September 2006

Environmental Technology Verification Report

PROTEIN-BIOSENSOR
OP-Stick Sensor

Prepared by
Battelle

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

ETV ETV ETV

September 2006

Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

Protein-Biosensor
OP-Stick Sensor

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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

AChe	acetylcholinesterase
AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
ATEL	Aqua Tech Environmental Laboratories
Ca	calcium
CWA	chemical warfare agent
DI	deionized
DPD	diethyl-p-phenylene diamine
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
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
µg/L	microgram per liter
mg/L	milligram per liter
mL	milliliter
MS	mass spectrometry
µMHO	micromho

NDR	negative differential resistance
NTU	nephelometric turbidity unit
OP	organophosphate
PE	performance evaluation
PPE	personal protective equipment
PT	performance test
QA	quality assurance
QC	quality control
QMP	quality management plan
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 Protein-Biosensor OP-Stick Sensor in detecting chemical agents, carbamate pesticides, and organophosphate (OP) 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-Stick Sensor. Following is a description of the OP-Stick Sensor, based on information provided by the vendor. The information provided below was not verified in this test.

The OP-Stick Sensor is an enzymatic colorimetric assay designed for detecting organophosphate (including thiophosphate) and carbamate (OP/C) pesticide residues in water, soil, and food. This technology had not been used to test for chemical warfare agents (CWA) prior to this verification test. This assay is a field diagnostic test that measures acetylcholinesterase (AChE) activity and is based on an enzyme engineered for increased sensitivity to OP and C pesticides.

When not in presence of inhibiting pesticides, AChE hydrolyzes acetylthiocholine to thiocholine, which reacts with a colorimetric substrate on a test stick (Figure 2-1) to produce a brown color. In the presence of OP/Cs (which are oxidized during the test to an "oxon" form), AChE is irreversibly inhibited and color formation is reduced or absent depending on the pesticide concentration. The intensity of the brown color is inversely proportional to OP/C concentration.

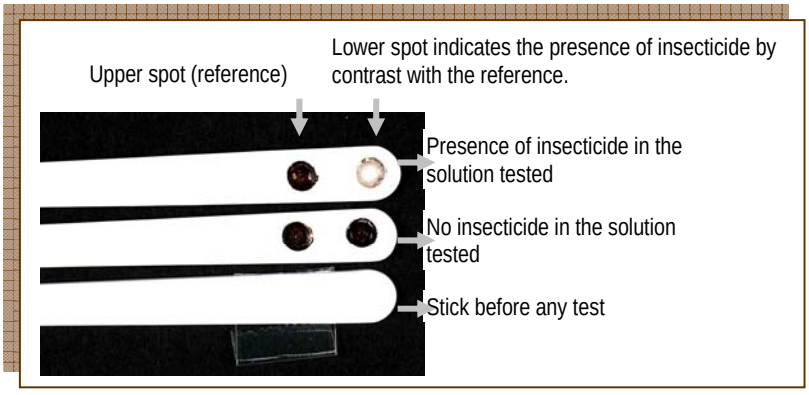

Figure 2-1. OP-Stick Sensor Results Analysis

Detection limits for the various OP/Cs differ depending on their ability to inhibit the enzyme. Combinations of various OP/Cs will have an additive effect on the inhibition assay. The test allows screening without any laboratory. Positive tests would need confirmation by further analysis for qualitative and quantitative assay.

One OP-Stick Sensor kit is composed of three tubes each labeled with a colored sticker and one test stick. Tube 1 (labeled yellow) contains an oxidizing agent for phosphorothioate activation in an “oxon” form. Tube 2 (labeled blue) contains a neutralizing agent to avoid denaturation of AChE by the reagent from Tube 1. Tube 3 (labeled red) contains the chromogen reagent. The OP-Stick Sensor kit is 10 by 5 by 2 centimeters. The price of the kit, which can be used for one test, is approximately $20.

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-Stick Sensor to detect chemical agents, carbamate pesticides, and OP pesticides in drinking water. This verification test assessed the performance of the OP-Stick Sensor 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-Stick Sensor to detect VX, sarin (GB), 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-Stick Sensor 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 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, with the exception of aldicarb, was added to these samples, along with the potential interferent, at a concentration consistent with a 10x dilution of the lethal dose, and the resulting samples were analyzed in triplicate. Table 3-2 lists the PT samples analyzed in this verification test for each contaminant. The vendor provided a limit of detection (LOD) of >100 mg/L for aldicarb, therefore interferent PT samples for aldicarb were fortified at the lethal dose concentration.

