THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

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ETV Joint Verification Statement

TECHNOLOGY TYPE:	**Enzymatic Test Kit**	
APPLICATION:	**Detecting Chemical Agents, Carbamate Pesticides, and Organophosphate Pesticides in Drinking Water**	
TECHNOLOGY NAME:	**Neuro-IQ Tox Test Kit™**	
COMPANY:	**Aqua Survey, Inc.**	
ADDRESS:	**469 Point Breeze Road** **Flemington, NJ 08822**	**PHONE: (908) 788-8700** **FAX: (908) 788-9165**
WEB SITE:	**www.aquasurvey.com**	
E-MAIL:	**hayes@aquasurvey.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 Aqua Survey's Neuro-IQ Tox Test 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 Neuro-IQ Tox Test Kit™ to detect chemical agents, carbamate pesticides, and organophosphate (OP) pesticides in drinking water (DW). This verification test assessed the performance of the Neuro-IQ Tox Test 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 Neuro-IQ Tox Test 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 control water samples, as prescribed by the Neuro-IQ Tox Test Kit™ protocol. 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 Neuro-IQ Tox Test 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 Neuro-IQ Tox Test 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 Neuro-IQ Tox Test 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 Neuro-IQ Tox Test 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.

TECHNOLOGY DESCRIPTION

The following description of the Neuro-IQ Tox Test Kit™ is based on information provided by the vendor. This technology description was not verified in this test.

The Neuro-IQ Tox Test Kit™ tests water supplies for the presence of contaminants in drinking water in sufficient concentrations to cause harm to humans. The Neuro-IQ-Tox Test Kit™ is acetocholine/cholinesterase based and detects contaminants of interest by interrupting an enzymatic reaction. The presence or absence of contaminants at significant concentrations is predicted by adding two reagents to water samples and measuring the drop in pH after three minutes. This test is generally performed in replicates of up to four. If the pH of the test samples is higher ($\geq$ 0.2 pH units) than the control water sample's three-minute pH reading, this indicates the possible presence of a significant threat contaminant concentration.

The test can be conducted by a technician with basic laboratory skills. Data are recorded on a scorecard provided with the kit.

Enough reagent is provided with the Neuro-IQ Tox Test Kit™ to assay up to 400 test water samples. The Neuro-IQ-Tox Test Kit™ retails for $300.

VERIFICATION RESULTS

To allow for testing of all of the samples prescribed for this verification test, differences in pH were calculated on a sample by sample basis. In addition, three samples were tested with each control water sample since each type of sample need only to be tested in triplicate for this verification test. These changes were recommended by the vendor.

Only qualitative (positive, negative) results were used to calculate the parameters presented in the following tables for the Neuro-IQ Tox Test Kit™. Qualitative results were determined based on the difference between the control water pH value and the sample pH value. If the sample pH value was $\geq$ 0.2 pH units above the control water's pH value, then the sample was concluded to be a positive result, indicating the presence of the contaminant in the sample. If the test sample pH value was < 0.2 pH units above the control water's pH value, then a negative result was recorded for that sample.

Accuracy was assessed by evaluating how often the Neuro-IQ Tox Test 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. LODs were not available for any of the contaminants tested in this verification test. Thus, all analyzed contaminant-only PT samples greater than the concentration level where consistent negative results were obtained were used for accuracy calculations. This level was defined at 1.4 mg/L for dicrotophos. Results for VX, GB, GD, and aldicarb 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 Neuro-IQ Tox Test 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 Neuro-IQ Tox Test 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 [(a)]	3/3
			0.21 mg/L	3/3
			0.021 mg/L	3/3
			0.0021 mg/L	3/3
			0.00021 mg/L	3/3
	Interferent PT Samples	Humic and Fulvic Acids	0.21 mg/L	6/6
		Ca and Mg	0.21 mg/L	5/6
	DW Samples	DW	0.21 mg/L	12/12
Accuracy		100% (15 out of 15) of the contaminant-only PT samples were positive.		
False Positives		Thirteen false positive responses were obtained. Seven positive responses were found across unspiked 1 mg/L and 5 mg/L humic and fulvic acids as well as unspiked 50 mg/L Ca and Mg samples. All six replicates for unspiked OH and FL DW yielded positive results.		
False Negatives		One false negative result was obtained for spiked PT and DW samples. One replicate of the spiked 250 mg/L Ca and Mg samples returned a negative result.		
Precision		90% (19 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

