September 2006

Environmental Technology Verification Report

ABRAXIS LLC

ORGANOPHOSPHATE/CARBAMATE SCREEN KIT

Prepared by
Battelle

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The Business of Innovation

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

ETV ETV ETV

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Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

Abraxis LLC

ORGANOPHOSPHATE/CARBAMATE SCREEN KIT

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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 permitters, 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.

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List of Abbreviations

ACh-E	acetyl cholinesterase
AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
ATC	acetylthiocholine
ATEL	Aqua Tech Environmental Laboratories, Inc.
Ca	calcium
DI	deionized
DPD	diethyl-p-phenylene diamine
DTNB	5,5′-dithio-bis(2-nitrobenzoic acid)
DW	drinking water
ECD	electron capture detection
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
GB	sarin
GC	gas chromatography
GD	soman
HAZWOPER	Hazardous Waste Operations and Emergency Response
HDPE	high density polyethylene
HMRC	Hazardous Materials Research Facility
ICP	inductively coupled plasma
ID	identification
kg	kilogram
L	liter
LC	liquid chromatography
LD_{50}	lethal dose for half of test subjects
LOD	limit of detection
LRB	laboratory record book
MB	method blank
Mg	magnesium
mg/L	milligram per liter
mL	milliliter
MS	mass spectrometry

µg/L	microgram per liter
µMHO	micromho
NaOH	sodium hydroxide
NDR	negative differential resistance
ng	nanogram
NTU	nephelometric turbidity unit
OP	organophosphate
OP/C	organophosphate/carbamate
PE	performance evaluation
PPE	personal protective equipment
PT	performance test
QA	quality assurance
QC	quality control
QMP	quality management plan
RPD	relative percent difference
SCBA	self-contained breathing apparatus
SM	standard method
SOP	standard operating procedure
TSA	technical systems audit

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 permitters; 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 Abraxis LLC, Organophosphate/Carbamate (OP/C) Screen Kit in detecting chemical agents, carbamate pesticides, and organophosphate pesticides in drinking water. Enzymatic test kits were identified as a priority technology category for verification through the AMS Center stakeholder process.

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 testing the OP/C Screen Kit. Following is a description of the OP/C Screen Kit, based on information provided by the vendor. The information provided below was not verified in this test.

The Organophosphate/Carbamate Screen Kit is an *in vitro* enzymatic test used to detect a wide range of organophosphates (including thiophosphate) and carbamates in water and other environmental matrices. The test is a qualitative, colorimetric assay (modification of the Ellman method) for the detection of organophosphates and carbamates that is based on their inhibition of the enzyme acetyl cholinesterase (ACh-E). ACh-E hydrolyzes acetylthiocholine (ATC), which reacts with 5, 5'- dithio-bis(2-nitrobenzoic acid) (DTNB) to produce a yellow color that is read at 405 or 450 nanometers. Depending on their concentrations, OP or C compounds present in a sample will inhibit ACh-E and therefore color formation will be reduced or absent.

Figure 2-1. Abraxis LLC, Organophosphate/ Carbamate Screen Kit System

The OP/C Screen Kit is supplied with freeze-dried ACh-E and ATC in dropper bottles. Both are reconstituted with diluents supplied in the OP/C Screen Kit. The oxidizer solution is prepared by taking 200 microliters (µL) of the oxidizer and placing it into the dropper bottle containing the oxidizer diluent. All other reagents are ready to use and supplied in color-coded dropper bottles. A 5-minute incubation follows the oxidation of controls and samples. After adding neutralizer and Ach-E, an incubation of 15 to 30 minutes is required; and after adding the ATC (substrate) and DTNB (chromagen), a 30-minute incubation is required. Color development is curtailed by adding stop solution. The tubes are read in a colorimeter at 405 or 450 nanometers. Not supplied is a colorimeter capable of reading 405 or 450 nanometers; however, samples can also be read by visually comparing the sample to the negative control.

The OP/C Screen Kit contains 20 tubes with assay buffer, two test tubes (one to be used for the negative control and ATC diluent and the other for the ACh-E diluent). Dropper bottles with color-coded caps contain the freeze dried ATC and Ach-E and ready-to-use solutions of oxidizer diluent, neutralizer, chromagen (DTNB), and stopper solution. Also included are two 4-milliliter (mL) amber vials that contain the oxidizer and positive control (5 parts per million diazinon in deionized water). There are two 3-mL transfer pipettes and 22 exact-volume 100-µL disposable pipettes included in the kit. The assay incubations are performed at 70±20º F (21±7º C).

The box containing the OP/C Screen Kit is 17 by 10.5 by 9.5 centimeters and can be used as a work station. The price of the OP/C Screening Kit (20 tests) is $180, not including the colorimeter.

Chapter 3
Test Design

3.1 Introduction

Enzymatic test kits, generally designed to be handheld and portable, detect the presence of chemical agents, carbamate pesticides, and/or OP pesticides by relying on the reaction of the cholinesterase enzyme. Under normal conditions, the enzyme reacts as expected with other chemicals present in the test kit. The activity of the enzyme is inhibited, however, by chemical agents, carbamate pesticides, and OP pesticides. The effects of this inhibition will then generally lead to a color change, indicating the presence or absence of these compounds.

The objective of this verification test was to evaluate the ability of the OP/C Screen Kit to detect chemical agents, carbamate pesticides, and OP pesticides in drinking water. This verification test assessed the performance of the OP/C Screen Kit relative to

- Accuracy
- False positive and negative rates
- Precision
- Potential matrix and interference effects
- Operational factors (operator observations, ease of use, and sample throughput).

3.2 Test Samples

This test evaluated the ability of the OP/C Screen Kit to detect VX, sarin (GB), and soman (GD) (chemical agents); aldicarb (carbamate pesticide); and dicrotophos (OP pesticide) in performance test (PT) and drinking water (DW) samples. Quality Control (QC) samples were also included as part of the test matrix to ensure the integrity of the test. Contaminants were tested individually, and stock solutions of each contaminant were prepared separately in American Society for Testing and Materials (ASTM) Type II deionized (DI) water. Samples were prepared in the appropriate matrix using these stock solutions and analyzed on the same day. To minimize the loss of analytes to hydrolysis, contaminant stock solutions prepared in DI water were made on a daily basis. Chemical agent stock solutions were prepared twice daily, once in the morning and once in the afternoon. Aliquots of each stock solution were diluted to the appropriate

concentration using volumetric glassware and volumetric or calibrated pipettes. In some cases, reference solutions were prepared in ASTM Type II DI water using the stock solutions used to prepare the test samples. In other cases, the actual stock solutions were submitted for concentration confirmation by the respective reference analysis (Table 4-1). Aqua Tech Environmental Laboratories, Inc. (ATEL) of Marion, OH performed the physiochemical characterization for each type of DW sample along with reference analyses of the interferent solutions. All other reference analyses were performed at Battelle.

3.2.1 PT Samples

PT samples were prepared separately in ASTM Type II DI water for each contaminant. The first type of PT samples consisted of ASTM Type II DI water spiked with the contaminant at five different concentrations: the lethal dose concentration given in Table 3-1 for each contaminant, along with dilutions at approximately 10, 100, 1,000, and 10,000 times less than the lethal dose. The contaminants were added individually to each spiked sample. The lethal dose of each contaminant was determined by calculating the concentration at which 250 milliliters (mL) of water is likely to cause the death of a 70-kilogram (kg) person based on human oral LD_{50} (lethal dose for half of the test subjects) data.[1,2] Human oral LD_{50} data were not available for aldicarb, so rat oral LD_{50} data were used instead.[3] Each concentration level for the PT samples was analyzed in triplicate.

In addition to the contaminant-only PT samples described above, a second type of PT sample was a potential interferent sample. Three replicates of each interferent PT sample were analyzed to determine the susceptibility of the OP/C Screen Kit to these commonly found interferents in DW. One interferent PT sample contained calcium (Ca) and magnesium (Mg) from carbonates spiked into ASTM Type II DI water, and the other contained humic and fulvic acids isolated from the Elliot River (obtained from the International Humic Substances Society) spiked into ASTM Type II DI water. Each interferent mixture was prepared at two concentration levels: near the upper limit of what would be expected in drinking water (250 milligrams/liter (mg/L) total concentration for Ca and Mg, 5 mg/L total concentration for humic and fulvic acids) and at a mid-low range of what would be expected (50 mg/L total concentration for Ca and Mg, 1 mg/L total concentration for humic and fulvic acids). These spiked interferent levels were confirmed through analysis of aliquots by ATEL. Also, each contaminant was added to these samples, along with the potential interferent, at a concentration consistent with a 10x dilution of the lethal dose. The resulting samples were analyzed in triplicate. Table 3-2 lists the PT samples analyzed in this verification test for each contaminant.

