

## Detecting Chemical Agents and Pesticides in Water, Using Enzymatic Test Kits

### ***Four enzymatic test kits evaluated for determining the presence of chemical warfare agents in water***

In the past, people in the United States have largely taken the convenience of potable municipal water for granted. However, the threat of intentional contamination of our water supplies is becoming a concern because of a rise in the number of terrorist acts around the world. As a result, there is much interest in technologies that can be used to detect a contamination event as well as dispel or confirm the credibility of a threat. Such technologies include enzymatic test kits, which are generally designed to be handheld and portable. These kits can detect the presence of chemical agents, carbamate pesticides, and/or organophosphate pesticides through a cholinesterase enzyme reaction. Under contaminant-free conditions, the enzyme reacts with other reagents present in the test kit. However, the presence of contaminants from one or more of the above chemical classes causes the enzyme activity to be inhibited. Inhibition is indicated by a color change.

U.S. EPA's Homeland Security Research Program (HSRP) develops products based on scientific research and technology evaluations. Our products and expertise are widely used in preventing, preparing for, and recovering from public health and environmental emergencies that arise from terrorist attacks. Our research and products address biological, radiological, or chemical contaminants that could affect indoor areas, outdoor areas, or water infrastructure. HSRP provides these products, technical assistance, and expertise to support EPA's roles and responsibilities under the National Response Framework, statutory requirements, and Homeland Security Presidential Directives.

In late 2005 and early 2006, EPA evaluated four enzymatic test kits:

Eclox™-Pesticide Strips (Severn Trent Services)

- Neuro-IQ Tox Test Kit™ (Aqua Survey, Inc.)
- OP-Stick Sensor (Protein-Biosensor)
- Organophosphate/Carbamate Screen Kit (Abraxis LLC)

EPA tested each enzymatic test kit to determine the responsiveness to specific toxic compounds as well as to possible interfering compounds and matrices. Because enzymatic test kits are anticipated to serve mainly as screening tools in water monitoring scenarios, this testing produces only qualitative results (i.e., results indicate only the presence or absence of a contaminant). The kits cannot distinguish between contaminants; however, some can indicate the concentration range of contaminants (high, medium, low). Each kit was specifically evaluated for:

- Accuracy
- Precision
- False positive/negative rates
- Matrix and interference effects
- Operational factors

## Test Design

Table 1 identifies the enzymatic test kits that were tested using various water types fortified (spiked) separately with contaminants and interfering compounds.

**Table 1. Technologies, Contaminants, and Interfering Compounds**

Technologies	Contaminants (test range concentration, mg/L)	Interfering Compounds
Eclox™-Pesticide Strips	VX (0.021–2.1)	Humic Acid Fulvic Acid Calcium Magnesium
Neuro-IQ Tox Test Kit™	Sarin GB (0.02–20)	
OP-Stick Sensor	Soman GD (0.0014–1.4)	
Organophosphate/Carbamate Screen Kit	Aldicarb (0.026–260) Dicrotophos (0.14–1,400)	

Three types of water samples were tested in these evaluations: performance test (PT), drinking water (DW), and quality control (QC). PT samples were prepared with deionized (DI) water and fortified with the contaminant only, the interferent only, or both the contaminant and interferent. Contaminant-only PT samples were tested in a series of concentrations that included the accepted lethal dose concentration and dilutions at approximately 10; 100; 1,000; and 10,000 times less than the lethal dose.

DW samples were tested to determine the effects of matrix-specific characteristics (e.g., location, filtering) on the technology being evaluated. DW samples were collected from four geographically diverse municipal sources that varied in source (ground water or surface water), treatment (filtered or unfiltered), and disinfection process (chlorination or chloramination). DW samples were either not spiked or spiked with a contaminant at a single concentration level 10 times more dilute than the lethal dose.

All PT and DW samples were analyzed in triplicate. The result of each replicate sample set was reported as a ratio of the number of positive results to the total number of replicates (e.g., 0/3, 1/3). Method blank QC samples consisted of 10% of all samples. The method blanks were not considered in evaluations of the various test parameters.

## Performance and Results

The accuracy of the enzymatic kits was determined by dividing the number of positive responses by the overall number of spiked contaminant-only PT samples. The precision of the technologies was determined by calculating the number of consistent responses for all sample sets.

Responses were considered consistent if all three replicates had similar results. A false positive rate was reported as the frequency of positive results out of the total number of unspiked samples. A false negative rate was reported as the frequency of negative results out of the total number of spiked PT (contaminant and interferent) samples and spiked DW samples. Table 2 summarizes the results of the evaluation parameters for each technology.

**Table 2. Summary of Results**

Technologies	Contaminant <sup>a</sup>	Accuracy	Precision	Total False Positives	Total False Negatives	Number of Matrix Effects	Number of Interferent Effects
Eclox™-Pesticide Strips	VX	100%	100%	0/24	0/30	0	0
	GB	100%	100%	0/24	0/36	0	0
	GD	78%	95%	0/24	0/33	0	0
	aldicarb	50%	100%	0/24	6/36	0	0
	dicrotophos	100%	95%	0/24	20/30	9	11
Neuro-IQ Tox Test Kit™	VX	100%	90%	13/24	1/39	6	8
	GB	93%	90%	13/24	1/39	6	7
	GD	87%	90%	13/24	2/39	6	7
	aldicarb	67%	95%	3/24	8/39	0	6
	dicrotophos	44%	90%	3/24	7/33	0	5
OP-Stick Sensor	VX	33%	62%	0/24	7/39	0	3
	GB	60%	71%	0/24	2/30	0	0
	GD	27%	57%	0/24	2/39	0	0
	aldicarb	100%	95%	1/24	0/27	0	1
	dicrotophos	100%	100%	0/24	0/33	0	0
Organophosphate/ Carbamate Screen Kit	VX	100%	90%	3/24	0/33	1	2
	GB	100%	90%	3/24	0/39	1	2
	GD	100%	90%	3/24	0/36	1	2
	aldicarb	100%	86%	4/24	0/39	0	4
	dicrotophos	100%	81%	5/24	5/39	1	9

<sup>a</sup>VX = VX nerve agents, GB = sarin, and GD = soman

The following is a summary of the testing of the 20 kit and contaminant combinations:

- 100% accuracy was achieved in 11 instances.
- Minimum accuracy across all contaminants was one instance of 27%.
- 100% precision was exhibited in four instances, while the minimum precision was 57%.
- There were no false positive results in 9 instances.
- Three of these combinations produced false positive results in 13 out of 24 replicates of unspiked DW or interferent PT samples.
- In one dicrotophos instance, 20 of 30 spiked samples were determined to be falsely negative due to matrix and interference effects. However, in most cases, the number of false positives and negatives was much lower.
- 100% accuracy, 100% precision, no false positives, and no false negatives were achieved for three combinations of kits and contaminants (Eclox™-Pesticide Strips and VX, Eclox™-Pesticide Strips and GB, OP-Stick Sensor and dicrotophos).

Note that the total number of matrix and interferent effects did not always equal the total number of false negatives and false positives because some false negatives occurred with contaminant-only PT samples.

## **CONTACT INFORMATION**

For more information, visit the EPA Web site at [www.epa.gov/nhsrc](http://www.epa.gov/nhsrc).

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