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: 2.0 mg/L GD: 0.14 mg/L aldicarb: 260 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-Stick Sensor 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, with the exception of aldicarb which was spiked at 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: 260 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. 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. All of 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 were summarized to describe the operational performance of the OP-Stick Sensor 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-Stick Sensor. 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-Stick Sensor by another Battelle staff person who was trained by the vendor. Since many of the contaminants being tested

are highly toxic and unsafe to be handled outside of a special facility, only MB samples were analyzed as part of the operational factors assessment. Because no samples spiked with the contaminants of interest were used, only the operational aspects of the OP-Stick Sensor were evaluated with the non-technical operator. As the OP-Stick Sensor may be used by first-responders, its performance was evaluated under simulated first-response conditions by having the operator don 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-Stick Sensor 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 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 100-mL quantities. Appropriate aliquots of this sample preparation were used for each test sample. Triplicate samples of 10 mL 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

The OP-Stick Sensor kit is composed of one plastic stick (with two spots at one end that contain the detecting agent) and three reagent containing tubes. Three test samples were analyzed in parallel. For each test sample, the operator used a pipette to introduce 10 mL of the test sample into a "tube 1" that is labeled with a yellow dot, which contains the activating agent. The operator carefully shook the tube to ensure that the pellet at the bottom of the tube was dissolved into solution. The sample in the tube was then incubated for 15 minutes. The operator recorded the time, kept by a stopwatch (timer), on the data sheet for each incubation step.

After the 15 minute incubation period, the operator transferred the solution from tube 1 into "tube 2," labeled with a blue dot. After shaking the tube to dissolve the powder at the bottom of this tube, the operator inserted the plastic stick into tube 2, without removing the adhesive tape

covering the upper spot. The stick was incubated for 1 hour for each sample. The vendor indicates that the longer incubation period will results in lower detection limits, though this was not verified during this test.

After the 1 hour incubation, the operator introduced 10 mL of contaminant-free water into "tube 3," labeled with a red or green dot. After shaking the tube to ensure that the pellet at the bottom of the tube was dissolved, the operator removed the plastic stick from tube 2. The operator then used a pair of tweezers to remove the adhesive tape covering the upper spot on the stick and dipped the stick into tube 3. The plastic stick was incubated in this solution for 15 minutes.

This upper spot is a reference for the OP-Stick Sensor. After the incubation period, the operator removed the stick from tube 3 and visually inspected the color of the two spots. Though the instructions provided with the kit indicate that the operator should observe a black or white color, which respectively indicates the absence or presence of a contaminant, the colors observed during testing were mostly not black or white, but also included shades of grey, green, and yellow. The operator compared the lower spot to the reference for the result. If the lower spot had the same color as the upper spot (black or dark), then no contaminant was detected by the OP-Stick Sensor. If the lower spot had a reduced color or white, then the test sample was considered to be positive for the presence of a contaminant.

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

MB samples were run as part of the verification test. Of the 70 method blank samples analyzed, 1 detect, 8 inconclusive, and 61 non-detect results were observed.

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 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.

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 found out of calibration over the course of testing.

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. The %R, listed in Table 4-1, represents the average of the %R across both concentration levels for those compounds. 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., Ca and Mg 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 are 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, 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-Stick Sensor. 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 were within the 25% acceptable tolerance.

Table 4-2. Performance Evaluation Samples and Percent Difference

Contaminant	Expected Concentration (mg/L)	Measured Concentration (mg/L)	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-Stick Sensor 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-Stick Sensor result is 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-Stick Sensor'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 ASTM Type II DI water (including interferent samples) or DW sample was not spiked with a 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-Stick Sensor'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-Stick Sensor'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). Inconclusive results were not considered positive or negative (so the total number of unspiked or spiked samples was decreased accordingly)."

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

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

5.3 Precision

Precision measures the repeatability and reproducibility of the OP-Stick Sensor'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-Stick Sensor 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).

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

5.4 Potential Matrix and Interferent Effects

The potential effect of the DW matrix on the OP-Stick Sensor'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-Stick Sensor'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-Stick Sensor from both the technical and non-technical operators' perspectives.

Chapter 6
Test Results

The results for the OP-Stick Sensor are discussed in the following sections. It is important to note that the ability of the operator to discern and compare the colors that appear on the stick at the end of the test is integral to the outcome of the test. As described in the Technology Description (Chapter 2), the degree to which the indicator spots change color depends on the concentration of the contaminant in a water sample. It was observed during the testing that the colors of the upper spot (reference) were often yellow and green (and not only degrees of brown or black as the instructions indicate). Comparison of the lower spot to assess reduced or absent color formation was necessary to conclude a test result. This led occasionally to inconclusive results, which could not be categorized by the operator as either positive or negative indications from the OP-Stick Sensor. Additionally, the same outcomes could have been interpreted by two operators in different ways, potentially leading to different test results.

