THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

ETV Joint Verification Statement

TECHNOLOGY TYPE: IMMUNOASSAY TEST KITS

APPLICATION: DETECTING BOTULINUM TOXIN

TECHNOLOGY NAME: EzyBot® A and EzyBot® B Test Kits

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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 in water. This verification statement provides a summary of the test results for the PharmaLeads EzyBot® A and B test kits.

VERIFICATION TEST DESCRIPTION

The verification test for the EzyBot® test kits was conducted at Battelle between November 2005 and January 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 EzyBot® test kits to detect various concentrations of botulinum toxin 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 EzyBot® test kits to lipopolysaccharide, a potentially cross-reactive compound for botulinum toxin. 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 both with and without the addition of botulinum toxin A and B at a concentration of 10 times the vendor-stated LOD. The EzyBot® A test kit was specific to botulinum toxin A, and the EzyBot® B test kit was specific to botulinum toxin B. 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 EzyBot® was provided by the vendor and was not verified in this test.

EzyBot® test kits provide a means for detecting botulinum toxins A (EzyBot® A) and B (EzyBot® B) in water. The technology is based on the PharmaLeads internal collision fluorescence quenching technology. A fluorogenic substance and a quenching substance in the substrate bracket an amino-acid sequence that, in the presence of botulinum toxin A or B, is cleaved, generating an intense fluorescence. This fluorescence is measured using either a laboratory or a field fluorimeter. Note that a laboratory fluorimeter is not provided by PharmaLeads with the EzyBot® kit; however, a field fluorimeter is available for purchase as part of the field case. The type of fluorimeter used for detection can affect the sensitivity of the analysis obtained with the EzyBot® test kit, therefore users may want to contact the vendor for recommended fluorimeters in order to achieve optimal sensitivity with the EzyBot® kits. The fluorescence generated by the EzyBot® test kit increases in intensity with time and with botulinum toxin concentration. Data can be read from the fluorimeter display or for the PharmaLeads field portable fluorimeter can be transferred to a computer through a cable provided with the fluorimeter. Note that a computer is not provided by PharmaLeads.

EzyBot® A and B are available individually in kits of 50 ready-to-use cuvettes containing freeze-dried reagents, which can be used in the laboratory or in the field. The PharmaLeads field case provides a field incubator which can be plugged into the auxiliary power outlet of a car to perform the 1-hour incubation at 37°C in the field. The field case also includes the PharmaLeads field portable fluorimeter. The price of an EzyBot® kit depends on the quantity ordered. For large quantities, unit price is approximately $30 per ready-to-use cuvette. Cost for the field case, including the field fluorimeter, the portable incubator, and 100 cuvettes, is less than $12,500.

VERIFICATION OF PERFORMANCE

The tables below summarize the performance of the EzyBot® test kits in detecting botulinum toxins A and B.

EzyBot® A Summary Table

Parameter	Sample Information	Botulinum Toxin A (mg/L)	Lab Bench Scale Fluorimeter[a]		Field Portable Fluorimeter[a]	
			30 min.	60 min.	30 min.	60 min.
Contaminant-only	DI water	0.01 (vendor-stated limit of detection)	0	0	0	0
		0.05	0	10	0	0
		0.1	0	3	0	0
		0.3 (lethal dose)	3	3	0	3
		0.5	3	3	1	3
Interferent	0.5 and 2.5 mg/L each humic/fulvic acids	0.1	NA	0	NA	
	50 and 250 mg/L each Ca/Mg	0.1		3		
DW-all locations	unconcentrated	0.1		3		
DW-California	concentrated	0.1		3		
DW-Florida	concentrated	0.1		3		
DW-New York	concentrated	0.1		0		
DW-Ohio	concentrated	0.1		3		
Lowest Detectable Concentration[b] (mg/L)			0.3	0.05	ND	0.3
False positives	There were no false positive results from interferents including a preservative blank, humic and fulvic acids, and Ca and Mg; DW from four locations using different water treatment technologies; or the potentially cross-reactive lipopolysaccharide (0.1 mg/L).					
False negatives	False negatives were obtained in the presence of both 0.5 and 2.5 mg/L each humic and fulvic acids. A false negative was also obtained in New York water which was concentrated by a factor of 400. A total of 3 false negative results were obtained out of the 12 solutions assessed at 60 minutes. The vendor informed Battelle after testing that the lab bench scale fluorimeter provided for testing may have had inconsistent functioning which could have caused the false negative results that were obtained.					
Consistency	Using the lab bench scale fluorimeter, results were consistent in 100% of the samples tested. Using the field portable fluorimeter, results were consistent in 90% of the samples tested.					
Other Performance Factors	Convenient ready-to use cuvettes. Easy to operate in the lab and easy to transport and operate in the field. No formal scientific education would be required to use the kit; however, general lab skills and training on fluorimeter use were helpful. Approximately 12-15 analyses were completed in one hour in the laboratory. Only five samples could be processed in one hour in the field due to size limitation of the field portable incubator. Each Ezybot ® kit contains 50 ready-to-use cuvettes.					

