toxin A (mg/L)	Raw Data Result	No. of Positive Results[a]
New York	none	556	0	none	469	0
		509			479	
		467			469	
	0.0005	1359	3	0.0005	1133	3
		1258			1116	
		1301			1271	
New York-Concentrated	none	895	0	none	522	0
		882			516	
		826			497	
	0.0005	1328	3	0.0005	1848	3
		1360			2001	
		1355			2198	
Ohio	none	559	0	none	488	0
		554			483	
		538			495	
	0.0005	1021	3	0.0005	1324	3
		1005			1466	
		977			1419	
Ohio-Concentrated	none	540	0	none	482	0
		495			463	
		548			465	
	0.0005	1260	3	0.0005	1271	3
		1179			1277	
		1231			1290	

[a] Number of positive results in each concentration level. Due to analysis on different days, the botulinum toxin A positive result raw data threshold was 1106 for unspiked samples and 945 for all other samples. Similarly, the ricin positive result raw data threshold was 893 for unspiked samples and 676 for all other samples.

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 chemical similar to each target contaminant was analyzed in the absence of any of the target contaminant. The number of positive results out of the number of replicates is given for each sample. As noted in Chapter 4, the cross-reactivity PT samples were prepared at a concentration greater than specified in the test/QA plan. No false positive results were obtained with the potentially cross-reactive compounds at the concentration prepared. Because there was no response to the higher concentration, which would be expected to cause greater interference than a lower concentration, the results of testing with the higher concentration solution are reported.

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

Cross-Reactivity Compound	Raw Data Result	No. of Positive Results[(a)]
Botulinum Toxin A: lipopolysaccharide (0.5 mg/L)	457	0
	407	
	469	
Ricin: Lectin from soybean (0.5 mg/L)	493	0
	487	
	502	

[(a)] Number of positive results out of three replicates. Due to analysis on different days, the positive result raw data threshold was 892 for botulinum toxin A and 925 for ricin.

6.3 Consistency

Using the BioVerify™ Botulinum Toxin A Test Kit, results were consistent (i.e., produced positive or negative results without variation among replicates) in 29 out of 29 sets of replicates or 100%. Using the BioVerify™ Ricin Test Kit, results were consistent in 28 out of 29 sets of replicates or 97%. Replicates included in the consistency calculation are the contaminant-only PT samples, the interferent PT samples and the DW samples.

6.4 Lowest Detectable Concentration

The lowest detectable concentration of each target contaminant was defined as the lowest concentration of contaminant-only PT sample to have at least two-thirds of the replicates generate positive results. For both botulinum toxin A and ricin, the lowest detectable concentration was 0.0005 mg/L (10 x LOD). It should be noted that the results for ricin at the 0.00025 mg/L level (5 x LOD) were very close to having two-thirds of the replicates generate a positive response (six out of 10 replicates were positive) and the four negative results (raw data values ranging from 627 to 669) were less than 10% different from the 676 threshold value for a positive result.

6.5 Other Performance Factors

6.5.1 Ease of Use

The BioVerify™ Botulinum Toxin A and Ricin Test Kits came with clearly written and informative instructions. In addition the M-SERIES® M1M analyzer came with a detailed operation manual. Contents of the test kits were color-coded for easy identification and storage requirements were readily available. Overall, the test kit packaging was easy to open. The M-SERIES® M1M analyzer required two people to move it into position for analysis due to its weight (approximately 80 pounds).

The analyzer system reagents used for all of the analyte-specific test kits were supplied ready to use and could be stored at room temperature. The analyte-specific test kits, however, required storage between 2-8º C. The shelf-life for analyzer system reagents is four to six months, while the shelf-life for the analyte-specific kits is one month once they are opened (note that if all kit reagents are not depleted during testing, they can be resealed and stored). Once a sample tube is prepared for analysis, it needs to be used immediately. The M-SERIES® M1M analyzer software is somewhat complex; however, verification testing staff found the software to be intuitive and were able to operate the system after a four hour training session. In addition to determining a raw data result value for each sample, the data system automatically identified if the sample result was positive or negative. An audible alarm was triggered each time a positive result was detected and needed to be turned off manually; this feature made it difficult for the operator to multi-task while multiple samples were being analyzed as the operator was continually turning the alarm off. The operator observed that the complex surface of the M-SERIES® M1M analyzer and the interior of the carrying case would be difficult to wipe clean; however, cleaning was not attempted during the course of testing. Pipette tips, sample tubes and spent reagent were generated as waste with each analysis. The M-SERIES® M1M analyzer contains a sealed waste container which is electronically monitored to ensure that its capacity is not exceeded.

No formal scientific education would be required for using the BioVerify™ Botulinum Toxin A and Ricin Test Kits and M-SERIES® M1M analyzer, but general good laboratory skills and reasonable computer skills are helpful. Because the kits are 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. Contact information for BioVeris, including phone, fax, and website address is included in the instruction manual for easy access to the vendor's contact information.