6.1 Accuracy

Accuracy was determined using contaminant-only PT samples that were equal to or above the vendor-provided LOD. If no LOD was known, only those concentration levels above which consistent negative results were obtained were used to determine accuracy. Results are provided in Table 6-1. No LODs were provided by the vendor for any of the target contaminants with the exception of aldicarb, for which an LOD of >100 mg/L for aldicarb was provided by the vendor. For this reason, accuracy was determined using the three PT samples at 260 mg/L aldicarb. For VX, GB, and GD, all contaminant-only PT samples were included in the calculation of accuracy since consistent negative responses were not established above the lowest set of PT samples. However, several inconclusive results were observed with these contaminants, primarily at the lower concentrations. Consistent negative results were obtained at and below 1.4 mg/L dicrotophos. Therefore, accuracy is determined for all replicates above this concentration.

Six inconclusive results were observed among the nine replicates at the 0.021 mg/L VX concentration level and below, though two positive results were also detected at the lowest concentration level for VX (0.00021 mg/L) to yield an overall accuracy of 33% for VX. Inconsistent results were also observed for GB and GD including two occurrences at the highest concentration level of 1.4 mg/L for GD. Overall accuracy for GB and GD was 60% and 27%, respectively. Accuracy for both aldicarb and dicrotophos was 100%, in the relatively high concentration levels for which accuracy was calculated for these contaminants. No inconclusive results were obtained for aldicarb and dicrotophos.

Table 6-1. Contaminant-Only PT Sample Results

Contaminant	Concentration (mg/L)	Positive Results Out of Total Replicates	Inconclusive Results Out of Total Replicates	Accuracy
VX	2.1 [a]	3/3	0/3	**33% (5/15)**
	0.21	0/3	0/3	
	0.021	0/3	2/3	
	0.0021	0/3	3/3	
	0.00021	2/3	1/3	
GB	20 [a]	3/3	0/3	**60% (9/15)**
	2.0	3/3	0/3	
	0.20	3/3	0/3	
	0.020	0/3	3/3	
	0.0020	0/3	1/3	
GD	1.4 [a]	1/3	2/3	**27% (4/15)**
	0.14	3/3	0/3	
	0.014	0/3	3/3	
	0.0014	0/3	3/3	
	0.00014	0/3	1/3	
aldicarb	260 [a]	3/3	0/3	**100% (3/3)**
	26 [b]	0/3	0/3	
	2.6 [b]	0/3	0/3	
	0.26 [b]	0/3	0/3	
	0.026 [b]	0/3	0/3	
dicrotophos	1400 [a]	3/3	0/3	**100% (9/9)**
	140	3/3	0/3	
	14	3/3	0/3	
	1.4 [c]	0/3	0/3	
	0.014	0/3	0/3	

[a] Lethal dose
[b] Vendor provided LOD of >100 mg/L; therefore concentrations below this limit were not used to calculate accuracy.
[c] Concentration at or below which consistent negative responses were observed

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 Protein Biosensor OP-Stick Sensor. 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 maximum 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.

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 for these unspiked samples 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.

As shown in Table 6-2a, seven false negative responses were observed for VX, in one replicate of the 0.021 mg/L VX in DI water PT sample, and three replicates each of the 0.21 mg/L VX in DI water PT sample and the 0.21 mg/L VX in 1 mg/L humic and fulvic acid solution interferent sample. No false positive results were observed for VX. For GB, two of 39 samples gave false negative results. These samples were the lowest concentration of GB only PT samples, fortified at 0.002 mg/L, as shown in Table 6-2b. No false positive results were observed for GB. For GD as well, two of 39 samples gave false negative results. These samples were also the lowest concentration of contaminant-only PT samples for GD: 0.00014 mg/L GD as shown in Table 6-2c. No false positives were observed for GD.

For aldicarb, the vendor-provided LOD is stated as < 100 ng/mL aldicarb in water. Therefore, no samples with lower concentrations of aldicarb were included in the calculations for false negatives rates, though these concentrations were included in the false positive calculation. One false positive result for aldicarb was observed for the Protein Biosensor OP-Stick Sensor with the 250 mg/L total Ca and Mg unfortified solution as shown in Table 6-2d. No false negatives were observed for aldicarb. Neither false negatives nor false positives were observed for dicrotophos as shown in Table 6-2e.

