

June 2006

# Environmental Technology Verification Report

STRATEGIC DIAGNOSTICS INC.  
RAPIDTOXKIT

Prepared by  
Battelle

**Battelle**  
*The Business of Innovation*

Under a cooperative agreement with

 **EPA** U.S. Environmental Protection Agency

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THE ENVIRONMENTAL TECHNOLOGY VERIFICATION  
PROGRAM



## ETV Joint Verification Statement

<b>TECHNOLOGY TYPE:</b>	<b>Rapid Toxicity Testing System</b>	
<b>APPLICATION:</b>	<b>Detecting Toxicity in Drinking Water</b>	
<b>TECHNOLOGY NAME:</b>	<b>RAPIDTOXKIT</b>	
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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](http://www.epa.gov/etv).

ETV works in partnership with recognized standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permittees), 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](http://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	<b>Interference</b>	<b>Conc. (mg/L)</b>	<b>Average Inhibition (%)</b>		<b>Standard Deviation (%)</b>			
	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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NOTICE: ETV verifications are based on an evaluation of technology performance under specific, predetermined criteria and the appropriate quality assurance procedures. EPA and Battelle make no expressed or implied warranties as to the performance of the technology and do not certify that a technology will always operate as verified. The end user is solely responsible for complying with any and all applicable federal, state, and local requirements. Mention of commercial product names does not imply endorsement.

June 2006

# **Environmental Technology Verification Report**

ETV Advanced Monitoring Systems Center

**Strategic Diagnostics Inc.  
RAPIDTOXKIT**

by

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## **Notice**

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, has financially supported and collaborated in the extramural program described here. This document has been peer reviewed by the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use.



## Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technology across all media and to report this objective information to permittees, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of six environmental technology centers. Information about each of these centers can be found on the Internet at <http://www.epa.gov/etv/>.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. Under a cooperative agreement, Battelle has received EPA funding to plan, coordinate, and conduct such verification tests for "Advanced Monitoring Systems for Air, Water, and Soil" and report the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at <http://www.epa.gov/etv/centers/center1.html>.

## **Acknowledgments**

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## List of Abbreviations

AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
ATEL	Aqua Tech Environmental Laboratories
DI	deionized water
DDW	dechlorinated drinking water from Columbus, Ohio
DPD	n,n-diethyl-p-phenylenediamine
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
HDPE	high-density polyethylene
LD	lethal dose
mM	millimolar
μL	microliter
mg/L	milligram per liter
mL	milliliter
mm	millimeter
NSDWR	National Secondary Drinking Water Regulations
%D	percent difference
PE	performance evaluation
QA	quality assurance
QC	quality control
QMP	quality management plan
SOP	standard operating procedure
TSA	technical systems audit



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## **Chapter 1 Background**

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative 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 testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permittees; and with the full participation of 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 EPA's National Exposure Research Laboratory and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of the Strategic Diagnostics Inc. RAPIDTOXKIT. Rapid toxicity technologies were identified as a priority verification category through the AMS Center stakeholder process.

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## Chapter 2 Technology Description

The objective of the ETV AMS Center is to verify the performance characteristics of environmental monitoring technologies for air, water, and soil. This verification report provides results for the verification testing of the RAPIDTOXKIT. Following is a description of the RAPIDTOXKIT, based on information provided by the vendor. The information provided below was not verified in this test.



**Figure 2-1. Strategic Diagnostics Inc.  
RAPIDTOXKIT**

The RAPIDTOXKIT (Figure 2-1) 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



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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.

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## Chapter 3 Test Design

The objective of this verification test of rapid toxicity technologies was to evaluate their ability to detect certain toxins and to determine their susceptibility to interfering chemicals in a controlled experimental matrix. Rapid toxicity technologies do not identify or determine the concentration of specific contaminants, but serve as a screening tool to quickly determine whether water is potentially toxic.

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. Table 3-1 shows the contaminants and potential interferences that were evaluated during this verification test.

This verification test was conducted from August to December 2005 according to procedures specified in the *Test/QA Plan for Verification of Rapid Toxicity Technologies* including Amendments 1 and 2.<sup>(1)</sup> The RAPIDTOXKIT was verified by analyzing a dechlorinated drinking water sample from Columbus, Ohio (hereafter in this report referred to as DDW), fortified with various concentrations of the contaminants and interferences shown in Table 3-1. Where possible, the concentration of each contaminant or potential interference was confirmed independently by Aqua Tech Environmental Laboratories (ATEL), Marion, Ohio, or by Battelle, depending on the analyte.

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

**Table 3-1. Contaminants and Potential Interferences**

<b>Category</b>	<b>Contaminant</b>
Biological toxins	Botulinum toxin complex B, ricin
Botanical pesticide	Nicotine
Carbamate pesticide	Aldicarb
Industrial chemical	Cyanide
Nerve agents	Soman, VX
Organophosphate pesticide	Dicrotophos
Pharmaceutical	Colchicine
Potential interferences	Aluminum, copper, iron, manganese, zinc, chloramination by-products, and chlorination by-products
Rodenticide	Thallium sulfate

- 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 less than 30% when present at lethal concentrations or negative background inhibition that caused falsely low inhibition results or negative background inhibition that caused falsely low inhibition
- Other performance factors (sample throughput, ease of use, reliability).

The RAPIDTOXKIT was used to analyze the DDW samples fortified with contaminants at concentrations ranging from lethal levels to concentrations up to one million times less than the lethal dose. The lethal dose of each contaminant was determined by calculating the concentration at which 250 milliliters (mL) of water would probably cause the death of a 154-pound person. These calculations were based on toxicological data available for each contaminant that are presented in Amendment 2 of the test/QA plan.<sup>(1)</sup> Inhibition (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of the RAPIDTOXKIT to detect toxicity at various concentrations of contaminants, as well as to measure the precision of the RAPIDTOXKIT results.

The response of the RAPIDTOXKIT to compounds used during the water treatment process (identified as potential interferences in Table 3-1) was evaluated by analyzing separate aliquots of DDW fortified with each potential interference at one-half of the concentration limit recommended by the EPA's National Secondary Drinking Water Regulations (NSDWR)<sup>(2)</sup> 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

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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.

### **3.1 Test Samples**

Test samples used in the verification test included drinking water and quality control (QC) samples. Table 3-2 shows the number and type of samples analyzed. QC samples included method blanks and positive and negative control samples. The fortified drinking water samples were prepared from a single drinking water sample collected from the Columbus, Ohio, system. The water was dechlorinated using sodium thiosulfate and then fortified with various concentrations of contaminants and interferences. The DDW containing the potential interferences was analyzed at a single concentration level, while at least four dilutions were analyzed for each contaminant using the RAPIDTOXKIT and additional dilutions of some contaminants were analyzed to better determine the toxicity threshold. Mixtures of contaminants and possible interfering compounds were not analyzed.

#### ***3.1.1 Quality Control Samples***

QC samples included method blanks, positive controls, negative controls, and preservative blanks. The method blank samples consisted of ASTM Type II DI water and were used to help ensure that no sources of contamination were introduced in the sample handling and analysis procedures. A positive control sample was included in the RAPIDTOXKIT and was used as provided from the vendor. While performance limits were not placed on the results, significant inhibition for the positive control sample indicated to the operator that the RAPIDTOXKIT was functioning properly. Two negative control samples were included. One was provided by the vendor. The second consisted of unspiked DDW and was used to set a background inhibition of the DDW, the matrix in which each test sample was prepared. To ensure that the preservatives in the contaminant solutions did not have an inhibitory effect, preservative blank samples were prepared. These preservative blanks consisted of DDW fortified with a concentration of preservative equivalent to that in the test solutions of botulinum toxin complex B, ricin, soman, and VX.