Table 3-1. Lethal Dose of Target Contaminants

Contaminant (common name)	Oral Lethal Dose Concentration	Contaminant Class
VX	2.1 milligrams/liter (mg/L)	Chemical agent
GB (sarin)	20 mg/L	Chemical agent
GD (soman)	1.4 mg/L	Chemical agent
aldicarb	260 mg/L	Carbamate pesticide
dicrotophos	1400 mg/L	Organophosphate pesticide

Table 3-2. Performance Test Samples

Type of PT Sample	Sample Characteristics	Concentrations
Contaminant-only	Contaminants in DI Water	VX: 2.1 to 0.00021 mg/L GB: 20 to 0.002 mg/L GD: 1.4 to 0.00014 mg/L aldicarb: 260 to 0.026 mg/L dicrotophos: 1400 to 0.14 mg/L
Interferent	Contaminants in 1 mg/L humic and fulvic acids	VX: 0.21 mg/L GB: 2 mg/L GD: 0.14 mg/L aldicarb: 26 mg/L dicrotophos: 140 mg/L
	Contaminants in 5 mg/L humic and fulvic acids	
	Contaminants in 50 mg/L Ca and Mg	
	Contaminants in 250 mg/L Ca and Mg	

3.2.2 DW Samples

Table 3-3 lists the DW samples analyzed for each contaminant in this test. DW samples were collected from four geographically distributed municipal sources (Ohio, New York, California, and Florida) to evaluate the performance of the OP/C Screen Kit with various DW matrices. These samples varied in their source, treatment, and disinfection process. All samples had undergone either chlorination or chloramination disinfection prior to receipt. Samples were collected from water utility systems with the following treatment and source characteristics:

- Chlorinated filtered surface water source
- Chlorinated unfiltered surface water source
- Chlorinated filtered groundwater source
- Chloraminated filtered surface water source

Approximately 175 liters (L) of each of the DW samples were collected in pre-cleaned, translucent, low-density polyethylene containers. After sample collection, an aliquot of each DW sample was sent to ATEL to determine the following water quality parameters: concentration of trihalomethanes, haloacetic acids, total organic halides, Ca and Mg, pH, conductivity, alkalinity, turbidity, organic carbon, and hardness. All DW samples were dechlorinated prior to their use with sodium thiosulfate pentahydrate to prevent the degradation of the target contaminants by chlorine. The dechlorination of the DW was qualitatively confirmed by adding a diethyl-p-phenylene diamine (DPD) tablet to an aliquot of DW. If the water did not turn pink, the dechlorination process was successful. If the water did turn pink, additional dechlorinating reagent was added and the dechlorination confirmation procedure repeated. Each DW sample was analyzed before addition of contaminant, as well as after

fortification with each individual contaminant at a single concentration level (10x dilution of the lethal dose). Aliquots of each contaminant stock solution were diluted with DW samples to the appropriate concentration. Each sample was tested in triplicate.

Table 3-3. Drinking Water Samples

Drinking Water Sample Description			Contaminant Concentrations
Water Utility	**Water Treatment**	**Source Type**	
Columbus, Ohio (OH DW)	chlorinated filtered	surface	VX: 0.21 mg/L GB: 2.0 mg/L GD: 0.14 mg/L aldicarb: 26 mg/L dicrotophos: 140 mg/L
New York City, New York (NY DW)	chlorinated unfiltered	surface	
Orlando, Florida (FL DW)	chlorinated filtered	ground	
Metropolitan Water District of Southern California (CA DW)	chloraminated filtered	surface	

3.2.3 QC Samples

QC samples included method blank (MB) samples consisting of ASTM Type II DI water and positive and negative control samples, as provided with each OP/C Screen Kit. Positive and negative control samples were prepared and used according to the protocol provided by the vendor. One set each of duplicate positive and negative control samples were tested with each kit. All MB QC samples were exposed to sample preparation and analysis procedures identical to the test samples. The MB samples were used to ensure that no sources of contamination were introduced in the sample handling and analysis procedures. At least 10% of the test samples (seven samples for each contaminant) were MB samples. For samples involving GD, only five MB samples were run. The test samples and MB samples were analyzed blindly by the operator in that the samples used for analysis were prepared by someone other than the operator and were marked with non-identifying numbers.

3.2.4 Operational Factors

3.2.4.1 Technical Operator

All of the test samples were analyzed by a technical operator who was trained by the vendor. Operational factors such as ease of use and sample throughput were evaluated based on observations recorded by the technical operator and the Verification Test Coordinator. Operational factors were noted during the laboratory portions of the verification test. These observations are summarized to describe the operational performance of the OP/C Screen Kit in this verification.

3.2.4.2 Non-Technical Operator

A subset of the samples was also tested by a non-technical operator using the OP/C Screen Kit. The non-technical operator was someone with little to no laboratory experience who would be

representative of a first responder. For this test, the non-technical operator was a State of Ohio certified firefighter with Hazardous Waste Operations and Emergency Response (HAZWOPER) training. The non-technical operator was trained in the use of the OP/C Screen Kit by another Battelle staff person who was trained by the vendor. Because many of the contaminants being tested are highly toxic and unsafe to be handled outside of a special facility, MB samples and non-toxic positive and negative control samples were analyzed as part of the operational factors assessment. The positive and negative control samples were provided by the vendor and prepared and used according to the vendor's protocol as described in the previous section. Because no samples spiked with the contaminants of interest were used, only the operational aspects of the OP/C Screen Kit were evaluated with the non-technical operator. As the OP/C Screen Kit may be used by first-responders, its performance was evaluated under simulated first-response conditions by having the operator dressed in a Level B protective suit, neoprene latex gloves, boots, and a self-contained breathing apparatus (SCBA). The operator had prior experience working in personal protective equipment (PPE). One set of MB samples was also tested without the use of PPE. Ease of use from the perspective of the operator was documented both with and without the PPE.

3.3 Verification Schedule

The verification test of the OP/C Screen Kit took place from November 2005 through February 2006 at Battelle facilities in Columbus and West Jefferson, Ohio.

3.4 Test Procedure

3.4.1 Test Sample Preparation and Storage

All testing for this verification test was conducted within Battelle laboratories. Aldicarb and dicrotophos samples were tested at Battelle's Columbus laboratories, while VX, GB, and GD samples were tested at Battelle's Hazardous Materials Research Center (HMRC) facility in West Jefferson, OH. Appropriate safety guidelines associated with each laboratory were followed throughout the verification test. Samples were prepared fresh each day from stock solutions in either DI water, an interferent matrix, or a DW matrix. Sample solutions were prepared to the specified concentration based on the concentration of the stock solution, which was confirmed through reference analysis. Test solutions were prepared in 1L quantities such that appropriate aliquots (100 μL) of the sample preparation could be used for each test sample. Triplicate samples of 100 μL each were taken from the same sample preparation. Each sample was placed in its own container and labeled only with a sample identification number that was also recorded in a laboratory record book (LRB) along with details of the sample preparation.

3.4.2 Test Sample Analysis Procedure

Before testing with the OP/C Screen Kit could begin, three reagents that were included in the kit had to be prepared: ACh-E, the oxidizer, and the substrate (ATC). Using a 3mL transfer pipette, 2 mL of the Ach-E diluent was transferred from the test tube to the ACh-E dropper bottle, which contained freeze-dried ACh-E. The bottle was then mixed by shaking it moderately. The ACh-E

solution was allowed to sit for at least 15 minutes to allow the ACh-E to go into solution. To prepare the oxidizer, 200 μL of the oxidizer was added to the appropriate dropper bottle and mixed. To prepare the substrate, 2 mL of substrate diluent were added to the appropriate dropper bottle and mixed.