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

Sample Type	Matrix	Concentration (mg/L)	Negative Results	Inconclusive Results	Positive Results out of Total Replicates[a]
Contaminant-only PT samples	DI water	2.1 [b]	0	0	3/3
	DI water	0.21	3	0	0/3
	DI water	0.021	1	2	0/3
	DI water	0.0021	0	3	0/3
	DI water	0.00021	0	1	2/3
Interferent samples [c]	1 mg/L humic and fulvic acids	Blank	3	0	0/3
	1 mg/L humic and fulvic acids	0.21	3	0	0/3
	5 mg/L humic and fulvic acids	Blank	2	1	0/3
	5 mg/L humic and fulvic acids	0.21	0	0	3/3
	50 mg/L Ca + Mg	Blank	0	3	0/3
	50 mg/L Ca + Mg	0.21	0	1	2/3
	250 mg/L Ca + Mg	Blank	3	0	0/3
	250 mg/L Ca + Mg	0.21	0	0	3/3
DW samples [c]	OH DW	Blank	1	2	0/3
	OH DW	0.21	0	1	2/3
	NY DW	Blank	3	0	0/3
	NY DW	0.21	0	0	3/3
	FL DW	Blank	3	0	0/3
	FL DW	0.21	0	1	2/3
	CA DW	Blank	1	2	0/3
	CA DW	0.21	0	0	3/3
	False Positive Rate				**0/24**
	False Negative Rate				**7/39**

[a] Shaded results indicate false negative observations
[b] Lethal dose
[c] Only one set of unspiked DW and PT interferent samples were run for VX, GB, and GD.

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

Sample Type	Matrix	Concentration (mg/L)	Negative Results	Inconclusive Results	Positive Results out of Total Replicates[a]
Contaminant-only PT samples	DI water	20 [b]	0	0	3/3
	DI water	2.0	0	0	3/3
	DI water	0.2	0	0	3/3
	DI water	0.02	0	3	0/3
	DI water	0.002	2	1	0/3
Interferent samples [c]	1 mg/L humic and fulvic acids	Blank	3	0	0/3
	1 mg/L humic and fulvic acids	2.0	0	0	3/3
	5 mg/L humic and fulvic acids	Blank	2	1	0/3
	5 mg/L humic and fulvic acids	2.0	0	0	3/3
	50 mg/L Ca + Mg	Blank	0	3	0/3
	50 mg/L Ca + Mg	2.0	0	2	1/3
	250 mg/L Ca + Mg	Blank	3	0	0/3
	250 mg/L Ca + Mg	2.0	0	0	3/3
DW samples [c]	OH DW	Blank	1	2	0/3
	OH DW	2.0	0	2	1/3
	NY DW	Blank	3	0	0/3
	NY DW	2.0	0	0	3/3
	FL DW	Blank	3	0	0/3
	FL DW	2.0	0	0	3/3
	CA DW	Blank	1	2	0/3
	CA DW	2.0	0	0	3/3
	False Positive Rate				**0/24**
	False Negative Rate				**2/30**

[a] Shaded results indicate false negative observations
[b] Lethal dose
[c] Only one set of unspiked DW and PT interferent samples were run for VX, GB, and GD.

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

Sample Type	Matrix	Concentration (mg/L)	Negative Results	Inconclusive Results	Positive Results out of Total Replicates[a]
Contaminant-only PT samples	DI water	1.4 [a]	0	2	1/3
	DI water	0.14	0	0	3/3
	DI water	0.014	0	3	0/3
	DI water	0.0014	0	3	0/3
	DI water	0.00014	2	1	0/3
Interferent samples [c]	1 mg/L humic and fulvic acids	Blank	3	0	0/3
	1 mg/L humic and fulvic acids	0.14	0	2	1/3
	5 mg/L humic and fulvic acids	Blank	2	1	0/3
	5 mg/L humic and fulvic acids	0.14	0	0	3/3
	50 mg/L Ca + Mg	Blank	0	3	0/3
	50 mg/L Ca + Mg	0.14	0	0	3/3
	250 mg/L Ca + Mg	Blank	3	0	0/3
	250 mg/L Ca + Mg	0.14	0	0	3/3
DW samples [c]	OH DW	Blank	1	2	0/3
	OH DW	0.14	0	1	2/3
	NY DW	Blank	3	0	0/3
	NY DW	0.14	0	0	3/3
	FL DW	Blank	3	0	0/3
	FL DW	0.14	0	2	1/3
	CA DW	Blank	1	2	0/3
	CA DW	0.14	0	1	2/3
	False Positive Rate				**0/24**
	False Negative Rate				**2/39**

[a] Shaded results indicate false negative observations
[b] Lethal dose
[c] Only one set of unspiked DW and PT interferent samples were run for VX, GB, and GD.