#### ***3.1.2 Drinking Water Fortified with Contaminants***

Approximately 50 liters of Columbus, Ohio, tap water were collected in a low-density polyethylene container. The water was dechlorinated with sodium thiosulfate. Dechlorination was confirmed by adding an n,n-diethyl-p-phenylenediamine (DPD) tablet to a 10-mL aliquot of the water. Lack of color development in the presence of DPD indicated that the water was dechlorinated. All subsequent test samples were prepared from this water sample.

A stock solution of each contaminant was prepared in DDW at concentrations at or above the lethal dose level. The stock solution was further diluted to obtain one sample containing the lethal dose concentration for each contaminant and three additional samples with concentrations 10, 100, and 1,000 times less than the lethal dose. Additional concentrations of some contaminants were prepared and analyzed for two reasons: one was because of the large difference in response between two concentration levels. For example, if only one dilution level was almost completely inhibitory and the next dilution level was non-inhibitory, several intermediate

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concentrations were analyzed to better determine the toxicity threshold of that contaminant. The other reason was because sometimes the lowest concentration analyzed was mostly inhibitory, thus, not providing even an estimate of the toxicity threshold. For these contaminants, additional tenfold dilutions were analyzed to more accurately determine the toxicity threshold. Table 3-2 lists each concentration level and the number of samples analyzed at each level.

### ***3.1.3 Drinking Water Fortified with Potential Interferences***

Individual aliquots of the DDW were fortified with one-half the concentration specified by the EPA's NSDWR for each potential interference. Table 3-2 lists the interferences, along with the concentrations at which they were tested. Four replicates of each of these samples were analyzed. To test the sensitivity of the RAPIDTOXKIT to by-products of the chlorination process as potential interferences, the unspiked DDW (same as the negative control) was used since the water sample originated from a utility that uses chlorination as its disinfectant procedure. In a similar manner, by-products of the chloramination process were evaluated using a water sample from the Metropolitan Water District of Southern California. The residual chlorine in both of these samples was removed using sodium thiosulfate, and then the samples were analyzed in replicate with no additional fortification of contaminants.

## **3.2 Test Procedure**

The procedures for preparing, storing, and analyzing test samples and confirming stock solutions are provided below.

### ***3.2.1 Test Sample Preparation and Storage***

A drinking water sample was collected as described in Section 3.1.2 and, because free chlorine kills the larval crustaceans within the RAPIDTOXKIT reagent and can degrade the contaminants during storage, was immediately dechlorinated with sodium thiosulfate. Dechlorination of the water sample was qualitatively confirmed by adding a DPD tablet to a 10-mL aliquot of the DDW. All the contaminant samples, potential interference samples, preservative blanks, and negative control QC samples were made from this water sample, while the method blank sample was prepared from ASTM Type II DI water. The positive control samples were made by adding the vendor-specified positive control solution to ASTM Type II DI water using calibrated auto-pipettes. All QC samples were prepared prior to the start of the testing and stored at room temperature. The stability of each contaminant for which analytical methods are available was confirmed by analyzing it three times over a two-week period. Throughout this time, each contaminant maintained its original concentration to within approximately 25%. Therefore, the aliquots of DDW containing the contaminants were prepared within two weeks of testing and were stored at room temperature without chemical preservation. The contaminants without analytical methods were analyzed within 48 hours of their preparation. To maintain the integrity of the test, test samples provided to the operators were labeled only with sample identification numbers so that the operators did not know their content.

### ***3.2.2 Test Sample Analysis Procedure***

Prior to test sample analysis, the larval *T. platyurus* was required to be hatched from cysts. This was done by hydrating the cysts for 1 hour in 1 mL of fresh water and then transferring them to

**Table 3-2. Summary of Quality Control and Contaminant Test Samples**

Type of Sample	Sample Characteristics	Concentration Levels	No. of Sample Analyses
Quality control	Method blank (ASTM Type II water)	NA	20
	Positive control (potassium iodide/iodine solution)	Used as provided in kit	20
	Negative control (standard freshwater: moderately hard EPA medium)	Used as provided in kit	76
	Negative control (unspiked DDW)	NA	76
	Preservative blank: botulinum toxin complex B	0.015 millimolar (mM) sodium citrate	4
	Preservative blank: VX and soman	0.21% isopropyl alcohol	4 with VX, 4 with soman
	Preservative blank: ricin	0.00024% NaN <sub>3</sub> , 0.00045 M NaCl, 0.03mM phosphate	4
DDW fortified with contaminants	Aldicarb	260; 26; 2.6; 0.26; 0.026; 0.0026; 0.00026 milligrams/liter (mg/L)	4 per concentration level
	Botulinum toxin complex B	0.30; 0.030; 0.0030; 0.00030 mg/L	4 per concentration level
	Colchicine	240; 24; 2.4; 0.24 mg/L	4 per concentration level
	Cyanide	250; 25; 2.5; 0.25; 0.1875; 0.1250; 0.0625 mg/L	4 per concentration level
	Dicrotophos	1,400; 140; 14; 1.4; 0.140; 0.014; 0.0014 mg/L	4 per concentration level
	Nicotine	2,800; 280; 28; 2.8; 0.28; 0.028; 0.0028 mg/L	4 per concentration level
	Ricin	15; 1.5; 0.15; 0.015 mg/L	4 per concentration level
	Soman	1.4; 0.14; 0.014; 0.0105; 0.0035; 0.0014; 0.007 mg/L	4 per concentration level
	Thallium sulfate	2,800; 280; 28; 2.8 mg/L	4 per concentration level
	VX	2.0; 1.5; 1.0; 0.5; 0.2; 0.02; 0.002; mg/L	4 per concentration level
DDW fortified with potential interferences	Aluminum	0.5 mg/L	4
	Copper	0.6 mg/L	4
	Iron	0.15 mg/L	4
	Manganese	0.25 mg/L	4
	Zinc	2.5 mg/L	4
Disinfectant by-products	Chloramination by-products	NA	4
	Chlorination by-products	NA	76

NA = not applicable, samples not fortified with any preservative, contaminant, or potential interference.