The test tubes supplied with the OP/C Screen Kit were labeled. Then 100 μL of the control solution or the test sample was added to the appropriate test tubes. Two drops of the oxidizer were then added to each test tube and the tubes were shaken. The tubes were then allowed to incubate at room temperature for five minutes. Two drops of neutralizer were then added to each test tube and the tubes were shaken. Next, two drops of the ACh-E solutions were added to each test tube, the tubes were shaken, and the tubes were then allowed to incubate at room temperature for 30 minutes. After 30 minutes, two drops of the ATC substrate solution were added to each test tube and the tubes were shaken. Then, two drops of chromogen were added to each test tube, the tubes were shaken and then allowed to incubate for 30 minutes. Finally, two drops of the stopping solution were added to each tube to stop the reaction. Each tube was then placed in the colorimeter (Hach Company) provided by the vendor and the reading was recorded. The color of the sample was also recorded on the data sheet.

To determine if the sample was positive or negative, a percent inhibition had to be calculated for each sample (see Chapter 6). A sample was considered positive if it had reduced color development when compared to the negative control. Specifically, 20% or more inhibition of the color, obtained through the inhibition calculations, indicated a positive sample. Less than 20% inhibition indicated a negative or non-contaminated sample.

Per the kit instructions, duplicate samples were run for each test sample. Positive and negative controls were also run with each batch of samples. A batch consisted of up to 8 samples. Each of the dropper bottles used in the test was color-coded to coincide with the instructions. Actual solution names are presented here instead of the colors used in the kit instructions.

3.4.3 Drinking Water Characterization

An aliquot of each DW sample, collected as described in Section 3.2.2, was sent to ATEL to determine the following water quality parameters: turbidity; concentration of dissolved and total organic carbon; conductivity; alkalinity; pH; concentration of Ca and Mg; hardness; and concentration of total organic halides, trihalomethanes, and haloacetic acids. Table 3-4 lists the characterization data from the four water sample types used in this verification test. Water samples were collected and water quality parameters were measured by ATEL in June 2005, while verification testing was tested with the DW between November 2005 and February 2006. The time delay between collection and testing was due to the fact that the water samples were collected for use during a separate ETV test conducted prior to this one. Because of this, an aliquot of each DW was tested by ATEL again in January 2006 to verify some of the parameters with the most potential to change over time. Note that dissolved organic carbon was not retested as this result was verified by the total organic carbon results, additionally the total organic halides and calcium and magnesium were not verified as there was no reason to expect a change in these parameters. The concentrations of most water quality parameters were similar; however,

there was a decrease in levels of volatile compounds such as trihalomethanes and haloacetic acids over this time-period.

Table 3-4. ATEL Water Quality Characterization of Drinking Water Samples

			Columbus, OH (OH DW)		New York City, NY (NY DW)		Orlando, FL (FL DW)		MWD [(b)], CA (CA DW)	
Parameter	**Unit**	**Method**	**2005**	**2006**	**2005**	**2006**	**2005**	**2006**	**2005**	**2006**
Turbidity	NTU[(a)]	EPA 180.1[(4)]	0.1	0.6	1.1	1.3	0.5	0.1	0.1	0.2
Dissolved Organic Carbon	mg/L	SM 5310[(5)]	2.1	NA	1.1	NA	1.6	NA	2.9	NA
Total Organic Carbon	mg/L	SM 5310[(5)]	2.1	2.3	1.6	4.1	1.7	2.1	2.5	2.7
Specific Conductivity	µMHO[(c)]	SM 2510[(5)]	572	602	84	78	322	325	807	812
Alkalinity	mg/L	SM 2320[(5)]	40	44	14	12	142	125	71	97
pH		EPA 150.1[(6)]	7.6	7.4	6.9	6.8	8.5	7.6	8.0	7.9
Calcium	mg/L	EPA 200.8[(7)]	33	NA	5.6	NA	8.8	NA	45	NA
Magnesium	mg/L	EPA 200.8[(7)]	7.7	NA	1.3	NA	43	NA	20	NA
Hardness	mg/L	EPA 130.2[(8)]	118	107	20	26	143	130	192	182
Total Organic Halides	µg/L	SM 5320[(5)]	220	NA	82	NA	300	NA	170	NA
Trihalomethanes	µg/L/ analyte	EPA 524.2[(9)]	74.9	16.6	39.0	23.1	56.4	41.8	39.2	24.1
Haloacetic Acids	µg/L/ analyte	EPA 552.2[(10)]	32.8	<6.0	39.0	<6.0	34.6	<6.0	17.4	<6.0

[(a)] NTU = Nephelometric turbidity unit.
[(b)] MWD = Metropolitan Water District of Southern California
[(c)] µMHO = micromho

Chapter 4
Quality Assurance/Quality Control

QA/QC procedures were performed in accordance with the quality management plan (QMP) for the AMS Center [11] and the test/QA plan [12] for this verification test.

QC procedures as noted in the reference methods or laboratory's operating procedures were followed in confirming analyses of stock or reference solutions of contaminants and interfering compounds and in characterizing the DW. The reference methods for this verification test are listed in Table 4-1. A summary of the QC samples and acceptance criteria associated with each method is presented in Table 7 in the test/QA plan. [12]

4.1 Sample Chain-of Custody Procedures

Sample custody was documented throughout collection, shipping, and analysis of the samples. Sample chain-of-custody procedures were in accordance with ASAT.I-009-DRAFT, *Standard Operating Procedure for Sample Chain of Custody*. The chain-of-custody forms summarized the samples collected and analyses requested and were signed by the person relinquishing samples once that person had verified that the custody forms were accurate. The original sample custody forms accompanied the samples; the shipper kept a copy. Upon receipt at the sample destination, sample custody forms were signed by the person receiving the samples once that person had verified that all samples identified on the custody forms were present in the shipping container.

4.2 QC Samples

The QC measures for the reference methods included the analysis of a MB sample with the analyses of the reference or stock solution. MB samples were analyzed to ensure that no sources of contamination were present. If the analysis of an MB sample indicated a concentration above the minimum detection limit for the confirmatory instrument, contamination was suspected. Any contamination source(s) were corrected, and proper blank readings were achieved, before proceeding with the analyses. In general, a matrix spike or laboratory fortified spike sample was also analyzed. Average acceptable recoveries for these samples were between 70 and 150%. Samples outside of the acceptable range were generally flagged and rerun once the QC acceptance criteria had been met. QC samples were run with every batch of 1 to 20 samples. Specific QC samples and acceptance criteria associated with each method can be found in the appropriate reference (Table 4-1).

Table 4-1. Reference Methods for Target Contaminants and Interferents

Target Analyte/Interferent	Reference Method (Instrumentation)	Number of Observations	Expected Concentrations (mg/L)	Average Measured Concentration (mg/L) ± SD	Recovery (%R) ± SD
VX	Battelle Internally Developed Method (LC-MS)	10	2.1	2.1 ± 0.1	101 ± 5
GB (sarin)	HMRC-IV-118-05 [13] (GC-MS)	4	20.0	17.0 ± 1.4	85 ± 7
GD (soman)	HMRC-IV-118-05 [13] (GC-MS)	4	1.4	1.7 ± 0.05	121 ± 4
aldicarb	SOP for Analysis of Water Sample Extracts for Type 1 Analytes by Liquid Chromatography/Mass Spectrometry [14] (LC-MS)	2	26.0	34	123 ±7 [a]
		2	260	303	
dicrotophos	SOP for Extracting and Preparing Water Samples for Analysis of Dicrotophos, Mevinphos, and Dichlorovos [15] (GC-MS)	4	140	157 ± 24	108 ± 17 [a]
		1	1400	1326	
calcium (Ca)	EPA 200.8 [7] (ICP-MS)	1	125	140	112
magnesium (Mg)	EPA 200.8 [7] (ICP-MS)	1	125	130	104
Humic and fulvic acids	Standard Method 5310 [5] Combustion Infrared NDR	1	1.0	0.9	90

[a] Average of two concentration levels.

QC samples as provided with the OP/C Screen Kit were also run per the vendor's instructions, and MB samples were run as part of the verification test (Section 3.2.3). At least seven MB samples were run with each set of chemical agent and pesticide samples except for GD, for which only five MB samples were run. For the pesticides, 17 MB samples were tested with aldicarb samples while 16 MB samples were tested with dicrotophos samples. Seven out of 17 and one out of 16 MB samples were positive for aldicarb and dicrotophos, respectively. There was no indication of contamination despite positive MB results on days when those samples were run. Eight MB samples were run with the GB sample set. All but one MB

returned a negative result. The positive GB MB sample had only a 23% inhibition and a pale yellow color. Only five MB samples were run for GD, and three out of five were positive. In one case, the pair of duplicates had significantly different colorimeter readings such that one of the duplicates was negative and one was positive. The average was negative. This discrepancy is believed to be related to issues regarding the colorimeter's reproducibility (see Chapter 6). Other samples tested in the same sample set as this MB produced no unexpected results, though the colorimeter readings for all but on set of duplicate samples in this sample set were significantly different from each other. Three of the seven MB samples tested with the VX sample set were positive. One sample was run in the same batch as two unspiked interferent PT sample replicates with positive results. However, other samples in that batch did not show signs of contamination. There were also no indications of contamination in sample batches where other positive MB samples were found for the chemical agent samples, despite the positive MB results.

