THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

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ETV Joint Verification Statement

TECHNOLOGY TYPE:	**Rapid Toxicity Testing System**	
APPLICATION:	**Detecting Toxicity in Drinking Water**	
TECHNOLOGY NAME:	**AbraTox Kit**	
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The U.S. Environmental Protection Agency (EPA) has established 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 the Abraxis AbraTox Kit. This verification statement provides a summary of the test results.

VERIFICATION TEST DESCRIPTION

Rapid toxicity technologies use various biological organisms and chemical reactions to indicate the presence of toxic contaminants. The toxic contaminants are indicated by a change or appearance of color or a change in intensity. As part of this verification test, the AbraTox Kit was subjected to various concentrations of contaminants such as industrial chemicals, pesticides, rodenticides, pharmaceuticals, nerve agents, and biological toxins. Each contaminant was added to separate drinking water samples and analyzed. In addition to determining whether the AbraTox Kit could detect the toxicity caused by each contaminant, its response to interfering compounds, such as water treatment chemicals and by-products in clean drinking water, was evaluated.

The AbraTox Kit was evaluated by

- Endpoints and precision—percent inhibition for all concentration levels of contaminants and potential interfering compounds and precision of replicate analyses
- Toxicity threshold for each contaminant—contaminant level at which higher concentrations generate inhibition significantly greater than the negative control and lower concentrations do not
- False positive responses—chlorination and chloramination by-product inhibition with respect to unspiked American Society for Testing and Materials Type II deionized water samples
- False negative responses—contaminants that were reported as producing inhibition similar to the negative control when present at lethal concentrations (the concentration at which 250 milliliters of water would probably cause the death of a 154-pound person) or negative background inhibition that caused falsely low inhibition
- Other performance factors (sample throughput, ease of use, reliability).

The AbraTox Kit was verified by analyzing a dechlorinated drinking water sample from Columbus, Ohio (DDW), fortified with contaminants (at concentrations ranging from lethal levels to concentrations up to 100,000 times less than the lethal dose) and interferences (metals possibly present as a result of the water treatment processes). Dechlorinated water was used because free chlorine kills the bacteria within the AbraTox reagent and can degrade the contaminants during storage. Inhibition results (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of the AbraTox Kit to detect toxicity, as well as to measure the precision of the AbraTox Kit results. The response of the AbraTox Kit to possible interferents was evaluated by analyzing them at one-half of the concentration limit recommended by the EPA's National Secondary Drinking Water Regulations guidance. For analysis of by-products of the chlorination process, the unspiked DDW was analyzed because Columbus, Ohio, uses chlorination as its disinfectant procedure. For the analysis of by-products of the chloramination process, a separate drinking water sample was obtained from the Metropolitan Water District of Southern California (LaVerne, California), which uses chloramination as its disinfection process. The samples were analyzed after residual chlorine was removed using sodium thiosulfate. Sample throughput was measured based on the number of samples analyzed per hour. Ease of use and reliability were determined based on documented observations of the operators.

Quality control samples included method blank samples, which consisted of American Society for Testing and Materials Type II deionized water; positive control samples (supplied by the vendor); and negative control samples, which consisted of the unspiked DDW.

QA oversight of verification testing was provided by Battelle and EPA. Battelle QA staff conducted a technical systems audit, a performance evaluation 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 test are all available at www.epa.gov/etv/centers/center1.html.

TECHNOLOGY DESCRIPTION

The following description of the AbraTox Kit is based on information provided by the vendor. This technology description was not verified in this test.

The AbraTox Kit is an *in vitro* testing system that uses a naturally occurring and non-pathogenic bioluminescent bacteria *Vibrio fischeri* (strain NRRL-B-11177) to determine the toxicity of water-soluble samples. *Vibrio fischeri*, when properly grown, emits light as part of its metabolic pathway; the emitted light is an indication of the metabolic status of the bacterium. Differences in the amount of light produced can therefore be correlated to bacterial metabolism. Toxic compounds interfere with the metabolic process, resulting in a reduction of light emission. The reduction of light emitted is proportional to the toxicity of the sample—the more toxic the sample, the greater percentage of light reduction.