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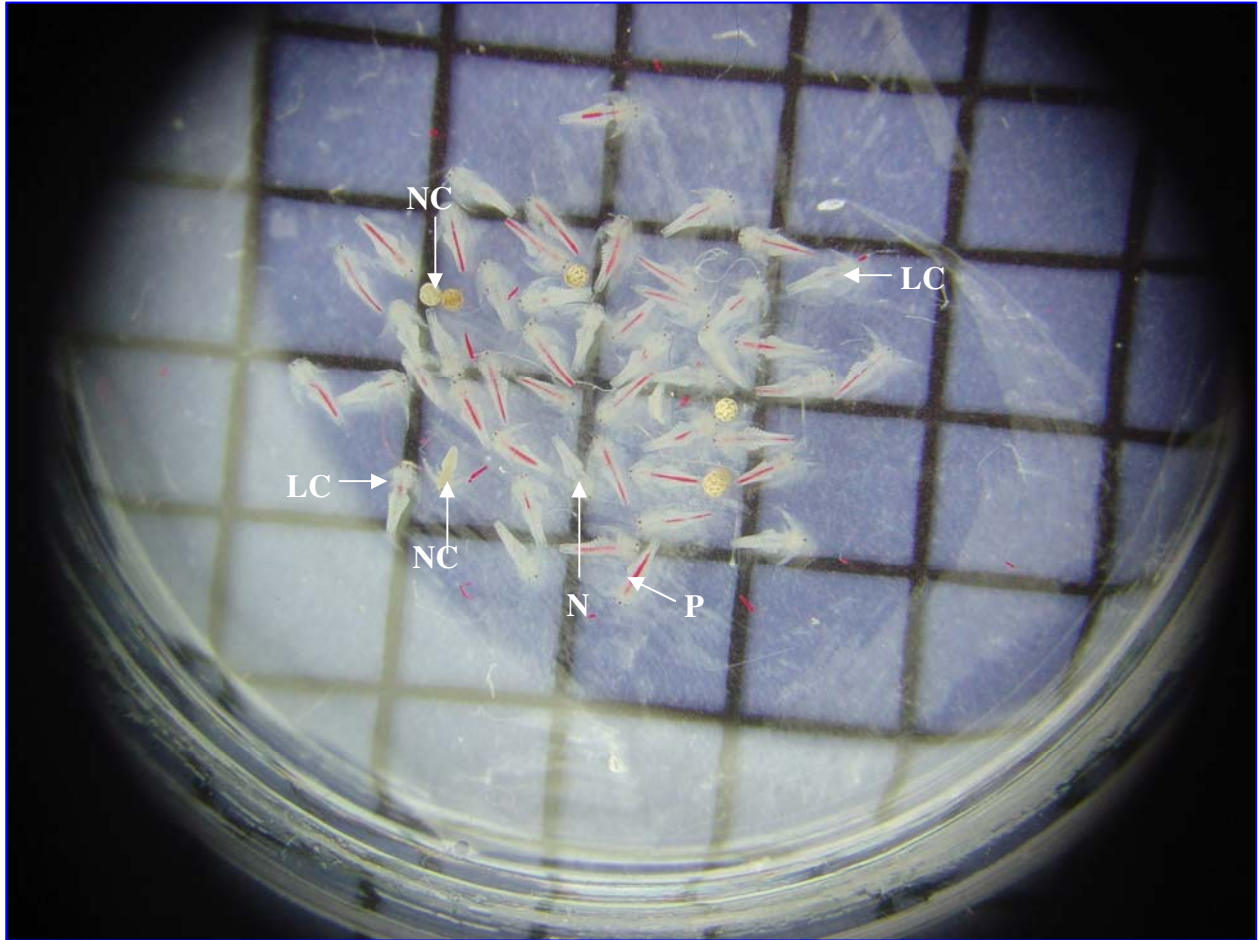
the hatching vessel with 8 mL of fresh water for an incubation time of 30 to 45 hours at 25°C with continuous illumination. Afterward, the test organisms were transferred into the sub-sampling tube until use. The test organisms must be used within 30 and 45 hours after the start of incubation.

Once the organisms were hatched, the test tubes were filled up to the horizontal mark with both control (positive and negative) and test sample solutions. The larvae were distributed evenly throughout the suspension in the sub-sampling tube by repeated aspiration and dispensation of the water/larvae suspension from a pipette, and 0.5 mL of larval suspension was transferred into each control and sample test tube. The samples were then incubated for 1 hour at 25°C. After an hour, 0.2 mL of the microsphere bead suspension (mixed well) was added to each sample test tube. The test tubes were then shaken gently to homogenize the contents. The tubes were incubated for 15 to 30 minutes at 25°C. Then, three drops of fixative (which kills the larvae) were added to each test tube, and the samples were mixed again.

After a 5-minute wait to allow the dead larvae to settle to the bottom of the test tubes, the micro-pipette was set to 0.2 mL; and all the larvae from the bottom of each test tube were transferred into wells on the observation plate. The observation plate was covered with a transparent cover and placed underneath the microscope. A magnification was selected that allowed a complete view of the well surface (example shown in Figure 3-1). The total number of larvae in the well (colored and not colored digestive tracts) was counted, as well as the number of larvae with distinct colored digestive tracts. In the figure, an example of a colored digestive tract is labeled “P” for positive microparticle uptake, indicating that the contaminant was not inhibiting the organism; and an example of a non-colored digestive tract that did not experience microparticle uptake is labeled “N” for negative. The uptake of colored particles may vary among larvae. Lightly colored larvae were still counted as positive. Two examples of these are labeled “LC” on the figure. In addition, because not all of the cysts hatch completely, among the larvae collected for the analysis, there will still be some at an early stage of development (smaller, orange in color, and not transparent). These opaque larvae were completely excluded from the scoring. In the figure, two larvae with these characteristics are labeled “NC” for not counted. The observation plate was placed on a grid to make counting the larvae easier. In general, fully developed larvae with colored digestive tracts have not been inhibited by a contaminant, while those without have. Two operators performed all the analyses using the RAPIDTOXKIT. One operator performed testing with contaminants that did not require special chemical and biological agent training and one performed testing with those that did. Both held bachelor’s degrees in the sciences and were trained by the vendor to operate the RAPIDTOXKIT.

### ***3.2.3 Stock Solution Confirmation Analysis***

The concentrations of the contaminant and interfering compound stock solutions were confirmed with standard analytical methods, with the exception of colchicine, ricin, and botulinum toxin complex B—contaminants without standard analytical methods. Aliquots to be analyzed by standard methods were preserved as prescribed by the method. In addition, the same standard methods were used to measure the concentration of each contaminant/potential interference in the unspiked DDW so that background concentrations of contaminants or potential interferences were accounted for within the displayed concentration of each contaminant/potential interference sample. Table 3-3 lists the standard methods used to measure each analyte; the results from the stock solution confirmation analyses (obtained by analyzing the lethal dose concentration for the



**Figure 3-1. Magnification of *T. platyurus* on Observation Plate.** P = positive, LC = lightly colored (still positive), N = negative, and NC = not counted.

contaminants and the single concentration that was analyzed for the potential interferences); and the background levels of the contaminants and potential interferences measured in the DDW sample, which were all non-detect or negligible.

Standard methods were also used to characterize several water quality parameters such as alkalinity; dissolved organic carbon content; specific conductivity; hardness; pH; concentration of haloacetic acids, total organic carbon, total organic halides, and trihalomethanes; and turbidity. Table 3-4 lists these measured water quality parameters for both the water sample collected in Columbus, Ohio, representing a water system using chlorination as the disinfecting process, and the water sample collected at the Metropolitan Water District of Southern California, representing a water system using chloramination for disinfection.



**Table 3-3. Stock Solution Confirmation Results**

	<b>Method</b>	<b>Average Concentration ± Standard Deviation N = 4 (mg/L)<sup>(b)</sup></b>	<b>Background in DDW (mg/L)</b>
<b>Contaminant</b>			
Aldicarb	Battelle method	260 ± 7	<0.005
Botulinum toxin complex B	<sup>(a)</sup>	NA	NA
Colchicine	<sup>(a)</sup>	NA	NA
Cyanide	EPA 335.3 <sup>(3)</sup>	249 ± 4 296 ± 26 (field portability)	0.006
Dicrotophos	Battelle method	1,168 ± 18	<3.0
Nicotine	Battelle method	2,837 ± 27	<0.01
Ricin	<sup>(a)</sup>	NA	NA
Soman	Battelle method	1.3 ± 0.1 (10/18/05) 1.16 ± 0.06 (10/21/05)	<0.025
Thallium sulfate	EPA 200.8 <sup>(4)</sup>	2,469 ± 31	<0.001
VX	<sup>(a)</sup> Battelle method	1.89 ± 0.08 (10/17/05) 1.77 ± 0.03 (10/20/05)	<0.0005
<b>Potential Interference</b>			
Aluminum	EPA 200.7 <sup>(5)</sup>	0.50 ± 0.02	<0.2
Copper	EPA 200.7 <sup>(5)</sup>	0.60 ± 0.03	<0.02
Iron	EPA 200.7 <sup>(5)</sup>	0.155 ± 0.006	<0.04
Manganese	EPA 200.7 <sup>(5)</sup>	0.281 ± 0.008	<0.01
Zinc	EPA 200.7 <sup>(5)</sup>	2.63 ± 0.05	0.27

NA = Not applicable.