4.3 Equipment/Calibration

The instruments used for the reference analyses were calibrated per the standard reference methods being used to make each measurement or the standard operating procedures (SOPs) of the analysis laboratory. Instruments used in the reference analyses for this test included gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), pH electrodes, inductively coupled plasma-mass spectrometry (ICP-MS), and gas chromatography with electron capture detector (GC-ECD). All calibrations were documented by Battelle in the project laboratory record book (LRB). Calibration of mass spectrometers involved a 4- to 8-point calibration curve covering the range of concentrations of the reference solutions to be analyzed. Calibration of each reference instrument was performed as frequently as required by the reference method guidelines.

The vendor provided the Battelle technical operator with instructions on how to properly maintain components of the OP/C Screen Kit requiring calibration, namely the colorimeter. The colorimeter was calibrated at the beginning of each day of testing.

Pipettes used during solution preparation were maintained and calibrated as required by Battelle SOPs (i.e., minimum of every 6 months). Pipettes were checked and either recalibrated or replaced if they were dropped over the course of testing. Pipettes supplied as part of the OP/C Screen Kit were used according to the vendor's instructions and could not be calibrated.

4.4 Characterization of Stock Solutions

During testing, aliquots of the stock solutions used for sample preparation were submitted for concentration confirmation via the respective methods. The results, along with the reference methods, are listed in Table 4-1. Averages and associated standard deviations are given in cases where more than two samples were tested. Recovery (%R) is calculated by the following equation:

$$\%R = \frac{C}{A} \times 100 \quad (1)$$

where *C* is the measured concentration (or average measured concentration if more than one sample was tested) and *A* is the expected concentration of the contaminant or interferent in solution. For aldicarb and dicrotophos, aliquots at two different concentration levels were confirmed through reference analysis. The %R, listed in Table 4-1, represents the average of the %R across both concentration levels for those compounds. Table 4-1 shows that %R values ranged from 85% to 123% across all analytes and interferents.

Contaminant stock solutions were prepared and tested individually. Interferent stock solutions contained multiple analytes in the same solution (e.g., calcium and magnesium or humic and fulvic acids together). Up to four aliquots of each stock solution were analyzed over the course of the verification test. In the case of VX, extra aliquots were analyzed and all were reported in Table 4-1. Aliquots were preserved or extracted on the day of preparation and stored as prescribed by the standard method.

4.5 Audits

4.5.1 Performance Evaluation Audit

The concentration of the standards used to prepare the samples fortified with contaminants and potential interfering compounds was confirmed by analyzing standards prepared in ASTM Type II DI water from two separate commercial vendors using the reference methods noted in Table 4-1. The standards from one vendor were used during the verification test, while the standards from the second vendor were used exclusively to confirm the accuracy of the standards from the first vendor.

Given the security requirements and lack of alternate sources for the chemical agents (VX, GB, and GD) used in this verification test, PE audits were not performed for these contaminants. PE audits were done for all remaining compounds when more than one source of the contaminant or potential interfering compounds was available. PE audits were performed only on compounds used to prepare test samples and not on any solutions supplied as part of the OP/C Screen Kit. Agreement of the standards within 25% (percent difference) was required for the measurements to be considered acceptable. 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:

$$\%D = \frac{M}{A} \times 100 \qquad (2)$$

where *M* is the absolute value of the difference between the measured and the expected concentration, and *A* is the expected concentration. The results of the PE samples are given in Table 4-2. All %D values calculated were within the 25% acceptable tolerance.

Table 4-2. Performance Evaluation Samples and Percent Difference

Contaminant	Expected Concentration (ng/mL)	Measured Concentration (ng/mL)	Percent Difference (%)
aldicarb	50	57	14
dicrotophos	1000	1103	10
Ca	1000	890	11
Mg	1000	990	1

4.5.2 Technical Systems Audit

The Battelle Quality Manager conducted technical systems audits (TSAs) in November 2005 (11/01, 11/11, 11/16, 11/18) , December 2005 (12/01, 12/29), and January 2006 (01/30) to ensure that the verification test was performed in accordance with the AMS Center QMP,[11] the test/QA plan,[12] published reference methods, and any SOPs used by Battelle. As part of the audit, the Battelle Quality Manager reviewed the reference methods, compared actual test procedures to those specified or referenced in the test/QA plan, and reviewed data acquisition and handling procedures. The Battelle Quality Manager also observed testing in progress and the reference method sample preparation and analysis, inspected documentation, and reviewed the LRBs used to record testing results. The Battelle Quality Manager also checked calibration certifications and conferred with Battelle staff. Observations and findings from this audit were documented and submitted to the Battelle Verification Test Coordinator for response. No major findings were reported from the audits. The records concerning the TSA are permanently stored with the Battelle Quality Manager.

4.5.3 Audit of Data Quality

At least 10% of the data acquired during the verification test was audited. The Battelle Quality Manager traced the data from initial acquisition, through reduction and statistical comparisons, to final reporting. All calculations performed on the data undergoing the audit were checked.

4.6 QA/QC Reporting

Each assessment and audit was documented in accordance with Section 3.3.4 of the AMS Center QMP.[11] Once the assessment report was prepared, the Battelle Verification Test Coordinator responded to each 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.

4.7 Data Review

Records generated in the verification test were reviewed before they were used to calculate, evaluate, or report verification results. Table 4-3 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 initials and the date to a hard copy of the record being reviewed.

Table 4-3. Summary of Data Recording Process

Data to Be Recorded	Responsible Party	Where Recorded	How Often Recorded	Disposition of Data
Dates, times, and details of test events	Battelle	ETV laboratory record book or data recording forms	Start/end of test procedure, and at each change of a test parameter	Used to organize and check test results and manually incorporated into data spreadsheets as necessary
Sample preparation (dates, concentrations, etc.)	Battelle	ETV laboratory record books	When each solution was prepared	Used to confirm the concentration and integrity of the samples analyzed
Enzymatic test kit procedures and sample results	Battelle	ETV data sheets and laboratory record book	Throughout test duration	Manually incorporated into data spreadsheets for statistical analysis and comparisons
Reference method sample preparation	Battelle	ETV laboratory record book	Throughout sample preparation	Used to demonstrate validity of samples submitted for reference measurements
Reference method procedures, calibrations, QA, etc.	Battelle or subcontract laboratory	Laboratory record book or data recording forms	Throughout sampling and analysis processes	Retained as documentation of reference method performance
Reference method analysis results	Battelle or subcontract laboratory	Electronically from reference analytical method	Every sample analysis	Converted to spreadsheets for calculations

Chapter 5
Statistical Methods and Reported Parameters

The OP/C Screen Kit was evaluated for qualitative results (i.e., positive/negative responses to samples). All data analyses were based on these qualitative results. QC and MB samples were not included in any of the analyses.

5.1 Accuracy

Accuracy was assessed by evaluating how often the OP/C Screen Kit result was positive in the presence of a concentration above the limit of detection (LOD). Contaminant-only PT samples were used for this analysis. An overall percent agreement was determined by dividing the number of positive responses by the overall number of analyses of contaminant-only PT samples greater than the OP/C Screen Kit's LOD (see Equation 3). If the LOD was not known or available, then all analyzed contaminant-only PT samples greater than the concentration level where consistent negative results were obtained were used.

$$\textit{Accuracy (\% Agreement)} = \frac{\textit{\# of positive contaminant only PT samples}}{\textit{total \# of contaminant only PT samples}} \times 100 \qquad (3)$$

5.2 False Positive/False Negative Rates

A false positive response was defined as a response indicating the presence of a contaminant when the PT interferent or DW sample was not spiked with contaminant. A false positive rate was reported as the number of false positive results out of the total number of unspiked samples (Equation 4).