6.5.2 Field Portability

Field portability testing was accomplished by transporting the BioVerify™ Ricin Test Kits and M-SERIES® M1M analyzer to a well lit shipping/receiving area. The temperature and relative humidity were ambient (20 +/- 2 ºC and 40-50%, respectively). The equipment packs neatly into two separate cases for field portability. Due to the weight of the system (approximately 80 pounds) three people were used to transport the system to the field (two to carry the analyzer and the third to carry the reagents). While fewer people could transport the equipment if aided by a cart, lifting and orienting the analyzer would be difficult for one person. Once at the field testing

location, the equipment was set up within approximately 15 minutes. All analyzer system reagents were supplied ready-to-use. At this non-laboratory location, the M-SERIES® M1M analyzer was operated using a battery back-up. The only equipment needed for field transport that was not supplied with the BioVerify™ Ricin Test Kit and M-SERIES® M1M analyzer was a cooler to transport test kits while maintaining a temperature of 2-8 ºC. For short-term use a cooler is sufficient for transporting the test kits which need to be maintained between 2-8ºC; however, for long-term field deployment a refrigerator would be useful to keep the test kits cold.

The technology was field tested with a DI water method blank sample and the vendor-provided positive control for ricin processed in duplicate. Results in the field were similar to results obtained in the laboratory with the method blank raw data result values below the positive result raw data threshold and the positive control raw data result well above the positive result raw data threshold.

6.5.3 Throughput

Approximately one hour is needed to prime the analyzer plumbing and run system diagnostics. After this initial set up effort, a 96 tube sample set can be processed in less than two hours. The test kits are supplied in 96 tube format. Four tubes must be reserved for system-required controls. The remaining 92 tubes can be used for field samples with the number of samples being processed depending on the number of replicates the user chooses to process for each sample.

Chapter 7
Performance Summary

Table 7-1. Botulinum Toxin A Summary Table

Parameter	Sample Information	Botulinum Toxin A Concentration (mg/L)		No. of Positive Results[(a)]	
Contaminant-only PT samples	DI water	0.00005 (vendor-stated limit of detection)		0	
		0.00025		0	
		0.0005		3	
		0.0025		3	
		0.3 (lethal dose)		3	
Interferent PT samples	0.5 mg/L humic and fulvic	unspiked	0.0005	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	0
DW samples	Unconcentrated CA	unspiked	0.0005	0	3
	Concentrated CA			0	3
	Unconcentrated FL			0	0
	Concentrated FL			0	3
	Unconcentrated NY			0	3
	Concentrated NY			0	3
	Unconcentrated OH			0	3
	Concentrated OH			0	3
Cross-reactivity	0.5 mg/L lipopolysaccharide	unspiked		0	

[(a)] Number of positive results out of three replicates, except for the 0.00025 mg/L contaminant-only PT sample which is out of 10 replicates.
Shading indicates results for unspiked sample.

Table 7-1. Botulinum Toxin A Summary Table (Continued)

False positives	There were no false positive results.
False negatives	False negatives were observed in the presence of 250 mg/L Ca and Mg and in the unconcentrated FL drinking water samples.
Consistency	Results were consistent (i.e., produced positive or negative results without variation among replicates) in 29 out of 29 sets of replicates or 100%.
Lowest detectable concentration	The lowest concentration where at least two-thirds of the replicates generated a positive response was 0.0005 mg/L.
Other performance factors	Test kits require storage at 2-8º C. Analyzer software requires training. The M-SERIES® M1M analyzer uses electricity or battery backup and includes a rugged carrying case. Analyzer console weighs approximately 80 pounds. Test kits and analyzer were used inside and outside a laboratory by a trained operator; one 96 tube sample set can be processed in approximately two hours, provided the analyzer is primed and system diagnostics have already been performed.

Table 7-2. Ricin Summary Table

Parameter	Sample Information	Ricin Concentration (mg/L)		No. of Positive Results[a]	
Contaminant-only PT samples	DI water	0.00005 (vendor-stated limit of detection)		0	
		0.00025		6	
		0.0005		3	
		0.0025		3	
		15 (lethal dose)		3	
Interferent PT samples	0.5 mg/L humic and fulvic	unspiked	0.0005	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	0
DW samples	Unconcentrated CA	unspiked	0.0005	0	3
	Concentrated CA			0	3
	Unconcentrated FL			0	3
	Concentrated FL			0	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	There were no false positive results.				
False negatives	False negatives were observed only in the 250 mg/L Ca and Mg sample.				
Consistency	Results were consistent (i.e., produced positive or negative results without variation among replicates) in 28 out of 29 sets of replicates or 97%.				
Lowest detectable concentration	The lowest concentration where at least two-thirds of the replicates generated a positive response was 0.0005 mg/L, although the 0.00025 mg/L concentration was detected in 6 out of 10 replicates.				
Other performance factors	Test kits require storage at 2-8º C. Analyzer software requires training. The M-SERIES® M1M analyzer uses electricity or battery backup and includes a rugged carrying case. Analyzer console weighs approximately 80 pounds. Test kits and analyzer were used inside and outside a laboratory by a trained operator; one 96 tube sample set can be processed in approximately two hours, provided the analyzer is primed and system diagnostics have already been performed.				

[a] Number of positive results out of three replicates, except for the 0.00025 mg/L contaminant-only PT sample which is out of 10 replicates.

Shading indicates results for unspiked sample.

Chapter 8
References

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