THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

Battelle
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ETV Joint Verification Statement

TECHNOLOGY TYPE:	**Enzymatic Test Kit**	
APPLICATION:	**Detecting Chemical Warfare Agents, Carbamate Pesticides, and Organophosphate Pesticides in Drinking Water**	
TECHNOLOGY NAME:	**OP-Stick Sensor**	
COMPANY:	**Protein-Biosensor**	
ADDRESS:	**IPBS-CNRS** **205 rte de Narbonne** **31077 Toulouse cedex, France**	**PHONE: +33(0)561 175 837** **FAX: +33(0)561 175 994**
WEB SITE:	**www.protein-biosensor.com**	

The U.S. Environmental Protection Agency (EPA) has established the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies. Information and ETV documents are available at www.epa.gov/etv.

ETV works in partnership with recognized standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permitters), and with individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The Advanced Monitoring Systems (AMS) Center, one of six technology areas under ETV, is operated by Battelle in cooperation with EPA's National Exposure Research Laboratory. The AMS Center evaluated the performance of the Protein-Biosensor's OP-Stick Sensor. This verification statement provides a summary of the test results.

VERIFICATION TEST DESCRIPTION

The objective of this verification test was to evaluate the ability of the OP-Stick Sensor to detect chemical agents, carbamate pesticides, and organophosphate (OP) pesticides as contaminants in drinking water (DW). 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; and various operational factors including operator observations, ease of use, and sample throughput from both a technical and non-technical operators' perspective. The OP-Stick Sensor was evaluated using VX, sarin (GB), and soman (GD) (chemical agents); aldicarb (carbamate pesticide); and dicrotophos (OP pesticide) in performance test (PT) and DW samples. Quality Control (QC) samples were also included as part of the test matrix to ensure the integrity of the test. PT samples included individual contaminants spiked into American Society for Testing and Materials (ASTM) Type II deionized (DI) water at five different concentrations: the lethal dose concentration for each contaminant, along with dilutions at approximately 10, 100, 1,000, and 10,000 times less than the lethal dose. PT samples also included potential interferent samples containing a single concentration (10 times less than the lethal dose) of the contaminant of interest in the presence of calcium (Ca) and magnesium (Mg) spiked into ASTM Type II DI water, and humic and fulvic acids spiked into ASTM Type II DI water. The vendor provided a limit of detection (LOD) of <100 mg/L, which is less than one tenth of the LD_{50} (lethal dose for half of the test subjects) for aldicarb. Therefore, the potential interferent samples were fortified at the LD_{50} level for aldicarb (260 mg/L). Each interferent mixture was prepared at two concentration levels: near the upper limit of what would be expected in drinking water (250 milligrams per 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). Interferent PT samples were also analyzed without the addition of any contaminant. DW samples consisted of chlorinated filtered surface water, chlorinated unfiltered surface water, chlorinated filtered groundwater, and chloraminated filtered surface water collected from four geographically distributed municipal water sources (OH, NY, FL, and CA, respectively). DW samples were analyzed before adding any contaminant and after fortification with each individual contaminant at 10 times less than the lethal dose of that contaminant, with the exception of aldicarb. As explained above, the DW samples were fortified at the LD_{50} level for aldicarb (260 mg/L). All DW samples were dechlorinated prior to use. QC samples included method blank (MB) samples and positive and negative controls, as supplied with the OP-Stick Sensor. All samples were tested in triplicate.

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. Human oral LD_{50} data were not available for aldicarb, so rat oral LD_{50} data were used instead. Lethal dose values are provided in the contaminant results tables below. Samples were tested blindly by Battelle technical operators who were trained by the vendor in the use of the OP-Stick Sensor. Contaminants were tested individually, and stock solutions of each contaminant were prepared separately in ASTM Type II DI water. To minimize the loss of analytes to hydrolysis, contaminant stock solutions prepared in DI water were made on a daily basis. In some cases, reference solutions were prepared in ASTM Type II DI water using the stock solutions to prepare the test samples. In other cases, the actual stock solutions were submitted for concentration confirmation by the respective reference analysis.

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. Only MB samples and non-toxic control samples were analyzed as part of the operational factors assessment. As the OP-Stick Sensor 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.

QA oversight of verification testing was provided by Battelle and EPA. Battelle QA staff conducted a technical systems audit, a performance evaluation audit, and a data quality audit of 10% of the test data. Testing was conducted from November 2005 through February 2006. This verification statement, the full report on which it is based, and the test/QA plan for this verification test are all available at www.epa.gov/etv/centers/center1.html.

TECHNOLOGY DESCRIPTION

The following description of the OP-Stick Sensor is based on information provided by the vendor. This technology description 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 the presence of inhibiting pesticides, AChE hydrolyzes acetylthiocholine to thiocholine, which reacts with a colorimetric substrate on a test stick 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.

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 analysis of the sample. 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 is approximately $20.

VERIFICATION RESULTS

Accuracy was assessed by evaluating how often the OP-Stick Sensor result was positive in the presence of a concentration above the limit of detection (LOD). Contaminant-only PT samples were used for this analysis. For aldicarb, the vendor-provided LOD was >100 mg/L. LODs were not available for dicrotophos, VX, GB, and GD. For these compounds, all analyzed contaminant-only PT samples greater than the concentration level where consistent negative results were obtained were used for calculations. Results for VX, GB, and GD were not consistently negative at any concentration level; thus, all analyzed PT samples were included in the accuracy calculations. For dicrotophos, consistent negative results were observed at 1.4 mg/L; therefore only contaminant-only PT samples with concentrations above this level were used to calculate accuracy.

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 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 or consistent negative response level, as defined above. Spiked PT (contaminant and interferent) samples and spiked DW samples were included in the analysis. The precision of three replicates of each sample set was assessed by calculating the overall number of consistent responses for all the sample sets. 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 were qualitative observations of the verification staff from both the technical and non-technical operators' perspective.

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 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

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

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

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.		

[(a)] Lethal dose

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 technician throughout testing with the pesticides and by a different Battelle technician throughout testing with chemical warfare agents. The technicians were trained by the vendor in the operation of the test kit. Both technicians 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 kits showed spots that were various shades of yellow, grey, or green, not only 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 kits 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 by a non-technical operator both with and without PPE. During testing with the PPE on, the samples were analyzed while the operator wore full PPE, consisting of a Level B suit, neoprene latex gloves, boots and SCBA. 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 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. 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 make its use difficult for users wearing PPE, such as first responders.

Original signed by Gregory Mack 9/18/06

Gregory A. Mack Date
Vice President
Energy, Transportation, and Environment Division
Battelle

Original signed by Andrew Avel 10/2/06

Andrew P. Avel Date
Acting Director
National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency