

June 2006

# Environmental Technology Verification Report

ENVIRONMENTAL BIO-DETECTION  
PRODUCTS INC.  
TOXI-CHROMOTEST

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>Toxi-Chromotest</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 Environmental Bio-Detection Products, Inc. Toxi-Chromotest. This verification statement provides a summary of the test results.

## VERIFICATION TEST DESCRIPTION

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

The Toxi-Chromotest was evaluated by

- Endpoints and precision—color inhibition (as indicator of toxicity) with respect to that of the negative control for all concentration levels of contaminants and potential interfering compounds and consistency of the color change across replicate analyses
- Toxicity threshold for each contaminant—contaminant level at which higher concentrations generate inhibition significantly greater than the negative control and lower concentrations do not
- False positive responses—chlorination and chloramination by-product inhibition with respect to unspiked American Society for Testing and Materials Type II deionized water samples
- False negative responses—contaminants that, when present at lethal concentrations, did not produce any color inhibition with respect to the negative control
- Other performance factors (sample throughput, ease of use, reliability).

The Toxi-Chromotest was verified by analyzing a dechlorinated drinking water sample from Columbus, Ohio (DDW), fortified with contaminants (at concentrations ranging from lethal levels to concentrations up to 1,000 times less than the lethal dose) and interferences (metals possibly present as a result of the water treatment processes). Dechlorinated water was used because free chlorine kills the bacteria within the Toxi-Chromotest reagent and can degrade the contaminants during storage. Inhibition results (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of the Toxi-Chromotest to detect toxicity, as well as to measure the precision of the Toxi-Chromotest results. The response of the Toxi-Chromotest to possible interferents was evaluated by analyzing them at one-half of the concentration limit recommended by the EPA's National Secondary Drinking Water Regulations guidance. For analysis of by-products of the chlorination process, the unspiked DDW was analyzed because Columbus, Ohio, uses chlorination as its disinfectant procedure. For the analysis of by-products of the chloramination process, a separate drinking water sample was obtained from the Metropolitan Water District of Southern California (LaVerne, California), which uses chloramination as its disinfection process. The samples were analyzed after residual chlorine was removed using sodium thiosulfate. Sample throughput was measured based on the number of samples analyzed per hour. Ease of use and reliability were determined based on documented observations of the operators.

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

QA oversight of verification testing was provided by Battelle and EPA. Battelle QA staff conducted a technical systems audit, a performance evaluation audit, and a data quality audit of 10% of the test data.

This verification statement, the full report on which it is based, and the test/QA plan for this verification test are all available at [www.epa.gov/etv/centers/center1.html](http://www.epa.gov/etv/centers/center1.html).

## TECHNOLOGY DESCRIPTION

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

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

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

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

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

## VERIFICATION RESULTS

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

+ = Visually distinguishable color inhibition from that of the negative control was observed.

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

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June 2006

# **Environmental Technology Verification Report**

ETV Advanced Monitoring Systems Center

Environmental Bio-Detection Products Inc.  
Toxi-Chromotest

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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
cm	centimeter
DI	deionized water
DDW	dechlorinated drinking water from Columbus, Ohio
DPD	n,n-diethyl-p-phenylenediamine
EBPI	Environmental Bio-Detection Products Inc.
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.

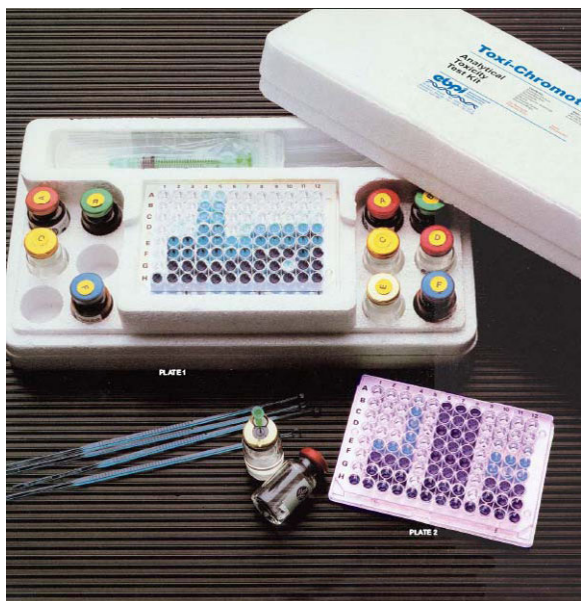
The EPA's National Exposure Research Laboratory and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of the Environmental Bio-Detection Products Inc. (EBPI) Toxi-Chromotest. Rapid toxicity 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 Toxi-Chromotest. Following is a description of the Toxi-Chromotest, based on information provided by the vendor. The information provided below was not verified in this test.

The Toxi-Chromotest (Figure 2-1) detects toxic substances in water, chemicals, pharmaceuticals, food, and body fluids. The Toxi-Chromotest is a bacterial assay based on the ability of toxic materials and antibiotics to inhibit the *de novo* synthesis of an inducible enzyme,  $\beta$ -galactosidase, in a strain of the bacteria, *E. coli* (K12 OR85). The bacteria in the Toxi-Chromotest are exposed to stressing conditions and freeze dried.



To test for toxicity, the bacteria are mixed with a rehydration cocktail containing inducers of the enzyme  $\beta$ -galactosidase and factors necessary for the recovery of the bacteria from their stressed condition. During the recovery phase, toxicants present at sufficient concentrations penetrate the cell walls of the bacteria and inhibit the *de novo* synthesis of the  $\beta$ -galactosidase. The rate of production of the induced enzyme is detected by a reaction of the excreted enzyme with a chromogenic substrate in the bacterial suspension that was exposed to the potential toxicant. Toxic materials above threshold levels interfere with the production of the enzyme and decrease color formation.

**Figure 2-1. EBPI Toxi-Chromotest**

The Toxi-Chromotest kit includes a reaction mixture (the cocktail containing an inducer for the enzyme  $\beta$ -galactosidase and co-factors required for the recovery of the bacteria from their stressed condition), lyophilized bacteria, rehydration solution, a positive control (4 micrograms per milliliter of mercuric chloride in water), a chromogenic substrate (blue chromogen cocktail, ready for use), and diluent for the positive control and test samples. In addition, the Toxi-Chromotest kit contains three 96-well microtiter plates and biohazard bags. The user must supply



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a micropipette for adding the test samples, rehydrated bacteria, and chromogenic substrates to the test wells and an incubator in which the plates containing the bacteria are allowed to recover and begin to produce the enzyme that reacts with the added chromogenic substrate. The incubator must maintain a constant temperature of 37 °C during the 90-minute incubation period.

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

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

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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 Toxi-Chromotest was subjected to various concentrations of contaminants such as industrial chemicals, pesticides, rodenticides, pharmaceuticals, nerve agents, and biological toxins. Each contaminant was added to separate drinking water samples and analyzed. In addition to determining whether the Toxi-Chromotest could detect the toxicity caused by each contaminant, its response to interfering compounds such as water treatment chemicals and by-products in clean drinking water, was evaluated. 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 Toxi-Chromotest 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 Toxi-Chromotest was evaluated by

- Endpoints and precision—color inhibition (as indicator of toxicity) with respect to that of the negative control for all concentration levels of contaminants and potential interfering compounds and consistency of the color change across replicate analyses
- Toxicity threshold for each contaminant— contaminant level at which higher concentrations generate inhibition significantly greater than the negative control and lower concentrations do not

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

Category	Contaminant
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 with respect to unspiked American Society for Testing and Materials (ASTM) Type II deionized (DI) water samples
- False negative responses—contaminants that, when present at lethal concentrations, did not produce any color inhibition with respect to the negative control
- Other performance factors (sample throughput, ease of use, reliability).