A false negative response was defined as a response indicating the absence of a contaminant when the sample was spiked with a contaminant at a concentration greater than the OP/C Screen Kit's LOD as defined above. Spiked PT (contaminant and interferent) samples and spiked DW samples were included in the analysis. Contaminant-only PT samples above the OP/C Screen Kit's LOD or the level at which consistent negative responses are obtained (when the LOD was not known) were included in the analysis. A false negative rate was evaluated as the number of false negative results out of the total number of spiked samples for a particular contaminant (Equation 5).

$$\textit{False Positive Rate} = \frac{\textit{\# of positive results}}{\textit{total \# of unspiked samples}} \qquad (4)$$

$$\text{False Negative Rate} = \frac{\text{\# of negative results}}{\text{total \# of spiked samples}} \quad (5)$$

5.3 Precision

Precision measures the repeatability and reproducibility of the OP/C Screen Kit's responses. The precision of three replicates of each sample set was assessed. Responses were considered inconsistent if one or more of the three replicates differed from the response of the other samples in the replicate set. The precision for the OP/C Screen Kit was assessed by calculating the overall number of consistent responses for all the sample sets. The results are reported as the percentage of consistent responses out of all replicate sets (Equation 6).

$$\text{Precision (\% Consistent results)} = \frac{\text{\# of consistent responses of replicate sets}}{\text{total \# of replicate sets}} \times 100 \quad (6)$$

5.4 Potential Matrix and Interferent Effects

The potential effect of the DW matrix on the OP/C Screen Kit's performance was evaluated qualitatively by comparing the results for the spiked and unspiked DW samples to those for the PT samples spiked with the contaminant at 10 times less than the lethal dose. Similarly, the potential effect of interferent PT samples was evaluated. The results indicating the correct or incorrect reporting of the presence of a contaminant were evaluated. The findings are reported and discussed in Section 6.4.

5.5 Operational Factors

Operational aspects of the OP/C Screen Kit's performance such as ease of use and sample throughput were evaluated through observations made during testing. Also addressed are the qualitative observations of the verification staff pertaining to the performance of the OP/C Screen Kit from both the technical and non-technical operators' perspective.

Chapter 6
Test Results

The OP/C Screen Kit is a qualitative, colorimetric detection technology. The test tubes in which the test is performed produce a color ranging from yellow (negative control) to clear (positive control). The absorbance of the sample was read on a 450 nm colorimeter. To determine whether or not a sample is positive, the absorbance of the sample was compared to that of the negative control by calculating the percent inhibition. Because duplicate samples were tested for each test and negative control sample, the average absorbance of the duplicates must first be calculated before the percent inhibition can be determined. Percent inhibition was then calculated using the following equation:

$$\%inhibition = \left[1 - \left(\frac{L_{sample}}{L_{control}}\right)\right] \times 100 \quad (7)$$

where L_{sample} is the average absorbance of the duplicate test sample and $L_{control}$ is the average absorbance of the duplicate negative control samples. A sample was considered positive if it had reduced color development when compared to the negative control. Specifically, 20% or more inhibition of the color, obtained through the inhibition calculation, indicated a positive sample. Less than 20% inhibition indicated a negative or non-contaminated sample. Based on these inhibition parameters, a qualitative (positive or negative) result was recorded for each sample. All of the test results presented in this chapter were calculated using the qualitative responses determined for the OP/C Screen Kit.

After the completion of testing, the vendor discovered reproducibility issues with the Hach colorimeter that was used during testing. Reproducibility is important for this test, particularly for the negative control samples, which are used as a baseline in determining percent inhibition. To denote the colorimeter problem, a relative percent difference (RPD) was calculated for each pair of duplicate negative control samples. RPD was calculated using the following equation:

$$RPD(\%) = \left|\frac{2 \times \left|NC_1 - NC_2\right|}{\left(NC_1 + NC_2\right)}\right| \times 100 \quad (8)$$

where NC_1 and NC_2 are the duplicate negative control samples. Based on the vendor's direction, any pair of negative control samples with a RPD of >20% were flagged. The vendor indicated that an RPD of >20% would lead to retesting for that set of samples associated with the negative

controls. Because testing was already completed when the colorimeter problem was discovered, suspect data were only flagged in this report. All data were used in calculating the results presented in this chapter. Results obtained from a set of samples where the RPD was >20% are marked accordingly in the tables 6-2a through 6-2e.

6.1 Accuracy

The accuracy results for the OP/C Screen Kit using the contaminant-only PT samples are discussed in this section. Table 6-1 presents the accuracy results for aldicarb, dicrotophos, VX, GB, and GD. The results for the lethal dose concentration of each contaminant are included in the table. Results are presented for all tested concentration levels; but, by definition, only those results above the kit's LOD are included in the calculation. The LOD for aldicarb is 0.010 mg/L. The LOD for dicrotophos is 0.004 mg/L. Both of these LODs are below the lowest concentration level tested for this test. Thus, all of the pesticide contaminant-only PT samples were included in the accuracy calculations for these compounds. LODs were not available for VX, GB, or GD. For these contaminants, only samples above the level where consistent negative responses were obtained were used in the accuracy calculations for that contaminant. For VX, consistent negative responses were found at a 1,000x dilution of the lethal dose, or 0.0021 mg/L. Consistent negative responses were not found for GB, so all contaminant-only PT samples were included in the accuracy calculations. For GD, consistent negative responses were found at the lowest tested concentration level (0.00014 mg/L); thus, those PT samples were not used in any accuracy calculations.

All concentration levels of VX and GD samples tested above the level of consistent negative responses for each contaminant generated 3 out of 3 positive responses, resulting in 100% accuracy for each chemical agent. All concentration levels analyzed for GB generated positive responses for all replicates, resulting in 100% accuracy. Results for contaminant-only PT samples containing aldicarb and dicrotophos were all positive across all concentration levels tested resulting in 100% accuracy for both pesticides.

6.2 False Positive/False Negative Rates

Contaminant-only PT samples, interferent PT samples, and DW samples were evaluated to determine false positive and false negative results for the OP/C Screen Kit. A false positive response was defined as a positive result when the contaminant was not spiked into the sample. A false negative response was defined as a negative result when the sample was spiked with a contaminant at a concentration greater than the level where consistent negative responses were obtained (see Section 6.1). Tables 6-2a through 6-2e present the false positive and false negative responses for VX, GB, GD, aldicarb, and dicrotophos, respectively. The number of positive samples out of the total replicates analyzed is presented in each table. Also presented in each table are the RPD values for the negative controls associated with that particular set of replicates. Only RPDs >20% are presented in the table as a means of flagging suspect data (see Chapter 6 introduction). These data were still used in the false positive/negative calculations for the table.

Table 6-1. Contaminant-Only PT Sample Results

Contaminant	Concentration (mg/L)	Positive Results Out of Total Replicates	Overall Accuracy
VX	2.1 [a]	3/3	**100% (9/9)**
	0.21	3/3	
	0.021	3/3	
	0.0021	0/3 [b]	
	0.00021	0/3 [b]	
GB	20 [a]	3/3	**100% (15/15)**
	2.0	3/3	
	0.20	3/3	
	0.020	3/3	
	0.0020	3/3	
GD	1.4 [a]	3/3	**100% (12/12)**
	0.14	3/3	
	0.014	3/3	
	0.0014	3/3	
	0.00014	0/3 [b]	
Aldicarb	260 [a]	3/3	**100% (15/15)**
	26	3/3	
	2.6	3/3	
	0.26	3/3	
	0.026	3/3	
Dicrotophos	1400 [a]	3/3	**100% (15/15)**
	140	3/3	
	14	3/3	
	1.4	3/3	
	0.14	3/3	

[a] Lethal dose.
[b] Not used in accuracy calculations because samples are at or below LOD or level or consistent negative responses.

Table 6-2a. VX False Positive/Negative Results

Sample Type	Matrix	Concentration (mg/L)	Positive Results Out of Total Replicates [a]	RPD of Negative Controls Associated with Sample [b]
Contaminant-only PT samples	DI water	2.1 [c]	3/3	37%
	DI water	0.21	3/3	23%
	DI water	0.021	3/3	37%
Interferent PT samples [d]	1 mg/L humic and fulvic acids	Blank	2/3	69%
	1 mg/L humic and fulvic acids	0.21	3/3	28%
	5 mg/L humic and fulvic acids	Blank	0/3	30%
	5 mg/L humic and fulvic acids	0.21	3/3	
	50 mg/L Ca and Mg	Blank	0/3	
	50 mg/L Ca and Mg	0.21	3/3	69%
	250 mg/L Ca and Mg	Blank	0/3	88%
	250 mg/L Ca and Mg	0.21	3/3	
DW samples [d]	OH DW	Blank	1/3	
	OH DW	0.21	3/3	37%
	CA DW	Blank	0/3	
	CA DW	0.21	3/3	
	FL DW	Blank	0/3	
	FL DW	0.21	3/3	
	NY DW	Blank	0/3	88%
	NY DW	0.21	3/3	
False Positive Rate			**3/24**	
False Negative Rate			**0/33**	

[a] Boxed results indicate false positive responses.
[b] RPD provided only when >20%, indicating suspect data, according to vendor, because of colorimeter lack of reproducibility.
[c] Lethal dose.
[d] Only one set of unspiked DW and PT interferent samples were run for VX, GB, and GD.

Table 6-2b. GB False Positive/Negative Results

Sample Type	Matrix	Concentration (mg/L)	Positive Results Out of Total Replicates [a]	RPD of Negative Controls Associated with Sample [b]
Contaminant-only PT samples	DI water	20 [c]	3/3	61%
	DI water	2.0	3/3	30%
	DI water	0.20	3/3	
	DI water	0.020	3/3	61%
	DI water	0.0020	3/3	
Interferent PT samples [d]	1 mg/L humic and fulvic acids	Blank	2/3	69%
	1 mg/L humic and fulvic acids	2.0	3/3	
	5 mg/L humic and fulvic acids	Blank	0/3	30%
	5 mg/L humic and fulvic acids	2.0	3/3	
	50 mg/L Ca and Mg	Blank	0/3	
	50 mg/L Ca and Mg	2.0	3/3	
	250 mg/L Ca and Mg	Blank	0/3	88%
	250 mg/L Ca and Mg	2.0	3/3	
DW samples[d]	OH DW	Blank	1/3	
	OH DW	2.0	3/3	
	CA DW	Blank	0/3	
	CA DW	2.0	3/3	
	FL DW	Blank	0/3	
	FL DW	2.0	3/3	
	NY DW	Blank	0/3	88%
	NY DW	2.0	3/3	
False Positive Rate			**3/24**	
False Negative Rate			**0/39**	

[a] Boxed results indicate false positive responses.
[b] RPD provided only when >20%, indicating suspect data, according to vendor, because of colorimeter lack of reproducibility.
[c] Lethal dose.
[d] Only one set of unspiked DW and PT interferent samples were run for VX, GB, and GD.

Table 6-2c. GD False Positive/Negative Results

Sample Type	Matrix	Concentration (mg/L)	Positive Results Out of Total Replicates [a]	RPD of Negative Controls Associated with Sample [b]
Contaminant-only PT samples	DI water	1.4 [c]	3/3	
	DI water	0.14	3/3	
	DI water	0.014	3/3	
	DI water	0.0014	3/3	
Interferent PT samples [d]	1 mg/L humic and fulvic acids	Blank	2/3	69%
	1 mg/L humic and fulvic acids	0.14	3/3	43%
	5 mg/L humic and fulvic acids	Blank	0/3	30%
	5 mg/L humic and fulvic acids	0.14	3/3	46%
	50 mg/L Ca and Mg	Blank	0/3	
	50 mg/L Ca and Mg	0.14	3/3	43%
	250 mg/L Ca and Mg	Blank	0/3	88%
	250 mg/L Ca and Mg	0.14	3/3	46%
DW samples[d]	OH DW	Blank	1/3	
	OH DW	0.14	3/3	
	CA DW	Blank	0/3	
	CA DW	0.14	3/3	
	FL DW	Blank	0/3	
	FL DW	0.14	3/3	
	NY DW	Blank	0/3	88%
	NY DW	0.14	3/3	
False Positive Rate			**3/24**	
False Negative Rate			**0/36**	

[a] Boxed results indicate false positive responses.
[b] RPD provided only when >20%, indicating suspect data, according to vendor, because of colorimeter lack of reproducibility.
[c] Lethal dose.
[d] Only one set of unspiked DW and PT interferent samples were run for VX, GB, and GD.

Table 6-2d. Aldicarb False Positive/Negative Results

Sample Type	Matrix	Concentration (mg/L)	Positive Results Out of Total Replicates [a]	RPD of Negative Controls Associated with Sample [b]
Contaminant-only PT samples	DI water	260 [c]	3/3	131%
	DI water	26	3/3	22%
	DI water	2.6	3/3	22%
	DI water	0.26	3/3	28%
	DI water	0.026	3/3	131%
Interferent PT samples	1 mg/L humic and fulvic acids	Blank	1/3	72%
	1 mg/L humic and fulvic acids	26	3/3	28%
	5 mg/L humic and fulvic acids	Blank	2/3	28%
	5 mg/L humic and fulvic acids	26	3/3	72%
	50 mg/L Ca and Mg	Blank	0/3	23%
	50 mg/L Ca and Mg	26	3/3	79%
	250 mg/L Ca and Mg	Blank	1/3	23%
	250 mg/L Ca and Mg	26	3/3	79%
DW samples	OH DW	Blank	0/3	
	OH DW	26	3/3	40%
	CA DW	Blank	0/3	
	CA DW	26	3/3	40%
	FL DW	Blank	0/3	71%
	FL DW	26	3/3	40%
	NY DW	Blank	0/3	71%
	NY DW	26	3/3	71%
False Positive Rate			**4/24**	
False Negative Rate			**0/39**	

[a] Boxed results indicate false positive responses.
[b] RPD provided only when >20%, indicating suspect data, according to vendor, because of colorimeter lack of reproducibility.
[c] Lethal dose.

Table 6-2e. Dicrotophos False Positive/Negative Results

Sample Type	Matrix	Concentration (mg/L)	Positive Results Out of Total Replicates [a]	RPD of Negative Controls Associated with Sample [b]
Contaminant-only PT samples	DI water	1400 [c]	3/3	
	DI water	140	3/3	
	DI water	14	3/3	24%
	DI water	1.4	3/3	24%
	DI water	0.14	3/3	24%
Interferent PT samples	1 mg/L humic and fulvic acids	Blank	0/3	44%
	1 mg/L humic and fulvic acids	140	2/3	382%
	5 mg/L humic and fulvic acids	Blank	0/3	79%
	5 mg/L humic and fulvic acids	140	0/3	382%
	50 mg/L Ca and Mg	Blank	1/3	161%
	50 mg/L Ca and Mg	140	2/3	382%
	250 mg/L Ca and Mg	Blank	3/3	
	250 mg/L Ca and Mg	140	3/3	
DW samples	OH DW	Blank	0/3	
	OH DW	140	3/3	
	CA DW	Blank	0/3	
	CA DW	140	3/3	
	FL DW	Blank	0/3	
	FL DW	140	3/3	
	NY DW	Blank	1/3	
	NY DW	140	3/3	
False Positive Rate			**5/24**	
False Negative Rate			**5/39**	

[a] Boxed results indicate false positive responses; shaded results indicate false negative responses.
[b] RPD provided only when >20%, indicating suspect data, according to vendor, because of colorimeter lack of reproducibility.
[c] Lethal dose.

For VX, GB, and GD, only one set of unspiked DW and PT interferent samples were run for all three chemical agents. Thus, the unspiked DW and PT-interferent sample results shown in Tables 6-2a through 6-2c are the same and from only one set of triplicate samples. For aldicarb and dicrotophos, sets of unspiked DW and PT interferent samples were run separately for each pesticide.

No false negative results were found for any of the contaminants except for dicrotophos. For dicrotophos, five false negatives were found, all of which were spiked interferent PT samples. Four negative results were obtained across the spiked humic and fulvic acid samples: three for 5 mg/L humic and fulvic acids and one for 1 mg/L humic and fulvic acids. One other negative result was obtained for spiked 50 mg/L Ca and Mg.

False positive results were found for each contaminant. For VX, GB, and GD, three false positive results were found: two for unspiked 1 mg/L humic and fulvic acids and one for unspiked OH DW. Four false positive results were found for aldicarb. All were in the interferent PT sample results. Two positive responses were obtained from the unspiked 5 mg/L humic and fulvic acid samples. One false positive was found in the 1 mg/L humic and fulvic acid replicates, and the other false positive occurred in the unspiked 250 mg/L Ca and Mg interferent PT samples.

Five false positive results were generated for dicrotophos samples. All three of the unspiked 250 mg/L Ca and Mg samples were positive. One replicate of the unspiked 50 mg/L Ca and Mg samples was positive as was one unspiked NY DW sample.

The RPD values presented in the tables indicate the difference between the duplicate negative controls for a particular set of samples. To determine if the colorimeter's lack of reproducibility was affecting the results, the inhibition was calculated for each sample replicate using the duplicate negative control samples individually. This exercise was only done to gather further information on the effect of the colorimeter; these results were not used to calculate any of the parameters defined in Chapter 5 or generate any of the results presented in Chapter 6. For the most part, the difference between the duplicate negative control values did not affect the outcome of a replicate. That is, when each negative control was used individually to calculate the inhibition for each sample, the overall qualitative results (positive or negative) were the same as when the average of the negative controls were used. However, there were a few instances where this was not the case.

The individual negative controls run for the sample set containing the unspiked 1 mg/L humic and fulvic acids for the chemical agents produced different results for those replicates if the results were calculated based on each negative control individually. Calculations based on one negative control sample would result in two positive and one negative response for the replicate set, the same results that using the average negative control value produced. Calculations using the other negative control produced the exact opposite results (two negative and one positive). Similarly, the individual negative controls for unspiked 250 mg/L Ca and Mg interferent PT replicates for all chemical agent samples produced one positive and one negative result for one of the replicates.

The only other instance where using individual negative controls, as opposed to the average, would make a difference in the overall results was for dicrotophos samples where the RPD was 382%. As with the samples outlined above, inhibition calculations conducted with one negative control produced results exactly opposite of those found using the other negative control for all samples in that particular sample set. In this instance, one negative control had a positive colorimeter reading, while one had a negative colorimeter reading. The average of the two negative controls was negative, and led to the same qualitative results as using the individual negative control value (the same results shown in table 6-2e). Using only the positive negative control sample led to qualitative results opposite of those given in Table 6-2e. This would mean 0/3 positive results for unspiked 250 mg/L Ca and Mg samples, 3/3 positive results for spiked 5 mg/L humic and fulvic acids, 3/3 positive results for spiked 50 mg/L Ca and Mg samples (only one replicate was run with the suspect negative controls), and 3/3 positive results for spiked 1 mg/L humic and fulvic acid samples (only one replicate was run with the suspect negative controls). Despite these few cases, the average of the duplicate negative controls was used to calculate the inhibition, as indicated by the kit's protocol provided by the vendor, and because the colorimeter issue and the calculation/criteria for acceptable negative control results were not provided until after testing was completed.

6.3 Precision

The performance of the OP/C Screen Kit in measuring VX, GB, and GD within sets of three replicates was consistent in 19 out of 21 samples sets (for each compound), indicating that 90% of the samples sets showed consistent results for these contaminants. Two sets of samples were inconsistent, the unspiked 1 mg/L humic and fulvic acids and the unspiked OH DW replicates.

Sample sets were consistent 86% of the time for aldicarb samples, where 18 out of 21 sample sets had consistent results. Unspiked 1 mg/L and 5 mg/L humic and fulvic acid samples showed inconsistent results as did unspiked 250 mg/L Ca and Mg replicates.

Four sets of replicates of dicrotophos samples generated inconsistent results, generating a precision of 81% (17 out of 21 sets of samples were consistent). Two sets of spiked and two sets of unspiked sample responses were inconsistent. Both 1 mg/L humic and fulvic acids and 50 mg/L Ca and Mg spiked with dicrotophos at 140 mg/L produced results that were not consistent within the sample set. Unspiked 50 mg/L Ca and Mg replicates were also inconsistent in their responses, as were unspiked NY DW samples.

6.4 Potential Matrix and Interferent Effects

6.4.1 Interferent PT Samples

The OP/C Screen Kit was able to readily and consistently detect VX, GB, GD, aldicarb, and dicrotophos at 10 times less than the lethal dose in DI water (see Tables 6-2a – e). Across all three chemical agents and aldicarb, all interferent PT samples spiked with the contaminant at 10 times less than the lethal dose produced positive responses. One set of spiked interferent PT samples for dicrotophos produced consistent positive responses with the OP/C Screen Kit. All

other spiked interferent PT samples for dicrotophos had at least one negative response, indicating possible inhibitory effects to the OP/C Screen Kit for the interferents used in this test.

For all contaminants except dicrotophos, unspiked 1 mg/L humic and fulvic acids replicates had at least one false positive result, further supporting the sensitivity of the OP/C Screen Kit to this interferent. For both aldicarb and dicrotophos samples, unspiked interferent PT samples were, in general, troublesome in three of the four unspiked sample sets for aldicarb and two of the four unspiked sets for dicrotophos, producing at least one positive result. These results indicate potential interferent effects for these two pesticides.

6.4.2 DW Samples

For the chemical agent sample sets, unspiked OH DW produced one positive result. For dicrotophos sample sets, one unspiked NY DW replicate was positive. All other DW sample results for VX, GB, GD, aldicarb, and dicrotophos were as expected. The discrepancies with OH and NY DW samples could indicate that there could be potential confounding compounds in these DW samples that the OP/C Screen Kit is sensitive to.

6.5 Operational Factors

6.5.1 Technical Operator

The OP/C Screen Kit was operated by one Battelle technician throughout testing with the pesticides and by a different Battelle technician throughout testing with chemical agents. The technicians were trained by the vendor in the operation of the test kit. Training was conducted at Battelle for one half day by the vendor. Both technicians had extensive laboratory experience.

The instructions provided with the kit were color-coded to aid the operator and laid out the test in a step-by-step manner. The colors on the dropper bottles helped to guide the operator through the testing and made using multiple test solutions easier. The caps on the sample test tubes were difficult to remove such that the technicians had to be cautious in their removal so as not to spill any of the buffer contained in the tubes. It also seemed that the dropper bottles did not consistently deliver the same size droplet. The instructions indicate that the samples should incubate for 15 to 30 minutes at various points throughout testing. However, during the initial training phase of the verification test, it was determined that the samples had to incubate for 30 minutes to achieve the correct results. Overall, the OP/C Screen Kit was straightforward and easy to use.

The OP/C Screen Kit needs to be refrigerated until use, and then all of the reagents must come to room temperature before they can be used. Multiple testing solutions are required for the assay. All of the solutions are provided with the kit. Three of the reagents used in testing must be prepared before they can be used. One reagent (ACh-E) requires at least five minutes incubation before it can be used, though the vendor requested that Battelle allow 15 minutes.

Up to eight sets of duplicate samples can be tested at the same time using one OP/C Screen Kit. Overall, it took the technical operators an average of 94 minutes to test seven samples. The operators were able to test between one and five OP/C Screen Kits a day with four to eight samples per kit.

6.5.2 Non-Technical Operator

Unspiked DI water samples were tested on the OP/C Screen Kit by a non-technical operator both in and not in PPE (see Section 3.2.4). The non-technical operator was trained in the use of the kit by a technical operator who had been trained by the vendor. The SCBA apparatus, including the mask, was worn throughout the entire testing procedure when PPE was to be worn. However, the operator ran the air from the SCBA only part of the time during testing to conserve the tank. Figure 6-1 shows the full PPE as worn for this verification test. Figure 6-2 shows the testing of the OP/C Screen Kit with PPE. Because this portion of the test was designed to evaluate the operational aspects of the OP/C Screen Kit, the handheld colorimeter used in other portions of this verification test was not used by the non-technical operator. Only color observations were recorded for each MB sample. With the PPE on, all MB samples were yellow; without the PPE, all MB samples were also yellow, indicating that the samples tested negative when the non-technical ran the MB samples both in and out of PPE. Removing the dropper tips for the OP/C Screen Kit dropper bottles was difficult to do in PPE. Also, when transferring drops to the tubes during testing, it was difficult to see the drops through the SCBA mask. The 100 µL pipettes supplied with the OP/C Screen Kit were slightly difficult to handle while wearing gloves as part of the PPE, but manageable. The vendor recommends the use of a laboratory pipettor for use in the field.

Even without PPE, removal of the caps from some of the test tubes was quite difficult for the non-technical operator as it was for the technical operator (see 6.5.1), causing the solutions inside to nearly spill out. Using the provided work station box to hold the samples proved to be somewhat problematic as it was difficult to know which sample tubes had already been worked on and which had not since the sample solution is not visible when the test tube is in the box. Testing three MB samples in PPE using the OP/C Screen Kit took 82 minutes. Six MB samples were tested by the non-technical operator while not wearing PPE, which took 86 minutes.