The AbraTox *Vibrio fischeri* reagent vials are supplied freeze dried. To analyze the water samples, the vials are reconstituted with 2.5 milliliter (mL) of cold reconstitution solution and allowed to hydrate under refrigerated conditions for 30 minutes. Meanwhile, 800 microliters (μL) of the water sample to be analyzed are added to test cuvettes, followed by the addition of 100 μL of osmotic adjusting buffer, and allowed to incubate in the refrigerated incubation chamber for at least 15 minutes. Then, 100 μL of the diluted bacteria are added to a negative control and to each test sample and incubated in the refrigerated incubation chamber for 15 to 60 minutes. Luminescence is then measured using a portable luminometer. Significant changes in luminescence compared to the negative control (or reference sample) reflect the toxicity of the test sample.

The AbraTox Kit contains six vials of freeze-dried bacteria, two vials of reconstitution solution, one bottle of osmotic adjusting buffer, and one vial of positive and negative control. Test cuvettes, a repeater pipette (100 μL), and a 200 to 1,000 μL pipette and tips are required but not provided.

The box containing the AbraTox Kit has dimensions of 18 by 13 by 8 centimeters (cm). The AbraTox luminometer is 20 by 8 by 5 cm, uses 2 AA batteries, and weighs 0.3 kilograms. It can be integrated (although it was not during this test) with a personal computer for data acquisition, evaluation, and storage. The price of the AbraTox Kit (150 single tests) is $250, the luminometer is $2,000, and the incubation chamber is $250.

VERIFICATION RESULTS

Parameter	Compound	Lethal Dose (LD) Conc. (mg/L)	Average Inhibition at Concentrations Relative to the LD Concentration (%)				Range of Standard Deviations (%)	Toxicity Thresh. (mg/L)
			LD	LD/10	LD/100	LD/1,000		
Contaminants in DDW	Aldicarb	260	63	21	-3	12	2-12	260
	Botulinum toxin complex B	0.3	-10	-11	-40	-2	19-34	ND
	Colchicine	240	17	-39	2	-51	2-9	240
	Cyanide	250	82	71	43	25	3-20	25
	Dicrotophos	1,400	42	10	14	26	8-12	1,400
	Nicotine	2,800	43	-3	-2	4	2-7	700
	Ricin	15	11[(a)]	8[(a)]	-1[(a)]	7[(a)]	5-17	ND
	Soman	1.4	31[(a)]	-24	-10	-1	4-13	1.4[(a)]
	Thallium sulfate	2,800	14	20	5	6	1-7	280
	VX	2	-32	-15	2	-7	6-21	ND

Parameter	Interference	Conc. (mg/L)	Average Inhibition (%)	Standard Deviation (%)
Potential interferences in DDW	Aluminum	0.5	-4	10
	Copper	0.6	32	11
	Iron	0.15	7	3
	Manganese	0.25	1	6
	Zinc	2.5	15	10

False positive response	No false positive results were obtained because the inhibition of the chlorination and chloramination by-product water samples was not significantly different from that of the negative control samples.
False negative response	The AbraTox Kit generated false negative responses at the lethal dose concentration for botulinum toxin complex B, ricin, and VX.
Ease of use	The AbraTox Kit contained clearly written instructions and illustrations, and the contents were clearly labeled. Storage requirements were marked on the vial labels. The packaging was easy to open except for the pull-back tabs on some of the bottles. The most difficult aspect of using the AbraTox Kit was keeping the incubator at 15°C because there was no temperature control on the incubator. Because bacteria stock had to be refrigerated for 30 minutes, at least 30 minutes of advance notice is necessary before using the AbraTox Kit. No formal scientific training would be required to use the AbraTox Kit.
Field portability	The AbraTox Kit was transported from a laboratory to a storage room to simulate a situation in which it would be operated in a non-laboratory location. The luminometer was transported in a small box, and a small cooler was used to transport the reagents. Overall the AbraTox Kit was easy to transport to the field and was deployed in a matter of minutes. The AbraTox Kit was tested with cyanide at the lethal dose concentration. Results were obtained within 30 minutes of starting the test and were very similar to those obtained in the laboratory. In the laboratory, the inhibition for the lethal dose concentration of cyanide was 82% ± 5%; while at the non-laboratory location, the inhibition was 76% ± 2%.
Throughput	Approximately 25 sample analyses plus method blanks and controls were completed in one hour. Approximately 25 samples could be processed per vial of *Vibrio fischeri*.

ND = Significant inhibition was not detected.
[(a)] Inhibition calculated with respect to the preservative blank.

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