The Toxi-Chromotest was used to analyze the DDW samples fortified with contaminants at concentrations ranging from lethal levels to concentrations up to 1,000 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> The decrease in color intensity from four replicates of each contaminant at each concentration level was evaluated to assess the ability of the Toxi-Chromotest to detect toxicity at various concentrations of contaminants, as well as to evaluate the repeatability of the Toxi-Chromotest results.

The response of the Toxi-Chromotest 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 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.

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### **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 Toxi-Chromotest kit. 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 ensure that no sources of contamination were introduced in the sample handling and analysis procedures. A positive control sample was included in the Toxi-Chromotest and was used as provided from the vendor. While performance limits were not placed on the results, a steadily increasing color intensity across a serial dilution indicated to the operator that the Toxi-Chromotest was functioning properly. The negative control consisted of unspiked DDW and was used to set a background color intensity 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 DDW.

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. Table 3-2 lists each concentration level and the number of samples analyzed at each level.

**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	10
	Positive control	4 mg/L mercury chloride (used as provided in kit)	10 serial dilutions
	Negative control (unspiked DDW)	NA	52
	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 molar NaCl, 0.03mM phosphate	4
DDW fortified with contaminants	Aldicarb	260; 26; 2.6; 0.26 milligrams/liter (mg/L)	4 per concentration level
	Botulinum toxin complex B	0.3; 0.03; 0.003; 0.0003 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 mg/L	4 per concentration level
	Dicrotophos	1,400; 140; 14; 1.4; mg/L	4 per concentration level
	Nicotine	2,800; 280; 28; 2.8 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; 0.0014 mg/L	4 per concentration level
	Thallium sulfate	2,800; 280; 28; 2.8 mg/L	4 per concentration level
VX	2; 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	52

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

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### ***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 Toxi-Chromotest 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 bacteria within the Toxi-Chromotest 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 that so that the operators did not know their content.

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

The first step in analyzing the test samples was to reconstitute the lyophilized bacteria by adding the solution in Bottle C to Bottle B, inverting several times, and allowing it to sit for 15 minutes. In the meantime, 200  $\mu$ L of each test sample were added to a well within a 96-well plate. Typically, four wells of each concentration level were analyzed. After the bacteria sat for 15 minutes, 1 mL was transferred into Bottle A, the reaction mixture. This solution was capped and inverted to mix, and 100  $\mu$ L of the mixture were added to all the positive control and test samples. The 96-well plate was placed in an incubator at 37°C for 90 minutes, and then 100  $\mu$ L

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of blue chromogen were added to each of the wells. The samples were returned to the incubator for an additional 90 minutes to allow a period of time for color development. Afterward, each well containing a test sample was compared with the negative control; and, if the color was visibly less intense than the negative control, it was considered detectable. For the purpose of data collection, each detectable solution was compared to the column of positive control samples and assigned the dilution level that its color most closely matched.

For each contaminant, a minimum of the lethal dose concentration and three additional concentration levels were analyzed four times using the Toxi-Chromotest. Only one concentration of each potential interference was analyzed four times. Two operators performed all the analyses using the Toxi-Chromotest. 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 Toxi-Chromotest.

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

The concentrations of the contaminant and interfering compound stock solutions were verified 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 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	(a)	NA	NA
Colchicine	(a)	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	(a)	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	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 Toxi-Chromotest for every 96-well plate of samples that was analyzed. A positive control sample also was analyzed on every plate of samples. Specifically, one column on each plate was dedicated to a serial twofold dilution (consisting of eight concentration levels) of a 4-mg/L mercuric chloride sample provided by the vendor. On every plate analyzed, this increasing color intensity (proportional to the decrease in positive control concentration) was observed in the positive control column, indicating that the proper procedure for the analysis of samples was being followed and that the reagents were performing as expected. A negative control sample (unspiked DDW) was analyzed with approximately every four samples. The color of these samples was compared with that of the test samples to determine the toxic effect of the test sample.

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### 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.