The instructions for the OP/C Screen Kit indicate that the test should be performed within a specific temperature range (70±20° F/ 21±7° C) to achieve accurate results. Presumably this would be difficult for a first responder in the field to control and could cause significant problems in the ability of the kit to perform correctly, assuming there is a strong temperature dependency. Also, the 15-30 minute incubation times that must be performed at various points in the test would make it difficult on the operator if they had to spend that time in PPE. For all of these reasons, the OP/C Screen Kit was felt to be not very first-responder friendly for use in the field wearing PPE.

Figure 6-1. Side View of PPE Worn by Non-Technical Operator

Figure 6-2. Testing of the OP/C Screen Kit with the Non-Technical Operator Wearing PPE

Chapter 7
Performance Summary

The OP/C Screen Kit results for this verification test for samples containing VX, GB, GD, aldicarb, and dicrotophos are presented in Tables 7-1 through 7-5. The results for each contaminant are presented in a separate table. Qualitative responses for each set of sample replicates as well as accuracy, false negatives and positives, and precision are presented in each table. A summary of the other performance factors associated with the OP/C Screen Kit is presented at the end of this chapter. These performance factors apply across all contaminants.

Table 7-1. VX Summary Table

Parameter		Matrix	VX Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	2.1 mg/L [(a)]	3/3
			0.21 mg/L	3/3
			0.021 mg/L	3/3
			0.0021 mg/L	0/3 [(b)]
			0.00021 mg/L	0/3 [(b)]
	Interferent PT Samples	Humic and Fulvic Acids	0.21 mg/L	6/6
		Ca and Mg	0.21 mg/L	6/6
	DW Samples	DW	0.21 mg/L	12/12
Accuracy		100% (9 out of 9) of the contaminant-only PT samples were positive.		
False Positives		Three false positive responses were obtained. Two positive responses were found for unspiked 1 mg/L humic and fulvic acids. One replicate for unspiked OH DW returned a positive result.		
False Negatives		No false negative results were obtained for spiked PT and DW samples.		
Precision		90% (19 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

[(a)] Lethal dose.
[(b)] Not used in accuracy calculations because samples are at or below level of consistent negative response.

Table 7-2. GB Summary Table

Parameter		Matrix	GB Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	20 mg/L [(a)]	3/3
			2.0 mg/L	3/3
			0.20 mg/L	3/3
			0.020 mg/L	3/3
			0.0020 mg/L	3/3
	Interferent PT Samples	Humic and Fulvic Acids	2.0 mg/L	6/6
		Ca and Mg	2.0 mg/L	6/6
	DW Samples	DW	2.0 mg/L	12/12
Accuracy		100% (15 out of 15) of the contaminant-only PT samples were positive.		
False Positives		Three false positive responses were obtained. Two positive responses were found for unspiked 1 mg/L humic and fulvic acids. One replicate for unspiked OH DW returned a positive result.		
False Negatives		No false negative results were obtained for spiked PT and DW samples.		
Precision		90% (19 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

[(a)] Lethal dose.

Table 7-3. GD Summary Table

Parameter		Matrix	GD Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	1.4 mg/L [(a)]	3/3
			0.14 mg/L	3/3
			0.014 mg/L	3/3
			0.0014 mg/L	3/3
			0.00014 mg/L	0/3 [(b)]
	Interferent PT Samples	Humic and Fulvic Acids	0.14 mg/L	6/6
		Ca and Mg	0.14 mg/L	6/6
	DW Samples	DW	0.14 mg/L	12/12
Accuracy		100% (12 out of 12) of the contaminant-only PT samples were positive.		
False Positives		Three false positive responses were obtained. Two positive responses were found for unspiked 1 mg/L humic and fulvic acids. One replicate for unspiked OH DW returned a positive result.		
False Negatives		No false negative results were obtained for spiked PT and DW samples.		
Precision		90% (19 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

[(a)] Lethal dose.
[(b)] Not used in accuracy calculations because samples are at or below level of consistent negative response.

Table 7-4. Aldicarb Summary Table

Parameter		Matrix	Aldicarb Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	260 mg/L [(a)]	3/3
			26 mg/L	3/3
			2.6 mg/L	3/3
			0.26 mg/L	3/3
			0.026 mg/L	3/3
	Interferent PT Samples	Humic and Fulvic Acids	26 mg/L	6/6
		Ca and Mg	26 mg/L	6/6
	DW Samples	DW	26 mg/L	12/12
Accuracy		100% (15 out of 15) of the contaminant-only PT samples were positive.		
False Positives		Four false positive responses were obtained. Three positive responses were found across unspiked 1 mg/L and 5 mg/L humic and fulvic acids. One positive response was found for unspiked 250 mg/L Ca and Mg samples.		
False Negatives		No false negative results were obtained for spiked PT and DW samples.		
Precision		86% (18 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

[(a)] Lethal dose.

Table 7-5. Dicrotophos Summary Table

Parameter		Matrix	Dicrotophos Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	1400 mg/L [(a)]	3/3
			140 mg/L	3/3
			14 mg/L	3/3
			1.4 mg/L	3/3
			0.14 mg/L	3/3
	Interferent PT Samples	Humic and Fulvic Acids	26 mg/L	2/6
		Ca and Mg	26 mg/L	5/6
	DW Samples	DW	26 mg/L	12/12
Accuracy		100% (15 out of 15) of the contaminant-only PT samples were positive.		
False Positives		Five false positive responses were obtained. Positive responses were found for all replicates of the unspiked 250 mg/L Ca and Mg samples. One positive response was found for the unspiked 50 mg/L Ca and Mg samples. One other positive response was found for unspiked NY DW.		
False Negatives		Five false negative results were obtained for spiked PT and DW samples. All three replicates of the spiked 5 mg/L humic and fulvic acid samples and one replicate of the spiked 1 mg/L humic and fulvic acid samples returned negative results. One spiked 50 mg/L Ca and Mg sample was also negative.		
Precision		81% (17 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

[(a)] Lethal dose.

Operational Factors:

Technical Operators

The OP/C Screen Kit was operated by one Battelle technician throughout testing with the pesticides and a different Battelle technician throughout testing with chemical agents. The technicians were trained by the vendor in the operation of the test kit. Both technicians had extensive laboratory experience. The instructions provided with the kit were color-coded. The colors on the dropper bottles helped to guide the operator through the testing and made using multiple test solutions easier. The caps on the sample test tubes were difficult to remove. It also seemed that the dropper bottles did not consistently deliver the same size droplet. The instructions indicate that the samples should incubate for 15 to 30 minutes at various points throughout testing; however, during the initial training phase of the verification test, it was determined that the samples had to incubate for 30 minutes to achieve the correct results. Overall, the OP/C Screen Kit was straightforward and easy to use. The OP/C Screen Kit needs to be refrigerated until use, and then all of the reagents must come to room temperature before they can be used. Three of the reagents used in testing must be prepared before they can be used. Up to eight sets of duplicate samples can be tested at the same time using one OP/C Screen Kit. Overall, it took the technical operators an average of 94 minutes to test seven samples. The operators were able to test between one and five OP/C Screen Kits a day with four to eight samples per kit.

Non-Technical Operators

Unspiked DI water samples were tested on the OP/C Screen Kit by a non-technical operator both in and not in PPE. The non-technical operator was trained in the use of the kit by a technical operator who had been trained by the vendor. Removing the dropper tips for the OP/C Screen Kit dropper bottles was difficult to do in and out of PPE. Also, when transferring drops to the tubes during testing, it was difficult to see the drops through the SCBA mask. The 100 μL pipettes supplied with the OP/C Screen Kit were slightly difficult to handle while wearing gloves as part of the PPE. The vendor recommends the use of a laboratory pipettor for use in the field. Using the provided work station box to hold the samples proved to be somewhat problematic as it was difficult to know which sample tubes had already been worked on and which had not. Testing three MB samples in PPE using the OP/C Screen Kit took 82 minutes; six MB samples without PPE took 86 minutes. The instructions for the OP/C Screen Kit indicate that the test should be performed within a specific temperature range (70 ± 20°F) to achieve accurate results. Presumably, this would be difficult for a first responder in the field to control. Also, the 15-30 minute incubations that are performed at various points during the test would make it difficult on the operator if they had to spend that time in PPR. The OP/C Screen Kit was felt to be not very first-responder friendly for use in the field wearing PPE.

Chapter 8
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