THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

ETV

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:	**ToxTrak™**		
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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.

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 seven technology areas under ETV, is operated by Battelle in cooperation with EPA's National Exposure Research Laboratory. The AMS Center has recently evaluated the performance of rapid toxicity testing systems used to detect toxicity in drinking water. This verification statement provides a summary of the test results for ToxTrak™

VERIFICATION TEST DESCRIPTION

Rapid toxicity technologies use bacteria, enzymes, or small crustaceans that product light or use oxygen at a steady rate in the absence of toxic contaminants. Toxic contaminants in drinking water are indicated by a change in the color or intensity of light or by a change in the rate of oxygen use. As part of this verification test, which, for this technology, took place between July 14 and September 12, 2003, various contaminants were added to separate drinking water samples and analyzed by ToxTrak™. Response to interfering compounds in clean drinking water also was evaluated. Dechlorinated drinking water samples from Columbus, Ohio, (DDW) were fortified with contaminants at concentrations ranging from lethal levels to levels 1,000 times less than the lethal dose and analyzed. Endpoint and precision, toxicity threshold for each contaminant, false positive/negative responses, ease of use, and sample throughput were evaluated.

Inhibition results (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of the ToxTrak™ to detect toxicity at various concentrations of contaminants, as well as to measure the precision of the ToxTrak™ results. The response of ToxTrak™ to compounds used during the water treatment process (interfering compounds) was evaluated by analyzing separate aliquots of DDW fortified with each potential interferent at approximately 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, 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 from St. Petersburg, Florida, which uses chloramination as its disinfection process, was obtained. 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 and the verification test coordinator.

The test/QA plan for this verification test describes only a quantitative evaluation of the percent inhibition data generated by each technology. The ToxTrak™ manufacturer indicated during the review of this report that a qualitative data evaluation also should be performed to describe how a typical user is more likely to interpret and use the ToxTrak™ results. Specifically, the manufacturer suggested that the percent inhibition results for each concentration level of each contaminant also be evaluated as a qualitative indicator of whether or not a toxic contaminant is present. The manufacturer stated that the percent inhibition results for each contaminant do not necessarily increase linearly with the concentration of the contaminant but, depending on the contaminant, can at times be represented by a non-linear relationship that may exhibit parabolic functionality that increases in response, up to a certain concentration, but then begins to decrease. Therefore, in addition to the quantitative evaluation of the data, a qualitative evaluation was performed.

Quality control samples included method blank samples, which consisted of American Society for Testing and Materials Type II deionized water; positive control samples, which were provided 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. EPA QA staff also performed a technical systems audit while testing was being conducted.

TECHNOLOGY DESCRIPTION

The following description of ToxTrak™ was provided by the vendor and was not subjected to verification in this test.

ToxTrak™ system is a colorimetric test based on resazurin dye chemistry. Resazurin is a redox-active dye that, when reduced, changes color from blue to pink. Resazurin is in the oxidized, blue state at the beginning of the test. The bacteria oxidize the glucose added to the sample with the dye and reduce the resazurin. The resazurin is first reduced by two electrons to resorufin, which is pink. Resorufin can be further reduced by two electrons to dihydroresorufin, which is colorless. Dihydroresorufin can be reoxidized by atmospheric oxygen to resorufin. To prevent interference, readings must be taken before a significant amount of resorufin has been reduced. This inhibition or acceleration of resazurin reduction is taken as an indication of toxicity in the test. Substances that are toxic to bacteria can inhibit their metabolism and thus inhibit the rate of resazurin reduction. If the reaction time is too long, the indicator is too far reduced and interference will result. Percent inhibition results of several replicate results that are greater than 10% inhibition or more negative than -10% are indications of toxicity, according to the vendor's protocol. The presence/absence data trend among the four replicates was evaluated to determine if ToxTrak™ consistently indicated the presence (or absence) of the contaminants at the measured concentrations. Three out of four positive responses were required to indicate the presence of a contaminant at that concentration level. If two results were positive and two negative, the overall result was not considered a positive or a negative result.