#### 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.

**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

### **4.3.3 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 results from the Toxi-Chromotest were interpreted visually, in a qualitative manner. Overall, the negative control sample made up of DDW and the very dilute positive control samples generated an intense bluish color. Toxic contaminants inhibit the color development; therefore, shades of blue that were less intense than the negative control were detectable. If three out of four replicates exhibited detectable color inhibition, the overall sample was considered to have a positive result (indicated by a “+” in Chapter 6). If two or more of the replicate samples were not detectable, the overall sample was considered a negative result (indicated by a “-” in Chapter 6). The color of the detectable sample wells was compared with the colors of the positive control sample. The dilution level that it matched most was recorded as raw data. If the color was similar to or darker than the negative control, the sample was considered non-detectable (indicated by a “-” in Chapter 6).

Because of the qualitative nature of the results, there was no quantitative measure of reproducibility. Reproducibility was evaluated simply by noting the similarity of the colors that developed in the sample wells. The fraction of sample sets that produced the same color was reported. Examples of how the color development was interpreted are presented in Section 6.1.1.

#### **5.2 Toxicity Threshold**

The toxicity threshold was defined as the lowest concentration of contaminant to exhibit inhibited color development with respect to the negative control. Also, each concentration level higher than the toxicity threshold had to have a less intense color than the negative control.

#### **5.3 False Positive/Negative Responses**

A response would be considered false positive if an unspiked drinking water sample produced a color visibly less intense than the negative control. To test for this possibility, unspiked drinking

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water from a water utility using chlorination, as well as from a utility using chloramination, was analyzed. A response was considered false negative when the Toxi-Chromotest was subjected to a lethal concentration of some contaminant in the DDW, and the color intensity was not decreased with respect to the negative control.

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

Ease of use (including clarity of the instruction manual 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.

## Chapter 6 Test Results

### 6.1 Endpoints

The Toxi-Chromotest was evaluated by visually comparing a negative control and the test sample data. A positive control that consisted of a serial dilution of a 4-mg/L mercuric chloride solution was analyzed with each sample set to confirm the performance of the Toxi-Chromotest. Contaminant test samples in which the bacteria were not inhibited, such as the negative control, were bright blue; while samples containing contaminants that inhibited bacterial recovery exhibited decreased color production. Data collected during the test are summarized in Table 6-1. Contaminants and potential interferences are in the left column, and the concentrations of contaminants with respect to the lethal dose are across the top of the table. The lethal dose concentrations can be found in Table 3-2. A less intense blue than the negative control (indicating a toxic effect) in the majority (defined as three out of four) of four sample replicates is shown with a positive sign, and a color similar to or a more intense blue than the negative control (indicating a non-toxic effect) in the majority of the replicates is shown with a negative sign.

**Table 6-1. Inhibition of Bacterial Recovery in the Presence of Contaminants**

Contaminant	Lethal Dose (LD)	LD/10	LD/100	LD/1,000
Aldicarb	-	-	-	-
Botulinum toxin complex B	-	-	-	-
Colchicine	-	-	-	-
Cyanide	+	+	-	-
Dicrotophos	-	-	-	-
Nicotine	+	-	-	-
Ricin	-	-	-	-
Soman	-	-	-	-
Thallium sulfate	+	+	-	+
VX	-	-	-	-
All interferences	- <sup>(a)</sup>			

<sup>(a)</sup> Only one concentration of possible interferences was analyzed (see Table 3-2).



### 6.1.1 Contaminants and Potential Interferences

As shown in Figure 6-1, the Toxi-Chromotest detected toxic effects in three of the contaminants analyzed: cyanide, thallium sulfate, and nicotine. Cyanide was detected at the lethal dose concentration of 250 mg/L as well as the 25 mg/L level. Thallium sulfate was detected at concentrations of 2,800 mg/L, 280 mg/L, and 2.8 mg/L. The color of the 28-mg/L concentration was not significantly different from the negative control. There was no clear reason why this was the case. Nicotine was only detectable at the highest concentration analyzed. Figure 6-1 shows a fully developed 96-well plate that contains each of these contaminants.