<sup>(a)</sup> No standard method available. QA audits and balance calibration assured accurately prepared solutions.

<sup>(b)</sup> Target concentration was highest concentration for each contaminant or interference on Table 3-2.

**Table 3-4. Water Quality Parameters**

<b>Parameter</b>	<b>Method</b>	<b>Dechlorinated Columbus, Ohio, Tap Water (disinfected by chlorination)</b>	<b>Dechlorinated Southern California Tap Water (disinfected by chloramination)</b>
Alkalinity (mg/L)	SM 2320 B <sup>(6)</sup>	40	71
Specific conductivity (µmho)	SM 2510 B <sup>(6)</sup>	572	807
Hardness (mg/L)	EPA 130.2 <sup>(7)</sup>	118	192
pH	EPA 150.1 <sup>(7)</sup>	7.6	8.0
Total haloacetic acids (µg/L)	EPA 552.2 <sup>(8)</sup>	32.8	17.4
Dissolved organic carbon (mg/L)	SM 5310 B <sup>(6)</sup>	2.1	2.9
Total organic carbon (mg/L)	SM 5310 B <sup>(6)</sup>	2.1	2.5
Total organic halides (µg/L)	SM 5320B <sup>(6)</sup>	220	170
Total trihalomethanes (µg/L)	EPA 524.2 <sup>(9)</sup>	74.9	39.2
Turbidity (NTU)	SM 2130 <sup>(10)</sup>	0.1	0.1

NTU = nephelometric turbidity unit.

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## **Chapter 4**

### **Quality Assurance/Quality Control**

QA/QC procedures were performed in accordance with the quality management plan (QMP) for the AMS Center<sup>(11)</sup> and the test/QA plan for this verification test.<sup>(1)</sup>

#### **4.1 Quality Control of Stock Solution Confirmation Methods**

The stock solutions for the contaminants cyanide and thallium sulfate and for the potential interferences aluminum, magnesium, zinc, iron, and copper were analyzed at ATEL using standard reference methods. As part of ATEL's standard operating procedures (SOPs), various QC samples were analyzed with each sample set. These included matrix spike, laboratory control spike, and method blank samples. According to the standard methods used for the analyses, recoveries of the QC spike samples analyzed with samples from this verification test were within acceptable limits of 75% to 125%, and the method blank samples were below the detectable levels for each analyte. For VX, soman, aldicarb, nicotine, and dicrotophos, the confirmation analyses were performed at Battelle using a Battelle SOP or method. Calibration standard recoveries of VX and soman were always between 62% and 141%, and most of the time were between 90% and 120%. Dicrotophos standard recoveries ranged from 89% to 122%. Aldicarb standard recoveries ranged from 95% to 120%. Nicotine standard recoveries ranged from 96% to 99%. Standard analytical methods for colchicine, ricin, and botulinum toxin complex B were not available and, therefore, not performed. QA audits and balance calibrations assured that solutions for these compounds were accurately prepared.

#### **4.2 Quality Control of Drinking Water Samples**

A method blank sample consisting of ASTM Type II DI water was analyzed once by the RAPIDTOXKIT for approximately every 20 drinking water samples that were analyzed. Because inhibition has to be calculated with respect to a control sample, none were calculated for the method blank samples. The method blanks were used as the control for calculating the background inhibition of the DDW for the disinfection by-product evaluation. A positive control solution of potassium iodide and iodine also was analyzed once for approximately every 20 drinking water samples. While performance limits were not placed on the results of the positive control sample, the vendor informed Battelle that, if the positive control samples did not cause significant inhibition, it would indicate to the operator that the RAPIDTOXKIT was not functioning properly. For 20 positive control samples, inhibition was complete (100%) in each

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case. A negative control sample (unspiked DDW) was analyzed with approximately every four samples. The percent inhibition calculation for each sample incorporated the average inhibition of the negative control samples analyzed with that particular sample set; therefore, by definition, the average inhibition of four negative control samples was 0%. The negative control included in the kit was analyzed once for approximately every 20 drinking water samples. This freshwater control sample was analyzed to confirm the viability of the RAPIDTOXKIT organisms. Strategic Diagnostics Inc. recommended that in the freshwater control the organisms have a minimum survival rate of 50% before any samples were analyzed. This requirement was met for each sample set and, on average, the survival rate (ratio of surviving to total organisms) in the freshwater negative control was  $70\% \pm 10\%$  for the 76 freshwater samples that were analyzed. Results for this negative control were similar to those obtained for the method blank and the DDW negative control, which had average survival rates of  $67\% \pm 11\%$  (N=20) and  $57\% \pm 13\%$  (N=76), respectively.

### 4.3 Audits

A performance evaluation (PE) audit, a technical systems audit (TSA), and an audit of data quality were performed for this verification test.

#### 4.3.1 Performance Evaluation Audit

The accuracy of the reference method used to confirm the concentrations of the stock solutions of the contaminants and potential interferences was confirmed by analyzing solutions of each analyte from two separate commercial vendors. The standards from one source were used to prepare the stock solutions during the verification test, while the standards from a second source were analyzed as the PE sample. The percent difference (%D) between the measured concentration of the PE sample, and the nominal concentration of that sample was calculated using the following equation:

$$\%D = \frac{M}{A} \times 100\% \quad (1)$$

where  $M$  is the absolute value of the difference between the measured and the nominal concentration, and  $A$  is the nominal concentration. The %D between the measured concentration of the PE standard and the nominal concentration had to be less than 25% for the measurements to be considered acceptable. Table 4-1 shows the results of the PE audit for each compound. All %D values were less than 25.

PE audits were performed when more than one source of the contaminant or potential interference was commercially available and when methods were available to perform the confirmation; therefore, PE audits were not performed for all of the contaminants. To assure the purity of the other standards, documentation, such as certificates of analysis, was obtained for colchicine, botulinum toxin complex B, and ricin. In the cases of VX and soman, which were obtained from the U.S. Army, the reputation of the source, combined with the confirmation analysis data, provided assurance of the concentration analyzed.

**Table 4-1. Summary of Performance Evaluation Audit**

		<b>Measured Concentration (mg/L)</b>	<b>Nominal Concentration (mg/L)</b>	<b>%D</b>
Contaminant	Aldicarb	0.057	0.050	14
	Cyanide	1,025	1,000	3
	Dicrotophos	1.10	1.00	10
	Nicotine	0.120	0.100	20
	Thallium	1,010	1,000	1
Potential interference	Aluminum	960	1,000	4
	Copper	1,000	1,000	0
	Iron	960	1,000	4
	Manganese	922	1,000	8
	Zinc	1,100	1,000	10

#### **4.3.2 Technical Systems Audit**

The Battelle Quality Manager conducted a TSA to ensure that the verification test was performed in accordance with the test/QA plan<sup>(1)</sup> and the AMS Center QMP.<sup>(11)</sup> As part of the audit, the Battelle Quality Manager reviewed the contaminant standard and stock solution confirmation methods, compared actual test procedures with those specified in the test/QA plan, and reviewed data acquisition and handling procedures. Observations and findings from this audit were documented and submitted to the Battelle Verification Test Coordinator for response. No findings were documented that required any significant action. The records concerning the TSA are permanently stored with the Battelle Quality Manager.