ToxTrak™ works with different species of bacteria (including both Gram positive and Gram negative species) or mixed cultures. The ToxTrak™ kit includes 12 reusable sample cells with caps, several capsules of dried bacteria, lauryl tryptose broth for culturing the bacteria, 50 ToxTrak™ Reagent Powder Pillows, 15 milliliters (mLs) of ToxTrak™ accelerator solution, 20 sterile transfer pipettes, a test tube rack, forceps, five germicidal cloths, a lab marker, illustrated instructions, and a carrying case. For this verification test, the vendor provided a Hach DR/4000V spectrophotometer for the laboratory-based colorimeter measurements and a Hach DR890 handheld colorimeter for the field measurements. Any colorimeter that can analyze samples at a wavelength at or near 603 nanometers could be used in conjunction with the ToxTrak™ reagents. The ToxTrak™ kit costs $280, and reagent sets cost $100. The reagent kit can be used with the test kit, a spectrophotometer, or a colorimeter The DR/4000V spectrophotometer used in this verification test cost $3,950.

VERIFICATION OF PERFORMANCE

Endpoint and Precision/Toxicity Threshold: The table below presents ToxTrak™ percent inhibition data and the range of standard deviations for the contaminants and potential interferences that were tested. The toxicity thresholds also are shown for each contaminant tested.

Parameter	Compound	Lethal Dose (LD) Conc. (mg/L)	Average Percent Inhibitions at Concentrations Relative to the LD Concentration (Qualitative Result: "+" = present "-" = absent)				Range of Standard Deviations (%)	Toxicity Thresh. (mg/L)	
			LD	LD/10	LD/100	LD/1,000		Quan.	Qual.
Contaminants in DDW	Aldicarb	280	-16 (+)	-7 (-)	12 (+)	-11 (NC)[a]	3–24	ND[b]	280
	Colchicine	240	14 (+)	8 (NC)	-3 (-)	8 (NC)	3–24	ND	240
	Cyanide	250	72 (+)	11 (+)	-6 (-)	-10 (-)	7–17	250	25
	Dicrotophos	1,400	-60 (+)	-53 (+)	-37 (+)	-12 (NC)	14–82	ND	14
	Thallium sulfate	2,400	-104 (+)	-37 (+)	-21 (+)	-38 (+)	22–62	ND	2.4
	Botulinum toxin[c]	0.30	10 (NC)	5 (-)	6 (-)	18 (+)	6–16	ND	ND
	Ricin[d]	15	-32 (+)	-38 (+)	-33 (+)	-45 (+)	11–27	ND	0.015
	Soman	0.15[e]	-6 (NC)	-24 (+)	-21 (+)	-10 (NC)	3–13	ND	ND
	VX	0.22	-16 (+)	-5 (-)	-6 (NC)	9 (-)	8–12	ND	0.22
Potential interferences in DDW	**Interference**	**Conc. (mg/L)**	**Average Inhibitions at a Single Concentration (%)**				**Standard Deviation (%)**		
	Aluminum	0.36	-3 (-)				10		
	Copper	0.65	-6 (NC)				14		
	Iron	0.069	-36 (+)				23		
	Manganese	0.26	11 (NC)				10		
	Zinc	3.5	-17 (NC)				19		

[a] NC = Not consistently positive or negative.
[b] ND = Not detectable.
[c] Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.
[d] Lethal dose solution also contained 3 mg/L phosphate, 26 mg/L sodium chloride, and 2 mg/L sodium azide.
[e] Due to the degradation of soman in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 51% of the expected concentration of 0.30 mg/L.

False Positive/Negative Responses: Inhibition was 45% ± 14% in dechlorinated water from the system disinfected by chlorination for samples analyzed in July. Samples analyzed in September were non-inhibitory. The water sample from a water system disinfected by chloramination was non-inhibitory (-11% ± 11%). Qualitative results were consistent with quantitative results (i.e., both interpretation methods indicated false positive responses with these matrices). According to the quantitative data interpretation, inhibition greater than the negative control was not detected for lethal doses of any contaminant except cyanide (i.e., all contaminants except cyanide produced false negative responses). According to the qualitative data interpretation, botulinum toxin and soman exhibited false negative responses.

Field Portability: ToxTrak™ performance in the field was similar to its performance in the laboratory both qualitatively and quantitatively for the one contaminant (cyanide) that was tested in both locations. The carrying case was not provided by the vendor. A Hach DR890 handheld colorimeter was used for field measurements. Overnight incubation of bacteria may be inconvenient for field deployment.

Other Performance Factors: The pictorial manual was useful, sample handling was easy, and sample throughput was approximately 25 samples per hour. Although the operators had scientific backgrounds, based on the observations of the verification test coordinator, operators with little technical training would probably be able to analyze sample using only the instruction manual as a guide.

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