THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

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**
COMPANY:	**BioVeris Corporation**
ADDRESS:	**16020 Industrial Drive** **PHONE: 800-336-4436** **Gaithersburg, Maryland 20877** **FAX: 301-230-0158**
WEB SITE:	**www.bioveris.com**
E-MAIL:	**bvcorp@bioveris.com**

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
Gregory A. Mack Date
Vice President
Energy, Transportation, and Environment Division
Battelle

Original signed by Jonathan G. Herrmann 11/12/2006
Jonathan G. Herrmann Date
Director
National Homeland Security Research Center
U.S. Environmental Protection Agency