#### **4.3.3 Audit of Data Quality**

At least 10% of the data acquired during the verification test were audited. Battelle's Quality Manager traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting, to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

#### **4.4 QA/QC Reporting**

Each internal assessment and audit was documented in accordance with Sections 3.3.4 and 3.3.5 of the QMP for the ETV AMS Center.<sup>(11)</sup> Once the assessment report was prepared, the Battelle Verification Test Coordinator ensured that a response was provided for each adverse finding or

potential problem and implemented any necessary follow-up corrective action. The Battelle Quality Manager ensured that follow-up corrective action was taken. The results of the TSA were sent to the EPA.

#### 4.5 Data Review

Records generated in the verification test were reviewed before they were used to calculate, evaluate, or report verification results. Table 4-2 summarizes the types of data recorded. The review was performed by a technical staff member involved in the verification test, but not the staff member who originally generated the record. The person performing the review added his/her signature or initials and the date to a hard copy of the record being reviewed.

**Table 4-2. Summary of Data Recording Process**

<b>Data to be Recorded</b>	<b>Responsible Party</b>	<b>Where Recorded</b>	<b>How Often Recorded</b>	<b>Disposition of Data<sup>(a)</sup></b>
Dates, times of test events	Battelle	Laboratory record books	Start/end of test, and at each change of a test parameter	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Sample preparation (dates, procedures, concentrations)	Battelle	Laboratory record books	When each sample was prepared	Used to confirm the concentration and integrity of the samples analyzed; procedures entered into laboratory record books
Test parameters (contaminant concentrations, location, etc.)	Battelle	Laboratory record books	When set or changed	Used to organize/check test results, manually incorporated in data spreadsheets as necessary
Stock solution confirmation analysis, sample analysis, chain of custody, and results	Battelle or contracted laboratory	Laboratory record books, data sheets, or data acquisition system, as appropriate	Throughout sample handling and analysis process	Transferred to spreadsheets/agreed upon report

<sup>(a)</sup> All activities subsequent to data recording were carried out by Battelle.

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## Chapter 5

### Statistical Methods and Reported Parameters

The statistical methods presented in this chapter were used to verify the performance parameters listed in Section 3.

#### 5.1 Endpoints and Precision

The raw data for the RAPIDTOXKIT was collected by observing the test organisms underneath a microscope. The organisms affected by the toxicant did not take up the colored particles and, therefore, appeared completely colorless. As described in Section 3.2.2, organisms that were lightly colored were considered positive. The total number of organisms present and the number of affected organisms were counted. Each test sample containing contaminants was compared with a negative control sample that, for this verification test, was unspiked DDW. The negative control supplied with the kit (freshwater) was analyzed for QC purposes, but was not used in the percent inhibition calculation. This comparison was made by accounting for the inhibition of the negative control in the calculation of the percent inhibition. Therefore, the percent inhibition of the four negative control samples within each sample set always averaged zero. The percent uptake (%*U*) and percent inhibition (%*I*) for each sample was calculated using the following equations:

$$\% \text{ uptake} = \frac{\text{colored organisms}}{\text{total organisms}} \times 100\% \quad (2)$$

$$\% \text{ inhibition} = \left( 1 - \frac{\%U_{\text{sample}}}{\overline{\%U}_{\text{negative control}}} \right) \times 100\% \quad (3)$$

Where %*U*<sub>sample</sub> is the percent uptake for each test sample and  $\overline{\%U}_{\text{negative control}}$  is the average %*U* of the four negative control samples analyzed in the same sample set as the subject test sample. The negative control sample was always DDW, except when the inhibition of the disinfectant by-products was being determined, in that case, ASTM Type II DI water served as the control sample.

The standard deviation (*SD*) of the results for the replicate samples was calculated, as follows, and used as a measure of technology precision at each concentration. The standard deviation around the average negative control results represented the variability of the inhibition caused by the negative control water. Similarly, the standard deviation of the rest of the contaminant

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concentrations represented the precision of the inhibition caused by the background water combined with the contaminant.

$$SD = \left[ \frac{1}{n-1} \sum_{k=1}^n (I_k - \bar{I})^2 \right]^{1/2} \quad (4)$$

where  $n$  is the number of replicate samples,  $I_k$  is the percent inhibition measured for the  $k^{\text{th}}$  sample, and  $\bar{I}$  is the average percent inhibition of the replicate samples. Because the average inhibition was frequently near zero for this data set, relative standard deviations often would have greatly exceeded 100%, making the results difficult to interpret. Therefore, the precision results were left in the form of standard deviations of the percent inhibition so the reader could easily view the uncertainty around the average percent inhibition for results that were both near zero and significantly larger than zero.

## 5.2 Toxicity Threshold

The toxicity threshold was defined as the lowest concentration of contaminant to exhibit a percent inhibition significantly greater than the negative control. Also, each concentration level higher than the toxicity threshold had to be significantly greater than the negative control, and the inhibition produced by each lower concentration analyzed had to be significantly less than that produced by the toxicity threshold concentration. Since the inhibition of the test samples was calculated with respect to the inhibition of each negative control sample, the percent inhibition of the negative control was always zero. A significant difference in the inhibition at two concentration levels required that average inhibition at each concentration level, plus or minus its respective standard deviation, did not overlap.

Strategic Diagnostics Inc. suggests that a 30% inhibition be attained for a conclusive indication of toxicity; however, for this test, a more thorough evaluation of sensitivity was performed. Therefore, the toxicity threshold was determined as described here, and the 30% inhibition threshold was used for the false negative/false positive evaluation.

## 5.3 False Positive/Negative Responses

A response was considered false positive if an unspiked drinking water sample produced an inhibition exceeding 30% when determined with respect to DI water. Depending on the degree of background inhibition in a sample, toxicity from subsequent contamination of that sample may not be detectable or could be exaggerated as a result of the baseline inhibition. Drinking water samples collected from water systems using chlorination and chloramination as the disinfecting process were analyzed in this manner.

A response was considered false negative if, when a lethal concentration of some contaminant was analyzed, the average inhibition did not exceed 30%, was not significantly different from the negative control, or was not significantly different from the other concentration levels analyzed (for lethal dose inhibition less than 100%). The inhibition of the lethal dose sample was required to be significantly greater than other concentration levels because it more thoroughly



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incorporated the uncertainty of all the measurements made by the RAPIDTOXKIT in determining a false negative result. A difference was considered significant if the average inhibition plus or minus the standard deviation did not encompass the value or range of values that were being compared.

#### **5.4 Other Performance Factors**

Ease of use (including clarity of the instruction manual, user-friendliness of software, and overall convenience) was qualitatively assessed throughout the verification test through documented observations of the operators and Verification Test Coordinator. Sample throughput was evaluated quantitatively based on the number of samples that could be analyzed per hour.

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## Chapter 6 Test Results

### 6.1 Endpoints and Precision

Tables 6-1 a-p present the percent inhibition data for 10 contaminants; and Table 6-2 gives the percent inhibition for preservatives with concentrations similar to what would be contained in a lethal dose of botulinum toxin complex B, ricin, soman, and VX. Given in each table are the concentrations analyzed, the percent inhibition for each replicate at each concentration, and the average and standard deviation of the inhibition of the four replicates at each concentration.

#### 6.1.1 Contaminants

The RAPIDTOXKIT produced a detectable inhibition for all the contaminants tested, with the exception of botulinum toxin complex B. Aldicarb, cyanide, dicrotophos, nicotine, soman, and VX all exhibited complete inhibition at some concentration level and required additional dilutions to more closely determine the toxicity threshold for each of those contaminants (Tables 6-1b, 6-1f, 6-1h, 6-1j, 6-1m, and 6-1p). As shown in the data tables throughout this chapter, aldicarb, cyanide, and nicotine were detectable in the top four concentrations analyzed; dicrotophos, thallium sulfate, and soman in the top three concentrations analyzed; and ricin and VX in the top one or two concentrations analyzed. The only contaminant that generated an inhibition that was not completely intuitive was colchicine, for which the highest concentration sample (240 mg/L) generated a  $56\% \pm 13\%$  inhibition, the next highest concentration (24 mg/L) generated an inhibition that was not significantly different from the negative control, and the lowest two concentrations generated an inhibition that was both significantly greater than the negative control. Because the 24 mg/L sample was not detectable, the lowest concentration of colchicine considered detectable was the lethal dose concentration.

