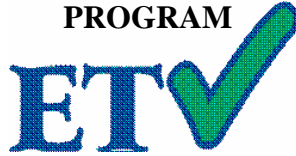


**THE ENVIRONMENTAL TECHNOLOGY VERIFICATION  
PROGRAM**



**ETV Joint Verification Statement**

**TECHNOLOGY TYPE:** Enzymatic Test Kit

**APPLICATION:** Detecting Chemical agents, Carbamate Pesticides, and Organophosphate Pesticides in Drinking Water

**TECHNOLOGY NAME:** Organophosphate/Carbamate Screen Kit

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

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

The Advanced Monitoring Systems (AMS) Center, one of six technology areas under ETV, is operated by Battelle in cooperation with EPA's National Exposure Research Laboratory. The AMS Center evaluated the performance of the Abraxis Organophosphate/Carbamate (OP/C) Screen Kit. 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/C Screen Kit to detect chemical agents, carbamate pesticides, and organophosphate (OP) pesticides in drinking water (DW). 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; and various operational factors including operator observations, ease of use, and sample throughput from both a technical and non-technical operators' perspective. The OP/C Screen Kit 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. 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. 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/C Screen Kit. 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<sub>50</sub> (lethal dose for half of the test subjects) data. Human oral LD<sub>50</sub> data were not available for aldicarb, so rat oral LD<sub>50</sub> data were used instead. Lethal dose values are provided in the contaminant results tables below. Samples were tested blindly by Battelle staff who were trained by the vendor in the use of the OP/C Screen Kit. 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/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. Only MB samples and non-toxic control samples were analyzed as part of the operational factors assessment. 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.

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](http://www.epa.gov/etv/centers/center1.html).

## **TECHNOLOGY DESCRIPTION**

The following description of the OP/C Screen Kit is based on information provided by the vendor. This technology description was not verified in this test.

The OP/C 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.

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 ( $\mu\text{L}$ ) 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 DI water). There are two 3-ml transfer pipettes and 22 exact-volume 100- $\mu\text{L}$  disposable pipettes included in the kit. The assay incubations are performed at  $70\pm 20^\circ\text{F}$  ( $21\pm 7^\circ\text{C}$ ).

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.

## **VERIFICATION RESULTS**

Only qualitative (positive, negative) results were used in the analysis of the test kit. Qualitative results were determined based on percent inhibition calculations from the OP/C Screen Kit sample results. Any sample with  $< 20\%$  inhibition was considered negative. Those with  $\geq 20\%$  inhibition were positive.

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. For aldicarb and dicrotophos, the LOD was 0.010 mg/L and 0.004 mg/L, respectively. LODs were not available for the chemical agents. For these compounds, all analyzed contaminant-only PT samples greater than the concentration level where consistent negative results were obtained were used for calculations. This level was defined at 0.0021 for VX and 0.00014 mg/L for GD. Results for GB were not consistently negative at any level; thus, all analyzed PT samples were included in the accuracy calculations.

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/C Screen Kit'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/C Screen Kit'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 <sup>(a)</sup>	3/3
			0.21 mg/L	3/3
			0.021 mg/L	3/3
			0.0021 mg/L	0/3 <sup>(b)</sup>
			0.00021 mg/L	0/3 <sup>(b)</sup>
	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.		

<sup>(a)</sup> Lethal dose.

<sup>(b)</sup> Not used in accuracy calculations because samples are at or below level of consistent negative response.

## GB Summary Table

Parameter		Sample Information	GB Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	20 mg/L <sup>(a)</sup>	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.		

## GD Summary Table

Parameter		Matrix	GD Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	1.4 mg/L <sup>(a)</sup>	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 <sup>(b)</sup>
	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.		

<sup>(a)</sup> Lethal dose.

<sup>(b)</sup> Not used in accuracy calculations because samples are at or below level of consistent negative response.

### Aldicarb Summary Table

Parameter		Sample Information	Aldicarb Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	260 mg/L <sup>(a)</sup>	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.		

<sup>(a)</sup> Lethal dose.

### Dicrotophos Summary Table

Parameter		Sample Information	Dicrotophos Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	1400 mg/L <sup>(a)</sup>	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.		

<sup>(a)</sup> 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 Operator*

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  $\mu$ L 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 ( $70\pm 20^{\circ}$  F/  $21\pm 7^{\circ}$  C) 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 operators if they had to spend that time in PPE. The OP/C Screen Kit was felt to be not very first-responder friendly for use in the field wearing PPE.

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