[(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)]	2/3
			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		93% (14 out of 15) of the contaminant-only PT samples were positive.		
False Positives		Thirteen false positive responses were obtained. Seven positive responses were found across unspiked 1 mg/L and 5 mg/L humic and fulvic acids as well as unspiked 50 mg/L Ca and Mg samples. All six replicates for unspiked OH and FL DW yielded positive results.		
False Negatives		One false negative result was obtained for spiked PT and DW samples. One replicate of the spiked DI water samples at the lethal dose returned a negative result.		
Precision		90% (19 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

[(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)]	3/3
			0.14 mg/L	3/3
			0.014 mg/L	3/3
			0.0014 mg/L	3/3
			0.00014 mg/L	1/3
	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		87% (13 out of 15) of the contaminant-only PT samples were positive.		
False Positives		Thirteen false positive responses were obtained. Seven positive responses were found across unspiked 1 mg/L and 5 mg/L humic and fulvic acids as well as unspiked 50 mg/L Ca and Mg samples. All six replicates for unspiked OH and FL DW yielded positive results.		
False Negatives		Two false negative results were obtained for spiked PT and DW samples. Two replicates of the spiked DI water samples at 10,000x less than the lethal dose (0.00014 mg/L) returned a negative result.		
Precision		90% (19 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

[(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	3/3
			2.6 mg/L	3/3
			0.26 mg/L	0/3
			0.026 mg/L	1/3
	Interferent PT Samples	Humic and Fulvic Acids	26 mg/L	6/6
		Ca and Mg	26 mg/L	3/6
	DW Samples	DW	26 mg/L	12/12
Accuracy		67% (10 out of 15) of the contaminant-only PT samples were positive.		
False Positives		Three false positive responses were obtained. Positive responses were found for all replicates of the unspiked 5 mg/L humic and fulvic acids samples.		
False Negatives		Eight false negative results were obtained for spiked PT and DW samples. Five samples of the spiked DI water samples returned a negative result. All three replicates of the spiked 250 mg/L Ca and Mg samples yielded negative results.		
Precision		95% (20 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

[(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	0/3
			14 mg/L	1/3
			1.4 mg/L	0/3 [(b)]
			0.14 mg/L	0/3 [(b)]
	Interferent PT Samples	Humic and Fulvic Acids	140 mg/L	4/6
		Ca and Mg	140 mg/L	6/6
	DW Samples	DW	140 mg/L	12/12
Accuracy		44% (4 out of 9) of the contaminant-only PT samples above the level of consistent negative responses were positive.		
False Positives		Three false positive responses were obtained. Positive responses were found for all replicates of the unspiked 5 mg/L humic and fulvic acids samples.		
False Negatives		Seven false negative results were obtained for spiked PT and DW samples. Five samples of the spiked DI water samples returned a negative result. Two replicates of the spiked 1 mg/L fulvic and humic acid samples yielded negative results.		
Precision		90% (19 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

[(a)] Lethal dose.

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

Operational Factors:

Technical Operators

The Neuro-IQ Tox Test Kit™ was operated by one Battelle technician throughout testing with the pesticides and by a different Battelle technician throughout testing with chemical agents. Both technicians had extensive laboratory experience. Multiple problems were encountered with the test kit operation, including a faulty pH probe and unstable pH readings after adding Reagent B and when trying to reach a pH of 8.30. Two reagents are used to test a water sample with the Neuro-IQ Tox Test Kit™. Reagent A is frozen and must come to room temperature before it can be used. Reagent B has to be reconstituted with DI water before use. Individual vials of each reagent were provided with the kit to make daily testing easier. Between the two operators, it took an average of 64 ± 18 minutes to complete testing on a set of three samples using the Neuro-IQ Tox Test Kit™. The operators were able to analyze between three and six sets of samples a day.

Non-Technical Operator

Unspiked DI water samples were tested on the Neuro-IQ Tox Test Kit™ by a non-technical operator both with and without PPE. Adjusting the pH to 8.30 was not easy for the operator to accomplish and many times that pH was exceeded. Reagent A was hard to handle with the gloves on, and the magnetic stir plate was difficult to adjust while in full PPE. The pipettes needed for the test were cumbersome, confusing, and difficult to use for a non-technical operator. The 50-mL beakers used for each sample were small, and the level of the liquid in them was shallow, making it difficult, particularly while in PPE, to correctly place the pH probe and magnetic stirrer. Testing of three MB samples while in PPE took 52 minutes, while testing of three MB samples without PPE took 40 minutes. The test kit would be very difficult for the operator to set up and use if no table-top surface was available in the field. A control water sample, or a water sample that is the same matrix as the test sample but not contaminated, is needed for the Neuro-IQ Tox Test Kit™ protocol. Obtaining such a sample could be problematic in the field. Overall, the Neuro-IQ Tox Test Kit™ would be hard for a first-responder with no experience with the kit and no laboratory skills to use if the operator is donned in the level of PPE used in this verification test.

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