It is important to note that the botulinum toxin complex B, ricin, soman, and VX stock solutions used to prepare the test samples were stored in various preservatives that included sodium azide, sodium chloride, and sodium phosphate for ricin; sodium citrate only for botulinum toxin complex B; and isopropyl alcohol for soman and VX. During the previous ETV test of this technology category, the preservatives were not accounted for in the negative control; therefore, the results from each test should be interpreted accordingly. The results for this test are more thorough because they show the sensitivity (or lack thereof) to both the preservative and the

**Table 6-1a. Aldicarb Percent Inhibition Results**

<b>Concentration (mg/L)</b>	<b>Inhibition (%)</b>	<b>Average (%)</b>	<b>Standard Deviation (%)</b>
Negative Control	3	0	18
	21		
	-23		
	5		
0.26	57	53	10
	48		
	65		
	44		
2.6	100	100	0
	100		
	100		
	100		
26	100	100	0
	100		
	100		
	100		
260 (Lethal Dose)	100	100	0
	100		
	100		
	100		

**Table 6-1b. Aldicarb Percent Inhibition Results—Additional Dilutions**

<b>Concentration (mg/L)</b>	<b>Inhibition (%)</b>	<b>Average (%)</b>	<b>Standard Deviation (%)</b>
Negative Control	-14	0	21
	16		
	19		
	21		
0.00026	12	-5	24
	4		
	-41		
	4		
0.0026	-29	-26	9
	-38		
	-15		
	-24		
0.026	-17	-12	8
	-18		
	-11		
	-1		
0.26	35	54	13
	62		
	63		
	56		

**Table 6-1c. Botulinum Toxin Complex B Percent Inhibition Results**

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	0	0	9
	-12		
	3		
	9		
0.0003	-28	-32	6
	-32		
	-40		
	-26		
0.003	-47	-40	23
	-60		
	-7		
	-47		
0.03	-61	-51	16
	-33		
	-41		
	-68		
0.3 (Lethal Dose)	-2	4	9
	-4		
	8		
	14		
Preservative Blank	14	12	16
	27		
	-10		
	20		

**Table 6-1d. Colchicine Percent Inhibition Results**

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-17	0	12
	1		
	13		
	2		
0.24	43	28	12
	24		
	15		
	31		
2.4	22	26	9
	28		
	17		
	38		
24	5	13	11
	19		
	26		
	2		
240 (Lethal Dose)	48	56	13
	42		
	69		
	64		

**Table 6-1e. Cyanide Percent Inhibition Results**

<b>Concentration (mg/L)</b>	<b>Inhibition (%)</b>	<b>Average (%)</b>	<b>Standard Deviation (%)</b>
Negative Control	8	0	9
	-12		
	5		
	-1		
0.25	53	51	17
	31		
	73		
	46		
2.5	100	100	0
	100		
	100		
	100		
25	100	100	0
	100		
	100		
	100		
250 (Lethal Dose)	100	100	0
	100		
	100		
	100		

**Table 6-1f. Cyanide Percent Inhibition Results—Additional Dilutions**

<b>Concentration (mg/L)</b>	<b>Inhibition (%)</b>	<b>Average (%)</b>	<b>Standard Deviation (%)</b>
Negative Control	7	0	16
	-19		
	19		
	-7		
0.0625	0	13	18
	31		
	25		
	-4		
0.125	-23	-2	15
	4		
	2		
	11		
0.1875	-6	3	8
	6		
	12		
	1		
0.25	49	53	14
	43		
	45		
	74		

**Table 6-1g. Dicrotophos Percent Inhibition Results**

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-12	0	20
	27		
	-17		
	2		
1.4	-3	-4	6
	-8		
	-9		
	4		
14	100	100	0
	100		
	100		
	100		
140	100	100	0
	100		
	100		
	100		
1,400 (Lethal Dose)	100	100	0
	100		
	100		
	100		

**Table 6-1h. Dicrotophos Percent Inhibition Results—Additional Dilutions**

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	17	0	12
	-4		
	-3		
	-9		
0.0014	11	14	3
	17		
	16		
	13		
0.014	12	17	7
	19		
	12		
	27		
0.14	21	14	13
	-3		
	14		
	26		
1.4	20	6	12
	-7		
	1		
	11		

**Table 6-1i. Nicotine Percent Inhibition Results**

<b>Concentration (mg/L)</b>	<b>Inhibition (%)</b>	<b>Average (%)</b>	<b>Standard Deviation (%)</b>
Negative Control	-21	0	14
	5		
	11		
	6		
2.8	100	100	0
	100		
	100		
	100		
28	100	100	0
	100		
	100		
	100		
280	100	100	0
	100		
	100		
	100		
2,800 (Lethal Dose)	100	100	0
	100		
	100		
	100		

**Table 6-1j. Nicotine Percent Inhibition Results—Additional Dilutions**

<b>Concentration (mg/L)</b>	<b>Inhibition (%)</b>	<b>Average (%)</b>	<b>Standard Deviation (%)</b>
Negative Control	-25	0	28
	31		
	15		
	-22		
0.0028	-42	-47	36
	-30		
	-16		
	-98		
0.028	-7	-1	20
	29		
	-18		
	-6		
0.28	75	56	20
	48		
	69		
	32		
2.8	100	100	0
	100		
	100		
	100		

**Table 6-1k. Ricin Percent Inhibition Results**

<b>Concentration (mg/L)</b>	<b>Inhibition (%)</b>	<b>Average (%)</b>	<b>Standard Deviation (%)</b>
Negative Control	1	0	4
	-6		
	3		
	2		
0.015	10	6	4
	4		
	2		
	11		
Lethal Dose/1,000 Preservative Blank	3	1	8
	8		
	-10		
	3		
0.15	-3	-2	1
	0		
	-2		
	-1		
Lethal Dose/100 Preservative Blank	13	3	14
	17		
	-11		
	-6		
1.5	18	14	6
	12		
	21		
	7		
Lethal Dose/10 Preservative Blank	22	4	13
	2		
	-9		
	1		
15 (Lethal Dose)	26	27	4
	29		
	30		
	22		
Lethal Dose Preservative Blank	3	3	3
	2		
	-9		
	1		



**Table 6-11. Soman Percent Inhibition Results**

<b>Concentration (mg/L)</b>	<b>Inhibition (%)</b>	<b>Average (%)</b>	<b>Standard Deviation (%)</b>
Negative Control	9	0	7
	3		
	-5		
	-7		
0.0014	-2	-2	6
	-6		
	-7		
	6		
0.014	100	100	0
	100		
	100		
	100		
0.14	100	99	3
	95		
	100		
	100		
1.4 (Lethal Dose)	100	100	0
	100		
	100		
	100		
Lethal Dose Preservative Blank	16	26	10
	20		
	31		
	37		

**Table 6-1m. Soman Percent Inhibition Results—Additional Dilutions**

<b>Concentration (mg/L)</b>	<b>Inhibition (%)</b>	<b>Average (%)</b>	<b>Standard Deviation (%)</b>
Negative Control	8	0	7
	-4		
	-7		
	2		
0.0014	4	-18	16
	-27		
	-16		
	-32		
0.0035	5	6	5
	-1		
	7		
	12		
0.007	100	98	3
	100		
	94		
	100		
0.0105	100	100	0
	100		
	100		
	100		
0.014	100	100	0
	100		
	100		
	100		
Lethal Dose Preservative Blank	-5	-3	2
	-1		
	-4		
	-2		