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

Sample Type	Matrix	Concentration (mg/L)	Negative Results	Inconclusive Results	Positive Results out of Total Replicates [a]
Contaminant-only PT samples	DI water	260 [b]	0	0	3/3
	DI water	26	3	0	0/3
	DI water	2.6	3	0	0/3
	DI water	0.26	3	0	0/3
	DI water	0.026	3	0	0/3
Interferent samples	1 mg/L humic and fulvic acids	Blank	3	0	0/3
	1 mg/L humic and fulvic acids	260	0	0	3/3
	5 mg/L humic and fulvic acids	Blank	3	0	0/3
	5 mg/L humic and fulvic acids	260	0	0	3/3
	50 mg/L Ca + Mg	Blank	3	0	0/3
	50 mg/L Ca + Mg	260	0	0	3/3
	250 mg/L Ca + Mg	Blank	2	0	1/3
	250 mg/L Ca + Mg	260	0	0	3/3
DW samples	OH DW	Blank	3	0	0/3
	OH DW	260	0	0	3/3
	NY DW	Blank	3	0	0/3
	NY DW	260	0	0	3/3
	FL DW	Blank	3	0	0/3
	FL DW	260	0	0	3/3
	CA DW	Blank	3	0	0/3
	CA DW	260	0	0	3/3
	False Positive Rate				**1/24**
	False Negative Rate				**0/27**

[a] Boxed results indicate respectively false negative or false positive observations
[b] Lethal dose

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

Sample Type	Matrix	Concentration (mg/L)	Negative Results	Inconclusive Results	Positive Results out of Total Replicates
Contaminant-only PT samples	DI water	1400 [(a)]	0	0	3/3
	DI water	140	0	0	3/3
	DI water	14	0	0	3/3
	DI water	1.4	3	0	0/3
	DI water	0.14	3	0	0/3
Interferent samples	1 mg/L humic and fulvic acids	Blank	3	0	0/3
	1 mg/L humic and fulvic acids	140	0	0	3/3
	5 mg/L humic and fulvic acids	Blank	3	0	0/3
	5 mg/L humic and fulvic acids	140	0	0	3/3
	50 mg/L Ca + Mg	Blank	3	0	0/3
	50 mg/L Ca + Mg	140	0	0	3/3
	250 mg/L Ca + Mg	Blank	3	0	0/3
	250 mg/L Ca + Mg	140	0	0	3/3
DW samples	OH DW	Blank	3	0	0/3
	OH DW	140	0	0	3/3
	NY DW	Blank	3	0	0/3
	NY DW	140	0	0	3/3
	FL DW	Blank	3	0	0/3
	FL DW	140	0	0	3/3
	CA DW	Blank	3	0	0/3
	CA DW	140	0	0	3/3
	False Positive Rate				**0/24**
	False Negative Rate				**0/33**

[(a)] Lethal dose

6.3 Precision

During testing with VX, the Protein Biosensor OP-Stick Sensor gave inconsistent results. Eight of the 21 sample sets, each consisting of three replicates, had at least one replicate that differed from the other two replicates, resulting in consistent results in 13 out of 21 sets, or a precision of 62%. For GB, 15 of 21 sample sets yielded consistent results, giving a precision of 71%. For GD, 12 of 21 samples sets yielded consistent results, giving a precision of 57%. Note that only one set of unspiked interferent samples were tested for VX, GB, and GD. These sample sets were shared among the three contaminants. Three of these 8 sample sets had at least one replicate that differed from the other two replicates.

For aldicarb, 20 of the 21 sample sets had consistent results, yielding a precision of 95%. One unspiked interferent sample (250 mg/L total Ca and Mg) gave a false positive result. No inconsistent results were observed during testing for dicrotophos, yielding a precision of 100%.

6.4 Potential Matrix and Interferent Effects

The Protein Biosensor OP-Stick Sensor was able to consistently detect GB, GD, and dicrotophos at 10 times less than the respective LD_{50} concentrations in DI water, so testing of matrix and interferent effects for these contaminants was conducted at these respective concentration levels. For aldicarb, one-tenth of its LD_{50} was below the vendor provided detection limit of the technology, so the test of potential matrix and interferent effects for aldicarb was performed at the LD_{50} concentration (260 mg/L) in water. For VX, testing was conducted at 10 times less than the LD_{50} concentration (i.e., at 0.21 mg/L) even though the contaminant-only PT samples at this concentration yielded consistent negative results.