Only ASTM Type II DI water was added to the first column of wells (method blank). No chromogen was added to any of these wells, and no color development was expected. The second column (PC) is the serial dilution of the mercury chloride positive control. The highest concentration of mercury chloride (4 ppm) is at the top of the column, with twofold dilutions in each well down to the bottom (0.031 mg/L). It is clear that color is almost completely depleted in the top two wells, in the third well it is moderately depleted, and, thereafter, less than 0.5 mg/L, the colors in the wells are virtually indistinguishable. The top four rows of the next five columns contain the cyanide solutions. The color is almost completely depleted in the 250 mg/L sample, while the 25 mg/L sample produced a light blue color. For the 25 mg/L sample, the lower three replicates were visually clearly distinguishable from the negative control sample, but the top sample was very similar to the top two negative control samples, causing it to be categorized as negative. Nonetheless, because three out of four results for that concentration level were positive, the result for the 25-mg/L concentration was considered positive. The other two cyanide

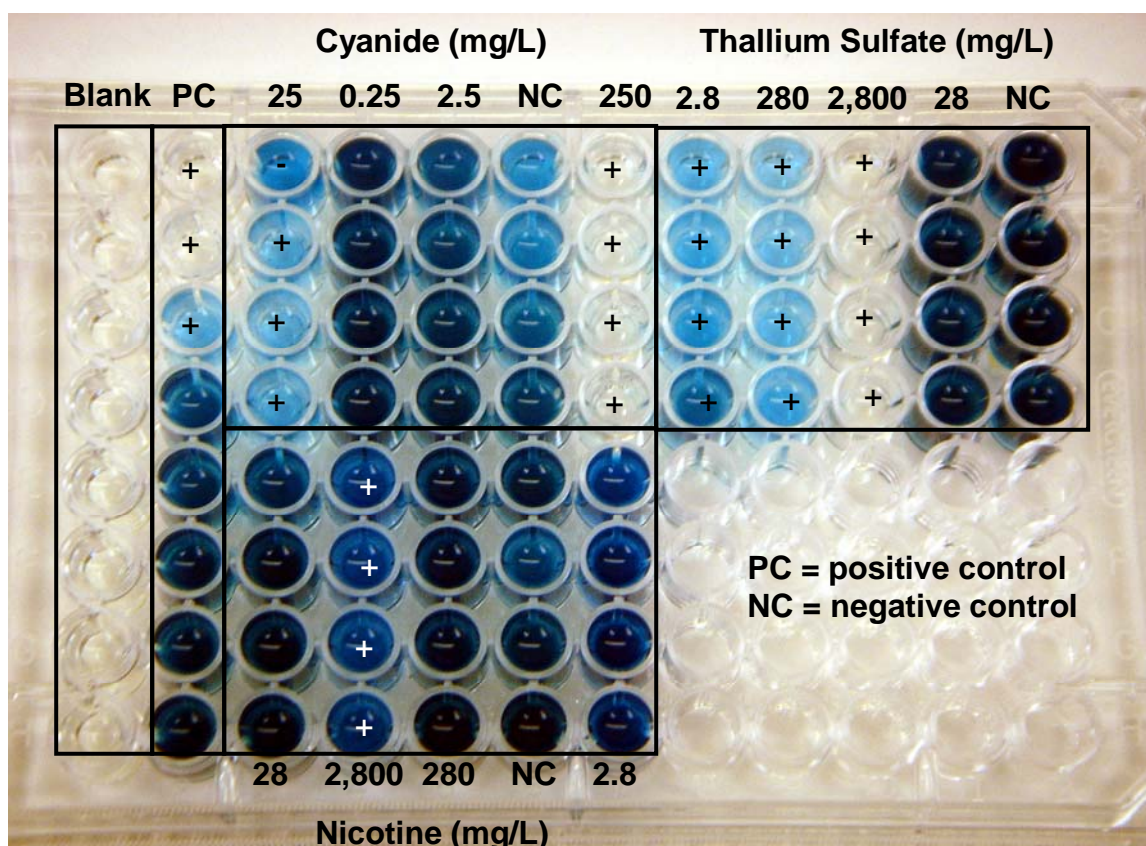


Figure 6-1. Effect of Contaminant on Test Color

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concentrations generated colors that were darker blue than the DDW negative control sample, indicating the lack of a toxic effect at these two concentrations. The top four rows of the next five columns contained the thallium sulfate solutions. The highest concentration of thallium sulfate inhibited all color production in the sample, while the 280-mg/L and 2.8-mg/L concentrations produced a light blue easily distinguishable from the DDW negative control. As mentioned previously, the 28-mg/L concentration, in the wells in the second column from the right, did not produce a change in color distinguishable from the negative control. The bottom four rows in columns three through seven contained the nicotine samples. The only concentration that produced any depleted color was 2,800 mg/L, which appeared to be just slightly less intense than the negative control and the other nicotine samples.

None of the other contaminants inhibited color production in any of the samples. In addition, none of the preservatives used in the stock solutions of ricin, botulinum toxin complex B, soman, and VX; or possible chemical interferences inhibited color production. However, the serial dilution of the mercury chloride positive control produced a gradual color gradient proportional to the concentration on each of the plates used to analyze these samples, indicating proper functioning of the test and reagents.

### **6.1.2 Precision**

Because of the qualitative nature of the Toxi-Chromotest data, no numerical calculation for repeatability was possible. The only measure of repeatability that could be evaluated was whether individual replicates of each sample represented positive or negative responses. All of the sample wells within four replicates of the same concentration had the same result with respect to the negative control except for 25 mg/L cyanide, which exhibited one negative and three positive responses (see discussion in Section 6.1).

