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:	**RAPIDTOXKIT**	
COMPANY:	**Strategic Diagnostics 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 Strategic Diagnostics Inc. RAPIDTOXKIT. 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 RAPIDTOXKIT 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 RAPIDTOXKIT 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 RAPIDTOXKIT 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. Note that Strategic Diagnostics Inc. recommends that a 30% inhibition is required for a conclusive indication of toxicity. During this test, a thorough evaluation of the toxicity threshold was performed. Therefore, the toxicity threshold was determined with respect to the negative control rather than the 30% inhibition threshold

- False positive responses—chlorination and chloramination by-product inhibition exceeding 30% with respect to unspiked American Society for Testing and Materials (ASTM) Type II deionized (DI) water samples

- False negative responses—contaminants that were reported as producing inhibition results less than 30% 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 RAPIDTOXKIT 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 one million 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 larval crustacean within the RAPIDTOXKIT 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 RAPIDTOXKIT to detect toxicity, as well as to measure the precision of the RAPIDTOXKIT results. The response of the RAPIDTOXKIT 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 (vendor-specified); 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 RAPIDTOXKIT is based on information provided by the vendor. This technology description was not verified in this test.

The RAPIDTOXKIT uses larvae of the anostracan crustacean *T. platyurus* to detect freshwater (including drinking water) contamination. The RAPIDTOXKIT bioassays are performed in disposable test tubes using *T. platyurus* hatched from cysts. Cyst hatching must begin 30 to 45 hours prior to performing the test. The *T. platyurus* are exposed to samples for 15 minutes to one hour, after which a suspension of red microspheres is added. The organisms ingest the microspheres, resulting in a deep red color in their digestive tracts. Stressed (intoxicated) organisms either fail to take up particles altogether or ingest at a much lower rate. The presence or the absence of colored microspheres in the digestive tract of the larval crustaceans is observed under a stereomicroscope, and data are recorded on a sheet supplied with the RAPIDTOXKIT. The total number of *T. platyurus* in the control (standard freshwater) well(s), and the number of *T. platyurus* that have taken up the red particles are counted, and the fraction of larval crustaceans affected by the contaminant is defined as the percent inhibition. As a guideline, 30 percent inhibition of particle uptake is considered a threshold for the presence of potentially toxic compounds in the water.

Each test kit includes three 1-milliliter test tubes containing cysts of *T. platyurus,* one bottle of standard freshwater, three hatching vessels, six sub-sampling tubes, 48 test tubes, six test tube holders, one vial with red microspheres, one vial with fixative, six observation plates, six transparent covers for observation plates, a blue plastic sheet and grid designed to be placed under plates to aid in observing and scoring test organisms, standard operating procedure booklet, bench protocol, six sheets for scoring test results and calculating mean inhibition of particle uptake, and a specification sheet containing batch numbers and shelf lives of kit components. Materials required but not provided as part of the kit include a 25°C incubator with 4,000-lux constant illumination, a dissection microscope with minimum 10X magnification, and an overhead light source for the microscope. The complete RAPIDTOXKIT, adequate for 7 to 15 water samples each, depending on the sample size, measures 30 centimeters by 25 centimeters by 10 centimeters and costs $196.

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	100	100	100	53	0–10	0.26
	Botulinum toxin complex B	0.3	4	-51	-40	-32	6–23	ND
	Colchicine	240	56	13	26	28	9–13	240
	Cyanide	250	100	100	100	51	0–17	0.25
	Dicrotophos	1,400	100	100	100	-4	0–6	14
	Nicotine	2,800	100	100	100	100	0	0.28
	Ricin	15	27	14	-2	6	1–6	15
	Soman	1.4	100	99	100	-2	0–6	0.007
	Thallium sulfate	2,800	100	100	79	29	0–19	28
	VX	2	99	10	4	22	1–6	1.5
Potential interferences in DDW	**Interference**	**Conc. (mg/L)**	**Average Inhibition (%)**		**Standard Deviation (%)**			
	Aluminum	0.5	29		6			
	Copper	0.6	100		0			
	Iron	0.15	20		4			
	Manganese	0.25	-11		11			
	Zinc	2.5	24		9			
False positive response	No results from the RAPIDTOXKIT were considered false positive because inhibition in the chlorinated and chloraminated drinking water samples was always less than 30%.							
False negative response	Only botulinum toxin complex B exhibited inhibition less than 30% when analyzed at a lethal dose concentration.							
Ease of use	The RAPIDTOXKIT contained clearly written instructions and illustrations. The contents of the RAPIDTOXKIT were well identified. The only problem, other than the difficulty opening some containers, was a slight difficulty getting the cysts out of the tubes with the recommended 1 mL of water. Manually counting the number of red organisms under the microscope was tedious when the results from many samples were determined one after the other over a few hours. Overall, the RAPIDTOXKIT was easy to use, making it likely that a person with no formal scientific training could conduct the tests.							
Field portability	The RAPIDTOXKIT was not evaluated for field portability.							
Throughput	Not including the 30 to 45-hour cyst-hatching period, approximately 25 analyses (including method blanks and positive and negative controls) were completed in three hours. A maximum of 45 samples could be processed per kit.							

ND = Significant inhibition was not detected.

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