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:	**Toxi-Chromotest**	
COMPANY:	**Environmental Bio-Detection Products, Inc.**	
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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 Environmental Bio-Detection Products, Inc. Toxi-Chromotest. 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 Toxi-Chromotest 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 Toxi-Chromotest 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 Toxi-Chromotest was evaluated by

- Endpoints and precision—color inhibition (as indicator of toxicity) with respect to that of the negative control for all concentration levels of contaminants and potential interfering compounds and consistency of the color change across 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, when present at lethal concentrations, did not produce any color inhibition with respect to the negative control
- Other performance factors (sample throughput, ease of use, reliability).

The Toxi-Chromotest 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 1,000 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 Toxi-Chromotest 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 Toxi-Chromotest to detect toxicity, as well as to measure the precision of the Toxi-Chromotest results. The response of the Toxi-Chromotest 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 (fortified with mercuric chloride); 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 Toxi-Chromotest is based on information provided by the vendor. This technology description was not verified in this test.

The Toxi-Chromotest detects toxic substances in water, chemicals, pharmaceuticals, food, and body fluids. The Toxi-Chromotest is a bacterial assay based on the ability of toxic materials and antibiotics to inhibit the *de novo* synthesis of an inducible enzyme, ß-galactosidase, in a strain of the bacteria, *E. coli* (K12 OR85). The bacteria in the Toxi-Chromotest are exposed to stressing conditions and freeze dried. To test for toxicity, the bacteria are mixed with a rehydration cocktail containing inducers of the enzyme ß-galactosidase and factors necessary for the recovery of the bacteria from their stressed condition. During the recovery phase, toxicants present at sufficient concentrations penetrate the cell walls of the bacteria and inhibit the *de novo* synthesis of the ß-galactosidase. The rate of production of the induced enzyme is detected by a reaction of the excreted enzyme with a chromogenic substrate in the bacterial suspension that was exposed to the potential toxicant. Toxic materials above threshold levels interfere with the production of the enzyme and decrease color formation.

The Toxi-Chromotest kit includes a reaction mixture (the cocktail containing an inducer for the enzyme ß-galactosidase and co-factors required for the recovery of the bacteria from their stressed condition), lyophilized bacteria, rehydration solution, a positive control (4 micrograms per milliliter of mercuric chloride in water), a chromogenic substrate (blue chromogen cocktail, ready for use), and diluent for the positive control and test samples. In addition, the Toxi-Chromotest kit contains three 96-well microtiter plates and biohazard bags. The user must supply a micropipette for adding the test samples, rehydrated bacteria, and chromogenic substrates to the test wells and an incubator in which the plates containing the bacteria are allowed to recover and begin to produce the enzyme that reacts with the added chromogenic substrate. The incubator must maintain a constant temperature of 37 ºC during the 90 minute incubation period.

The Toxi-Chromotest is supplied in a 25- by 13- by 8- centimeter (cm) Styrofoam box that contains the 96-well plates, the biohazard bags for disposal of test materials, and all of the necessary reagents to carry out three separate analytical test series. For field use, a 15-cm by 15-cm by 15-cm incubator can be supplied that runs off a 12-volt battery or 120-volt alternating current.

The output from the Toxi-Chromotest can be measured in the laboratory by absorbance at 615 nanometers using a plate reader. If the test is conducted in the field or a plate reader is not available (as during this test), the results can be read by visually recording the intensity of blue color produced against an internally run set of standards to obtain a relative toxicity reading. The standard Toxi-Chromotest kit, with reagent, bacteria, and plates to run the tests in the three 96-well microtiter plates provided, sells for $375.

VERIFICATION RESULTS

Parameter	Compound	Lethal Dose (LD) Conc. (mg/L)	Visual Observance of Color Inhibition at Concentrations Relative to the LD Concentration				Toxicity Threshold (mg/L)
			LD	LD/10	LD/100	LD/1,000	
Contaminants in DDW	Aldicarb	260	−	−	−	−	ND
	Botulinum toxin complex B	0.3	−	−	−	−	ND
	Colchicine	240	−	−	−	−	ND
	Cyanide	250	+	+	−	−	25
	Dicrotophos	1,400	−	−	−	−	ND
	Nicotine	2,800	+	−	−	−	2,800
	Ricin	15	−	−	−	−	ND
	Soman	1.4	−	−	−	−	ND
	Thallium sulfate	2,800	+	+	−	+	280
	VX	2	−	−	−	−	ND
Potential interferences in DDW	**Interference**	**Conc. (mg/L)**	**Visual Observance of Color Inhibition**				
	Aluminum	0.5	−				
	Copper	0.6	−				
	Iron	0.15	−				
	Manganese	0.25	−				
	Zinc	2.5	−				
False positive response	The Toxi-Chromotest did not generate any false positive results to water containing chlorination or chloramination by-products.						
False negative response	Aldicarb, botulinum toxin complex B, colchicine, dicrotophos, ricin, soman, and VX produced an inhibition that was not visually distinguishable from the negative control at the lethal dose concentrations.						
Ease of use	The Toxi-Chromotest requires two 1.5-hour incubation periods. After bacteria rehydration, the hydrated bacteria could be used only for one hour. In addition, the reaction of the Toxi-Chromotest was observed visually, which was difficult when there were only slight variations in color. No formal scientific training would be required to use the Toxi-Chromotest.						
Field portability	Overall the Toxi-Chromotest was easy to transport to the field and, with an incubator warmed ahead of time, was deployed in a matter of minutes. Results were obtained within 3 to 4 hours of starting the test. Each Toxi-Chromotest kit contained materials to process three 96-well plates.						
Throughput	The number of samples that can be processed depends on the number of replicates per sample and the number of dilutions per sample that are processed on each 96-well plate. One plate can be taken through the procedure in 3 to 4 hours. Each Toxi-Chromotest kit contained materials to process samples filling three 96-well plates.						

+ = Visually distinguishable color inhibition from that of the negative control was observed.
- = Visually distinguishable color inhibition from that of the negative control was not observed

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