## **6.2 Toxicity Threshold**

Table 6-2 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 color inhibition with respect to 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 cyanide at 25 mg/L. Thallium sulfate was also detected at 2.8 mg/L; but the 28-mg/L sample was not detectable, causing the toxicity threshold to become 280 mg/L.

## **6.3 False Positive/Negative Responses**

The Toxi-Chromotest did not generate any results that could be considered false positive. Neither the chlorination nor chloramination by-product samples generated detectable toxicity.

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## 6.4 Other Performance Factors

### 6.4.1 Ease of Use

The Toxi-Chromotest step-by-step instructions were easy to read; however, the letters with which the solutions were identified did not correspond to the solution names. For example, the diluent was labeled “G,” while mercury chloride was labeled “D,” which was confusing because there was a tendency to think of diluent as “D.” Storage conditions were marked on the vial labels. The reaction of the Toxi-Chromotest could be observed visually or with a plate reader. For this evaluation, color intensity was determined by visually comparing the samples with the

**Table 6-2. Toxicity Thresholds**

Contaminant	Concentration (mg/L)
Aldicarb	ND
Botulinum toxin complex B	ND
Colchicine	ND
Cyanide	25
Dicrotophos	ND
Nicotine	2,800
Ricin	ND
Soman	ND
Thallium sulfate	280
VX	ND

ND = Significant color depletion was not observed.

**Table 6-3. False Negative Responses**

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

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positive control. Visual observation of reactions was difficult when there were only slight variations in color intensity.

The Toxi-Chromotest requires two 1.5-hour incubation periods; and, after the 15-minute bacterial rehydration, the hydrated bacteria could be used for only one hour. Because all reagents started out colorless, it was somewhat difficult to tell whether reagents had been added and difficult to observe the level of reagents in the wells. All reagents were stored in a refrigerator. An expiration date was listed on the vial labels, but there was no indication how long they were good once they were opened.

All the necessary supplies were provided with the Toxi-Chromotest except for pipettes with tips. No formal scientific training would be required to use the Toxi-Chromotest. However, good laboratory skills, especially in pipetting, would be helpful. Verification testing staff were able to use the Toxi-Chromotest after a 30-minute training session.

The Toxi-Chromotest generated a small amount of aqueous waste, pipette tips, and 96-well plates as waste. It was not clear whether the bacteria or other reagents should be considered hazardous waste. Providing this information in the instructions or on reagent vials would be helpful.