6.4.1 Interferent PT Samples

For VX (Table 6-2a), testing with the 1 mg/L humic and fulvic acid matrix yielded consistent negative responses when the matrix was spiked with the contaminant at 10 times less than the LD_{50} (0.2 mg/L). One inconclusive result was obtained when VX was spiked into the 50 mg/L Ca + Mg solution, though the unspiked matrix gave three inconclusive results. With these exceptions, the remaining eight fortified interferent samples yielded positive results for VX, indicating that other matrix effects were minimal if present. For GB (Table 6-2b), one inconclusive result was observed in the unfortified 5 mg/L humic and fulvic acid. The 50 mg/L Ca + Mg solution produced three inconclusive results while the samples with a higher concentration of Ca + Mg (250 mg/L) indicated no interferent effects were present for GB. Two inconclusive results were also observed with the 50 mg/L Ca + Mg matrix spiked with 2.0 mg/L of GB. With these exceptions, the other ten fortified interferent samples yielded positive results for GB. For GD (Table 6-2c), two inconclusive results were observed for 0.14 mg/L GD in 1 mg/L humic and fulvic acid solution. One inconclusive result was observed for the unfortified 5 mg/L humic and fulvic acid solution, and three were observed for the unfortified 50 mg/L Ca + Mg solution. With these exceptions, the other ten fortified samples for GD yielded positive results. For dicrotophos, all fortified samples yielded positive results while unfortified samples yielded negative results, indicating that no matrix effects are present for these contaminants in the interferent solutions tested. For aldicarb, all fortified samples also yielded positive results

though one unfortified sample, 250 mg/L Ca + Mg, gave a positive response (as shown in Table 6-2d). All of the remaining unfortified samples yielded negative results for aldicarb.

6.4.2 DW Samples

For VX, GB, and GD, two inconclusive results were reported for both unfortified OH DW and unfortified CA DW samples. For VX, one inconclusive result was also reported for the fortified (0.21 mg/L) test samples in each of the OH and FL DW matrices. With these exceptions, ten of the 12 remaining fortified samples yielded positive results, indicating minimal matrix effects for VX in these DW samples. For GB, two inconclusive results were also observed for the 2 mg/L GB in OH DW. With these exceptions, 10 of 12 fortified samples yielded positive results for GB. One inconclusive result was also observed for GD in each of the fortified OH DW, FL DW, and CA DW matrices. With these exceptions, nine of 12 fortified samples yielded positive results for GD. For aldicarb and dicrotophos, all fortified samples yielded positive results while blank samples yielded negative results, indicating that no matrix effects were present for these contaminants in the DW matrices tested.

6.5 Operational Factors

6.5.1 Technical Operators

The Protein Biosensor OP-Stick Sensor was operated by one Battelle technical operator throughout testing with the pesticides and by a different Battelle technical operator throughout testing with CWA. The technical operators were trained by the vendor in the operation of the test kit. The half-day of face-to-face training was provided by a vendor representative. Both technical operators had extensive laboratory experience.

The operators commonly observed that the tape on the bottom of the sticks is extremely difficult to remove. Since the test samples may be potentially hazardous, it may not be acceptable to remove the tape by hand. Therefore, tweezers of some kind must be used and this requires dexterity that may not be achievable while gloved.

With the first lot of OP-Stick Sensors used, the spots were not black or white as the instructions indicated they should be. More often they were various shades of yellow, grey or green. This made it very difficult to discern the result for a particular sample, leading to inconclusive results. It appeared that the various lots used during the testing differed in the reactivity to the contaminants used during testing. The second lot of OP-Stick Sensors that were used toward the end of testing was much more reactive. The reference spot on these tubes showed a deep black color and the indicator spot was either a deep black or plain white. These results were less subjective and much easier to read.

Some kind of rack should be included with the test kit to hold the several tubes. The tubes should be more clearly labeled as they were only labeled with a colored dot which would occasionally fall off of the bag. Nothing is included in the kit to deliver the 10 mL of test sample needed in tube 1 or the 10 mL of water needed in tube 3. It would be helpful to include some

kind of pipette with the kit for this purpose. Tweezers also should be included with the kit for retrieving the OP-Stick Sensor from tube 2 and transferring it to tube 3. Sample throughput varied with the operator as multiple samples can be analyzed simultaneously. Physical accommodations (i.e., hood space or table space) and operator preference for sample size may affect sample throughput. Instructions that include a diagram of the various steps required of the test are included with the kit. Samples did not require any storage considerations and were kept at room temperature.