**Table 6-1n. Thallium Sulfate Percent Inhibition Results**

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	13	0	13
	-18		
	6		
	-1		
2.8	48	29	19
	7		
	20		
	40		
28	74	79	9
	91		
	70		
	80		
280	100	100	0
	100		
	100		
	100		
2,800 (Lethal Dose)	100	100	0
	100		
	100		
	100		

**Table 6-1o. VX Percent Inhibition Results**

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-9	0	8
	-2		
	8		
	3		
0.002	20	22	2
	25		
	22		
	20		
0.02	3	4	6
	-3		
	12		
	4		
0.2	5	10	4
	14		
	12		
	9		
2 (Lethal Dose)	100	99	1
	97		
	100		
	100		
Lethal Dose Preservative Blank	25	21	11
	21		
	31		
	5		

**Table 6-1p. VX Percent Inhibition Results—Additional Dilutions**

<b>Concentration (mg/L)</b>	<b>Inhibition (%)</b>	<b>Average (%)</b>	<b>Standard Deviation (%)</b>
Negative Control	-18	0	32
	-23		
	-6		
	47		
0.2	-19	-26	9
	-38		
	-28		
	-19		
0.5	2	6	5
	10		
	11		
	0		
1.0	25	10	10
	6		
	8		
	2		
1.5	100	100	0
	100		
	100		
	100		
2	100	100	0
	100		
	100		
	100		
Lethal Dose Preservative Blank	-21	-20	12
	-27		
	-3		
	-28		

contaminant. In the earlier verification test, toxicity could have been the result of either. Table 3-2 details the concentrations of preservatives in the lethal dose samples of each contaminant. These data could be evaluated in two ways to determine the sensitivity of the RAPIDTOXKIT to contaminants stored in preservatives. The first approach would be to determine the inhibition of the test samples containing preservatives with respect to the background negative control, as was the case for the contaminants not stored in preservatives. This technique, however, could indicate that the RAPIDTOXKIT was sensitive to the contaminant when, in fact, it was sensitive to one of the preservatives. Since these contaminants are only available (either commercially or from the government) in aqueous formulations with the preservatives, this may be appropriate. The second approach would be to fortify negative control samples with the same concentrations of preservative contained in all the samples so that the inhibition resulting from the preservatives could be subtracted from the inhibition caused by the contaminant. This approach would greatly increase the number of samples required for analysis. Therefore, for this test, aspects of both approaches were incorporated without substantially increasing the number of samples. Negative control samples fortified with a concentration of each preservative equivalent to the concentration in the lethal dose test samples (preservative blanks) were analyzed prior to and with every set of test samples. For those sets of test samples for which it was especially difficult to determine whether inhibitory effects were

from the contaminant or the preservative, the preservative blank was diluted identically to all the contaminant samples and analyzed so a background subtraction could take place if necessary.

Interestingly, when the preservative blanks were analyzed prior to the contaminant analysis, all of the preservatives generated detectable inhibition (Table 6-2). The contaminants were analyzed along with only the lethal dose equivalent preservative blank before moving forward with additional dilution levels of the preservative (except for ricin, where dilutions of the preservative blank were performed for each contaminant dilution level). In all four cases during contaminant testing, the inhibition caused by the lethal dose of the preservative blank was less than 30%, the minimum considered to indicate toxicity, according to Strategic Diagnostics Inc.; and, for ricin and botulinum toxin complex B, the inhibition of the lethal dose preservative blank was not significantly different from the negative control. The ricin test samples were analyzed initially along with preservative dilutions; but, for the other three contaminants, further analysis of the preservative blanks was unnecessary because of the lack of toxic effect. It was not clear why the preservative blanks exhibited a toxic effect initially but did not when analyzed with the test samples.

**Table 6-2. Lethal Dose Level Preservative Blank Percent Inhibition Results**

<b>Preservative Blank</b>	<b>Inhibition (%)</b>	<b>Average (%)</b>	<b>Standard Deviation (%)</b>
Negative Control	0	0	13
	-18		
	4		
	13		
Ricin	61	66	6
	70		
	72		
	61		
Soman/VX	49	52	9
	40		
	59		
	58		
Botulinum Toxin Complex B	14	19	5
	16		
	24		
	20		

For botulinum toxin complex B, the lethal dose preservative blank was not significantly different from the negative control, and all the test samples were either negative or not significantly greater than the negative control. Therefore, botulinum toxin complex B apparently does not generate toxic effects on the RAPIDTOXKIT organisms.

The lethal dose concentration of ricin ( $27\% \pm 4\%$ ) resulted in an inhibition that was significantly greater than the negative control ( $0\% \pm 4\%$ ) and the preservative blank ( $3\% \pm 3\%$ ), indicating a slight toxic effect. However, Strategic Diagnostics Inc. suggests that 30% inhibition is the lowest detectable inhibition, so the result is borderline detectable. As previously mentioned, preservative blanks diluted identically to concentrations of each of the other ricin test samples were analyzed. None of these ricin test samples generated an inhibition significantly different from their respective preservative blanks.

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VX and soman were similar in that both of their lethal dose preservative blanks analyzed with the contaminant samples generated an inhibition that was greater than 20% and significantly greater than the negative control. However, in neither case were additional dilutions of the preservative blank analyzed because, in the case of VX, the lethal dose contaminant sample generated an inhibition of nearly 100% and, for soman, the top three concentration levels generated an inhibition of nearly 100%. Therefore, it was clear that almost all of the inhibition exhibited by the test samples was caused by the contaminant and not the preservative. Because of the very strong inhibition, additional dilutions of the test samples were analyzed to more accurately determine the toxicity threshold of each contaminant. The lethal dose preservative blanks were analyzed along with the additional dilutions of VX and soman to confirm whether the preservatives cause inhibitory effects. In neither case did the preservative blanks generate an inhibition greater than the negative control. Again, it is not clear why the preservative blanks caused inhibition upon initial analysis and did not when analyzed with the contaminants. For VX, two of the additional dilutions generated complete inhibition; and, for soman, three of the additional concentration levels also generated complete inhibition.

### ***6.1.2 Potential Interferences***

All of the potential interference samples were prepared in DDW and compared with the negative control to determine the level of inhibition. This determination is crucial because the ability of the RAPIDTOXKIT to detect toxicity is dependent on the organism's ability to take up particles in whatever drinking water matrix is being used. If the background drinking water sample completely inhibits the uptake of particles, inhibition caused by contaminants could not be detected. Table 6-3 presents the results from the samples analyzed to test the effect of potential interferences on the RAPIDTOXKIT organisms. Of the five metal solutions evaluated as possible interferences with the RAPIDTOXKIT, four of them, zinc ( $24\% \pm 9\%$ ), copper ( $100\% \pm 0\%$ ), iron ( $20\% \pm 4\%$ ), and aluminum ( $29\% \pm 6\%$ ), exhibited an inhibition that was significantly different from the DDW negative control ( $0\% \pm 3\%$ ). Because zinc, iron, and aluminum exhibited an inhibition less than that considered a minimum detectable inhibition by Strategic Diagnostics Inc., they should be considered very slight interferences. Therefore, water samples containing similar concentration of metals could be used as a representative negative control sample because there was still enough particle uptake in the presence of these metals to detect any additional inhibition of particle uptake caused by contaminants. Copper, on the other hand, should be considered a possible interference because the organisms' particle uptake would be completely inhibited by the matrix if a similar copper concentration was present, leaving no residual particle uptake to be inhibited by contamination.