#### **6.4.2 Field Portability**

The Toxi-Chromotest was transported from a laboratory setting to a storage room for the field portability evaluation. The storage room contained several tables and light and power sources, but no other laboratory facilities. The Styrofoam container that reagents were shipped in was used to carry the Toxi-Chromotest and related supplies. The incubator was carried separately. One person could carry the kit and incubator; but, when laboratory peripherals, such as pipettes, were added, it became more convenient to use a cart. The Toxi-Chromotest was set up easily in only a few minutes. A source of electricity for the incubator and a flat surface of approximately 45 by 60 cm on which to fill the plate wells were required for analysis. The non-rehydrated reagents are good at room temperature for up to one week. A plastic bag in the Toxi-Chromotest was used to collect solid and liquid waste. Because the bacteria must be used within one hour of rehydration, reagents are best prepared in the field. Overall the Toxi-Chromotest was easy to transport to the field and, with an incubator warmed ahead of time, was deployed in a matter of minutes. Results were obtained within 3 to 4 hours of starting the test. At this location, the Toxi-Chromotest was tested using one concentration of cyanide at 250 mg/L. Apparently, there was a problem with the reagents or the procedure because very little color development took place; however, there is no reason to think that this had anything to do with the location where the test was performed.

#### **6.4.3 Throughput**

The number of samples that can be processed depends on the number of replicates per sample and the number of dilutions per sample that are processed on each 96-well plate. One plate can be taken through the procedure in 3 to 4 hours. Each Toxi-Chromotest kit contained materials to process samples filling three 96-well plates.

## Chapter 7 Performance Summary

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

+ = Visually distinguishable color inhibition from that of the negative control was observed.

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

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## Chapter 8

### References

1. *Test/QA Plan for Verification of Rapid Toxicity Technologies*, Battelle, Columbus, Ohio, June 2003; Amendment 1: June 9, 2005; Amendment 2: August 19, 2005.
2. United States Environmental Protection Agency, *National Secondary Drinking Water Regulations: Guidance for Nuisance Chemicals*, EPA/810/K-92/001, July 1992.
3. U.S. EPA Method 335.3, “Cyanide, Total—Colorimetric, Automated UV,” in *Methods for the Chemical Analysis of Water and Wastes*, EPA/600/4-79/020, March 1983.
4. U.S. EPA Method 200.8, “Determination of Trace Elements in Waters and Wastes by Inductively-Coupled Plasma Mass Spectrometry,” in *Methods for the Determination of Metals in Environmental Samples*, Supplement I, EPA/600/R-94/111, 1994.
5. U.S. EPA Method 200.7, “Trace Elements in Water, Solids, and Biosolids by Inductively Coupled Plasma—Atomic Emission Spectrometry,” EPA-821-R-01-010, January 2001.
6. American Public Health Association, et al. *Standard Methods for the Examination of Water and Wastewater*. 19th Edition, 1997. Washington, DC.
7. U.S. EPA, *Methods for Chemical Analysis of Water and Wastes*, EPA/600/4-79/020.
8. U.S. EPA Method 552.2, “Haloacetic Acids and Dalapon by Liquid-Liquid Extraction, Derivatization and GC with Electron Capture Detector,” *Methods for the Determination of Organic Compounds in Drinking Water—Supplement III* EPA/600/R-95/131.
9. U.S. EPA Method 524.2, “Purgeable Organic Compounds by Capillary Column GC/Mass Spectrometry,” *Methods for the Determination of Organic Compounds in Drinking Water—Supplement III*, EPA/600/R-95/131.
10. American Public Health Association, et al. *Standard Methods for the Examination of Water and Wastewater*, 20th edition, 1998, Washington, DC.

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11. *Quality Management Plan (QMP) for the ETV Advanced Monitoring Systems Center*, Version 5.0, U.S. EPA Environmental Technology Verification Program, Battelle, Columbus, Ohio, December 2004.