6.5.2 Non-Technical Operator

Unspiked DI water samples were tested on the Protein Biosensor OP-Stick Sensor by a non-technical operator both with and without PPE (see Section 3.2.4). The samples were analyzed while wearing full PPE, consisting of a Level B suit, neoprene latex gloves, boots and SCBA as shown in Figures 6-1 and 6-2. The SCBA was worn throughout the entire testing procedure by the non-technical operator (only during the tests in which PPE was to be donned) to represent the physical burden borne by a similarly outfitted first responder. However, the operator ran the air from the SCBA only part of the time during testing to conserve the tank.

Including set up and operation, the time required for a test is approximately 1.5 to 2 hours for each sample though multiple samples may be analyzed simultaneously. An operator equipped with SCBA would have to obtain a new tank of air for the duration of the test. A gloved operator would also have trouble removing the tape on the OP-Stick Sensor. The operator had to use tweezers to remove the tape.

Samples were also analyzed without PPE. All samples were negative for the unspiked DI water samples. While the individual test tubes containing the reagents (three separate kinds are required for a single test) and OP-stick Sensors are portable, the time required for incubation of the samples makes it necessary for the operator to have sufficient working space in which the test tubes may be placed for the duration of the test (which is over 1 hour). Adequate lighting is also required to read the color changes which may preclude the use of the test in environments with low lighting. A test tube rack and a pipetter are also recommended for use, so these items should be considered for field use of the Protein Biosensor OP-Stick Sensor.

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

Figure 6-2. Testing of the OP-Stick Sensor with the Non-Technical Operator Wearing PPE

Chapter 7
Performance Summary

The Protein Biosensor OP-Stick Sensor results from this verification test for samples containing VX, GB, GD, aldicarb, and dicrotophos are presented in Tables 7-1a-e, respectively. 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 Protein Biosensor OP-Stick Sensor is presented at the end of this chapter. These performance factors apply across all contaminants.

Table 7-1a. 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	0/3
			0.021 mg/L	0/3
			0.0021 mg/L	0/3
			0.00021 mg/L	2/3
	Interferent PT Samples	Humic and Fulvic Acids	0.21 mg/L	3/6
		Ca and Mg	0.21 mg/L	5/6
	DW Samples	DW	0.21 mg/L	10/12
Accuracy		33% (5 out of 15) of the contaminant-only PT samples gave positive results during testing at concentrations levels of 0.00021 to 2.1 mg/L VX. Six inconclusive results were observed in the nine replicates of the contaminant-only PT samples at and below the concentration level of 0.021 mg/L VX		
False Positive Rate		No false positive results (0 out of 24) were observed during the testing with VX.		
False Negative Rate		Seven false negative results out of 39 samples were observed during testing with VX: one replicate of the 0.021 mg/L VX in DI water PT sample, and three replicates each of the 0.21 mg/L VX in DI water PT sample and the 0.21 mg/L VX in 1 mg/L humic and fulvic acid solution interferent sample.		
Precision		62% (13 out of 21) of the sample sets showed consistent results among the individual replicates within each set during testing with VX.		

[(a)] Lethal dose

Table 7-1b. 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
			0.2 mg/L	3/3
			0.02 mg/L	0/3
			0.002 mg/L	0/3
	Interferent PT Samples	Humic and Fulvic Acids	2.0 mg/L	6/6
		Ca and Mg	2.0 mg/L	4/6
	DW Samples	DW	2.0 mg/L	10/12
Accuracy		60% (9 out of 15) of the contaminant-only PT samples gave positive results during testing with GB. Four inconclusive results were observed at the 0.02 and 0.002 mg/L GB concentration levels, with two negative results observed at the 0.002 mg/L GB concentration level.		
False Positive Rate		No false positive results (0 out of 24) were observed during testing with GB.		
False Negative Rate		Two false negative results out of 39 samples were observed during testing with GB. These samples were at the lowest concentration of the contaminant-only PT samples, fortified at 0.002 mg/L.		
Precision		71% (15 out of 21) of the sample sets showed consistent results among the individual replicates with each set during testing with GB.		