NA = Not tested. Testing concentration below detection in the contaminant only PT testing.
ND = not detectable at concentrations tested.
[a] Results out of 3 replicates except for the 0.05 mg/L contaminant only concentration for which results are out of 10 replicates
[b] The lowest concentration of contaminant-only PT samples to have at least two thirds of the measurements generate positive results.

EzyBot® B Summary Table

Parameter	Sample Information	Botulinum Toxin B (mg/L)	Lab Bench Scale Fluorimeter[a]		Field Portable Fluorimeter[a]	
			30 min.	60 min.	30 min.	60 min.
Contaminant-only	DI water	0.01(vendor-stated limit of detection)	0	3	0	0
		0.05	7	10	0	0
		0.1	3	3	0	0
		0.3 (lethal dose)	3	3	3	3
		0.5	3	3	0	3
Interferent	0.5 mg/L each humic/fulvic acids	0.1	3	3	NA	
	2.5 mg/L each humic/fulvic acids	0.1	1	3		
	50 and 250 mg/L each Ca/Mg	0.1	0	0		
DW- all but New York	unconcentrated	0.1	0	3		
DW- New York	unconcentrated	0.1	3	3		
DW-California	concentrated	0.1	0	3		
DW-Florida	concentrated	0.1	3	3		
DW-New York	concentrated	0.1	0	3		
DW-Ohio	concentrated	0.1	3	3		
Lowest Detectable Concentration [b] (mg/L)			0.05	0.01	ND	0.3
False positives	There were no false positive results from interferents including a preservative blank, humic and fulvic acids, and Ca and Mg; DW from four locations using different water treatment technologies; or the potentially cross-reactive lipopolysaccharide (0.1 mg/L).					
False negatives	False negative results were obtained in the presence of both 50 and 250 mg/L Ca and Mg using both a 30 minute and 60 minute incubation time. The 30 minute incubation time also generated false negative results in unconcentrated water from California, Florida, and Ohio; and in concentrated water from California and New York. A total of 8 false negative results were obtained out of the 12 solutions assessed at 30 minutes. A total of 2 false negative results were obtained out of the 12 solutions assessed at 60 minutes. The vendor informed Battelle after testing that the lab bench scale fluorimeter provided for testing may have had inconsistent functioning which could have caused the false negative results that were obtained.					
Consistency	For the lab bench scale fluorimeter, results were consistent in 97% of the samples tested. With the field portable fluorimeter, results were consistent in 100% of the samples tested.					
Other Performance Factors	Convenient ready-to use cuvettes. Easy to operate in the lab and easy to transport and operate in the field. No formal scientific education would be required to use the kit; however, general lab skills and training on fluorimeter use were helpful. Approximately 12-15 analyses were completed in one hour in the laboratory. Only five samples could be processed in one hour in the field due to size limitation of the field portable incubator. Each Ezybot ® kit contains 50 ready-to-use cuvettes.					

NA = Not tested. Testing concentration below detection in the contaminant only PT testing.
ND = not detectable at concentrations tested.
[a] Results out of 3 replicates except for the 0.05 mg/L contaminant only concentration for which results are out of 10 replicates.
[b] The lowest concentration of contaminant-only PT samples to have at least two thirds of the measurements generate positive results.

Original signed by Gregory A. Mack 10/26/2006	Original signed by Jonathan G. Herrmann 11/12/2006
Gregory A. Mack Date	Jonathan G. Herrmann Date
Vice President	Director
Energy, Transportation, and Environment Division	National Homeland Security Research Center
Battelle	U.S. Environmental Protection Agency

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