To investigate whether the RAPIDTOXKIT is sensitive to by-products of disinfecting processes, DDW samples from water systems that use chlorination and chloramination were analyzed and compared with ASTM Type II DI water as the control sample. In the absence of a background water sample, it seems likely that DI water may be used as a "clean water" control; therefore, it would be helpful to know what the results would be if this is done. The sample from the water supply disinfected by chlorination (N=76) exhibited an average inhibition of  $12 \pm 18\%$ , while the sample from the water supply disinfected by chloramination exhibited an inhibition of  $3\% \pm 8\%$  on four replicates. The difference in the number of replicates is because the dechlorinated water was used as the negative control with each sample set; therefore, much more data were collected

**Table 6-3. Potential Interferences Results**

Potential Interferences	Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative control (Metals)	NA	-2	0	3
		-3		
		4		
		1		
Aluminum	0.5	30	29	6
		30		
		20		
		35		
Copper	0.6	100	100	0
		100		
		100		
		100		
Iron	0.15	19	20	4
		22		
		24		
		14		
Manganese	0.25	-27	-11	11
		-6		
		-10		
		-1		
Zinc	2.5	35	24	9
		15		
		18		
		26		
Negative control (By-products)	NA	4	0	3
		-4		
		-1		
		1		
Chlorination by-products	NA	(a)	12	18
Chloramination by-products	NA	-5	3	8
		2		
		14		
		0		

NA = Not applicable.

<sup>(a)</sup> Average inhibition across all DDW negative control samples (N=76).

on that water. These inhibition data suggest that samples disinfected by either process are not likely to interfere with the RAPIDTOXKIT results because the inhibition caused by the two disinfected drinking water matrices left most of the organisms able to take up particles to potentially be inhibited by subsequent contamination.

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### 6.1.3 Precision

Across all the contaminants and potential interferences, the standard deviation (not relative standard deviation) was measured and reported for each set of four replicates to evaluate the precision of the RAPIDTOXKIT. Out of 105 opportunities, the standard deviation of the four replicate inhibition measurements was less than 10% inhibition 68 times (65% of the time), between 10% and 20% inhibition 29 times (27% of the time), and greater than 20% inhibition just 8 times (8%). As described in Section 3.2.2, the analysis procedure required that each replicate undergo the entire analysis process; therefore, the measurement of precision represents the precision of the analysis method performed on a single water sample on a given day. The precision does not reflect the repeatability of the method across more than one day or more than one preparation of reagents or more than one operator.

### 6.2 Toxicity Threshold

Table 6-4 gives the toxicity thresholds, as defined in Section 5.2, for each contaminant. Note the difference between detectability with respect to the negative control and the toxicity threshold with respect to the other concentration levels analyzed. A contaminant concentration level can have an inhibition significantly different from the negative control (thus detectable), but if its inhibition is not significantly different from the concentration levels below it, it would not be considered the toxicity threshold because in the context of this test, its inhibition would not be distinguishable from that of the lower concentrations. The lowest toxicity threshold concentration was for soman at 0.007 mg/L. Only botulinum toxin complex B did not generate detectable inhibition at some concentration level.

**Table 6-4. Toxicity Thresholds**

Contaminant	Concentration (mg/L)
Aldicarb	0.26
Botulinum toxin complex B	ND
Colchicine	240
Cyanide	0.25
Dicrotophos	14
Nicotine	0.28
Ricin	15
Soman	0.007
Thallium sulfate	28
VX	1.5

ND = Significant inhibition was not detected.



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### 6.3 False Positive/Negative Responses

No results from the RAPIDTOXKIT were considered false positive because the chlorination and chloramination by-product samples did not inhibit particle uptake in 30% of the exposed organisms. Since the background inhibition is not complete, it can be accounted for by using negative control samples that are very similar to the water being analyzed. If samples are analyzed daily, a good practice would be to archive a negative control sample each day in case of contamination the next day.

Table 6-5 shows the RAPIDTOXKIT false negative responses, which are described in Section 5.3. Only botulinum toxin complex B did not exhibit a detectable inhibition at the lethal concentration. Ricin's average inhibition at the lethal dose was  $27\% \pm 4\%$ , not meeting the minimum requirement set by the vendor. It was not, however, considered false negative because the relatively small uncertainty around the average encompassed 30%.

**Table 6-5. False Negative Responses**

<b>Contaminant</b>	<b>Lethal Dose Concentration (mg/L)</b>	<b>False Negative</b>
Aldicarb	260	No
Botulinum toxin complex B	0.30	Yes
Colchicine	240	No
Cyanide	250	No
Dicrotophos	1,400	No
Nicotine	2,800	No
Ricin	15	No <sup>(a)</sup>
Soman	1.4	No
Thallium sulfate	2,800	No
VX	2.0	No

<sup>(a)</sup> Inhibition was  $27\% \pm 4\%$ ; vendor suggests 30% as minimum indicator of toxicity, but it was not considered false negative because relatively small uncertainty encompassed 30%.

### 6.4 Other Performance Factors

#### 6.4.1 Ease of Use

RAPIDTOXKIT contained clearly written instructions and illustrations. The detailed information on which organisms to count and which to disregard was especially useful. Microscope slides with a grid facilitated the process of counting the organisms. Contents of the RAPIDTOXKIT were well identified. The tubes in which the cysts were stored before hatching were somewhat difficult to open, while the test tubes for the test waters were extremely difficult to open. The vendor indicated that a design change for these tubes was underway. The only problem, other

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than the difficulty opening some containers, was a slight difficulty getting the cysts out of the tubes with the recommended 1 mL of water. The cysts tended to sink to the bottom of the tube and get stuck, often requiring more than 1 mL to transfer. Additionally, the test organisms could be used only 30 to 45 hours after starting the hatching process. This also may make spontaneous testing problematic. According to Strategic Diagnostics Inc., a programmable incubator is now available to pre-program cyst hatching to make the use of living test organisms more convenient.

Reagents were easy to prepare. Storage conditions were not indicated on the reagent containers, but were noted on a warning label on the RAPIDTOXKIT and also in the manual. The RAPIDTOXKIT warning label indicated that the cysts, microspheres, and fixative needed to be refrigerated, while the manual indicated that only the cysts needed to be refrigerated.

All the necessary supplies were provided with the kit except for pipettes with tips, an incubator used to hatch the cysts, and a microscope. 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. Highly colored organisms were easy to identify; but, if only a small amount of red spheres had been ingested, identification was more difficult. The microscope was easily wiped clean and did not require significant routine maintenance.

No formal scientific education would be required to use the RAPIDTOXKIT. However, good laboratory skills, especially pipetting, would be beneficial. Basic math skills are required for interpreting results. Verification testing staff were able to use the RAPIDTOXKIT after a two-hour training session. Test tubes, observation plates, and pipette tips were generated as solid waste. It was not stated whether the organisms or the fixative solution should be considered hazardous waste.

#### ***6.4.2 Field Portability***

The RAPIDTOXKIT was not evaluated for field portability because the vendor indicated that it was not intended to be used in the field at this time.

#### ***6.4.3 Throughput***

Approximately 25 analyses were completed in three hours. The 25 analyses included method blanks and positive and negative controls, as well as test samples. Note that additional lead time (30 to 45 hours) is required to hatch the cysts. The hatching process took approximately one hour of labor prior to the extended incubation. Throughput evaluations assumed that the cysts were already hatched. A maximum of 45 samples, without replicates, could be processed per kit.

## Chapter 7 Performance Summary

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	<b>Interference</b>	<b>Conc. (mg/L)</b>	<b>Average Inhibition (%)</b>		<b>Standard Deviation (%)</b>			
	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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## Chapter 8 References

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