[(a)] Lethal dose

Table 7-1c. 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)]	1/3
			0.14 mg/L	3/3
			0.014 mg/L	0/3
			0.0014 mg/L	0/3
			0.00014 mg/L	0/3
	Interferent PT Samples	Humic and Fulvic Acids	0.14 mg/L	4/6
		Ca and Mg	0.14 mg/L	6/6
	DW Samples	DW	0.14 mg/L	8/12
Accuracy		27% (4 out of 15) of the contaminant-only PT samples gave positive results during testing at concentrations of 0.00014 to 1.4 mg/L GD. Seven inconclusive results were observed at the concentration level of 0.014 mg/L GD and below. Two negative results were observed at the lowest concentration level tested, 0.00014 mg/L GD.		
False Positive Rate		No false positive results (0 out of 24) were observed during testing with GD.		
False Negative Rate		Two false negative results (2 out of 39) were observed during testing with GD. These results were observed at the lowest concentration level tested, 0.00014 mg/L GD.		
Precision		57% (12 out of 21) of the sample sets showed consistent results among the individual replicates within that set during testing with GD.		

[(a)] Lethal dose

Table 7-1d. 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	0/3
			2.6 mg/L	0/3
			0.26 mg/L	0/3
			0.026 mg/L	0/3
	Interferent PT Samples	Humic and Fulvic Acids	260 mg/L	6/6
		Ca and Mg	260 mg/L	6/6
	DW Samples	DW	260 mg/L	12/12
Accuracy		100% (3 out of 3) of the contaminant-only PT samples at 260 mg/L gave positive results during testing with aldicarb. The vendor provided an LOD of >100 mg/L, therefore none of the other concentration levels were included in the calculation of accuracy.		
False Positive Rate		One false positive result (out of 24 results) was observed during testing with aldicarb. This positive result was observed in a 250 mg/L Ca and Mg solution into which no aldicarb was spiked. The other two results for this sample set were two negative results.		
False Negative Rate		No false negative results (0 out of 27) were observed during testing with aldicarb.		
Precision		95% (20 out of 21) of the sample sets showed consistent results among the individual replicates with each set during testing with aldicarb. The one set which did not have consistent results was the unfortified 250 mg/L Ca and Mg solution described above under False Positive Rate.		

[(a)] Lethal dose

Table 7-1e. 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	0/3
			0.14 mg/L	0/3
	Interferent PT Samples	Humic and Fulvic Acids	140 mg/L	6/6
		Ca and Mg	140 mg/L	6/6
	DW Samples	DW	140 mg/L	12/12
Accuracy		100% (9 out of 9) of the contaminant-only PT samples gave positive results during testing with dicrotophos. Consistent negative results were observed at and below the concentration level of 1.4 mg/L dicrotophos, therefore only concentrations above this level were used to calculate accuracy.		
False Positive Rate		No false positive results (0 out of 24) were observed during testing with dicrotophos.		
False Negative Rate		No false negative results (0 out of 30) were observed during testing with dicrotophos.		
Precision		100% (21 out of 21) of the sample sets showed consistent results among the individual replicates within each set during the testing of dicrotophos.		

[(a)] Lethal dose

Operational Factors:

Technical Operators
The Protein Biosensor OP-Stick Sensor was operated by one Battelle technical operator throughout testing with the pesticides and by a different Battelle technical operator throughout testing with chemical warfare agents. The technical operators were trained by the vendor in the operation of the test kit. Both technical operators had extensive laboratory experience. The operators commonly observed that the tape on the bottom of the sticks is extremely difficult to remove. Since the test samples may be potentially hazardous, it may not be acceptable to remove the tape by hand.

Some variability within the production lots of kits was observed. The first lot of OP-Stick Sensors showed spots that were various shades of yellow, grey, or green, not black or white as the instructions indicated they should be. This made it very difficult to discern the result for a particular sample, leading to inconclusive results. The second lot of OP-Stick Sensors that were used toward the end of testing was much more reactive. The reference spot on these tubes showed a deep black color, and the indicator spot was either a deep black or plain white. These results were less subjective and much easier to read. Sample throughput varied with the operator as multiple samples can be analyzed simultaneously. Physical accommodations (i.e., hood space or table space) and operator preference for sample size may affect sample throughput.

Non-Technical Operator
Unspiked DI water samples were tested on the Protein Biosensor OP-Stick Sensor by a non-technical operator both with and without PPE. During testing with the PPE on, the samples were analyzed while the operator wore a full PPE, consisting of a Level B suit, neoprene latex gloves, boots and SCBA. Including set up and operation, the time required for a test was approximately 1.5 to 2 hours; an operator equipped with a SCBA would have to obtain a new tank of air for the duration of the test. A gloved operator would also have trouble removing the tape on the OP-Stick Sensor. The operator had to use tweezers to remove the tape. The length of time for the test and the need to manipulate the OP-Stick Sensor make its use difficult for users wearing PPE, such as first responders.

Chapter 8
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