

US EPA ARCHIVE DOCUMENT

THE ENVIRONMENTAL TECHNOLOGY VERIFICATION
PROGRAM



U.S. Environmental Protection Agency

Battelle
The Business of Innovation

ETV Joint Verification Statement

TECHNOLOGY TYPE: Atrazine Test Kit

APPLICATION: ANALYSIS OF ATRAZINE IN WATER

TECHNOLOGY NAME: RaPID Assay[®] Kit

COMPANY: Strategic Diagnostics Inc.

ADDRESS: 111 Pencader Drive **PHONE:** 302-456-6789
Newark DE 19702 **FAX:** 302-456-6782

WEB SITE: www.sdix.com
EMAIL: sales@sdix.com

The U.S. Environmental Protection Agency (EPA) has created 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 that consist of buyers, vendor organizations, and permittees; 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 Advanced Monitoring Systems (AMS) Center, one of seven technology areas under ETV, is operated by Battelle in cooperation with EPA's National Exposure Research Laboratory. The AMS Center has recently evaluated the performance of test kits for the analysis of atrazine in water. This verification statement provides a summary of the test results for the Strategic Diagnostics, Inc. RaPID Assay[®] Kit.

VERIFICATION TEST DESCRIPTION

The RaPID Assay[®] Kit was verified in terms of its performance on the following parameters: accuracy, precision, linearity, method detection limit (MDL), cross-reactivity of hydroxylatrazine and desethyl atrazine, matrix interference effects, and rate of false positives/false negatives. Qualitative factors including ease of use, reliability, and sample throughput were also evaluated. All preparation and analyses were performed according to the manufacturer's recommended procedures. The verification test involved challenging the RaPID Assay[®] Kit with seven performance test (PT) samples and four types of environmental samples. The PT samples consisted of ASTM

Type I water samples fortified with atrazine or an atrazine degradation product. Five of the PT samples contained atrazine at concentrations ranging from 0.1 to 5 parts per billion (ppb), and two of the samples contained 3 ppb of a cross-reactive compound, but no atrazine. Four types of environmental samples also were analyzed: fresh pond water, brackish pond water, groundwater, and chlorinated drinking water. Environmental samples were filtered prior to test kit analysis. The background atrazine concentration in each environmental sample was less than 0.062 ppb. Each environmental sample was fortified in the laboratory at concentrations of 1 ppb and 3 ppb atrazine. All laboratory-fortified samples were prepared using certified, commercially available standards. All samples were analyzed by the RaPID Assay[®] Kit and by gas chromatography/mass spectrometry (GC/MS) according to modified EPA Method 525.2. Each sample was analyzed in triplicate using the test kit (seven replicates of the MDL sample were analyzed). Samples were given to the analyst blind and in random order.

The verification test was conducted in September 2003 at the Battelle laboratory in Duxbury, Massachusetts. Environmental samples were provided by the National Oceanic and Atmospheric Administration, National Ocean Service's Center for Coastal Environmental Health and Biomolecular Research Center at Charleston, and the University of Missouri - Rolla. Reference laboratory analyses were provided by the EPA's Office of Pesticide Programs, Environmental Chemistry Branch at the John C. Stennis Space Center. Test kit analyses were conducted by the Texas Commission on Environmental Quality.

The RaPID Assay[®] Kit and reference method results were used to assess accuracy and linearity. Replicate sample results were used to assess precision. Results for replicates of a low-level spiked sample were used to evaluate the MDL. Cross-reactivity of hydroxyatrazine and desethyl atrazine were assessed by evaluating the RaPID Assay[®] Kit results for samples that contained only one degradation compound, but not atrazine. Potential matrix effects were assessed by comparing accuracy and precision results for environmental samples (i.e., chlorinated drinking water, fresh surface water, brackish surface water, and groundwater) to those for ASTM Type I water samples. Performance parameters, such as ease of use and reliability, were based on documented observations of the analyst. Sample throughput was estimated based on the time required to analyze a sample set.

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

TECHNOLOGY DESCRIPTION

The following description of the RaPID Assay[®] Kit is based on information provided by the vendor. This information was not verified in this test. The RaPID Assay[®] Kit is a magnetic-particle immunoassay for detecting the presence of atrazine in soil, food, or water samples. Testing can be done in the field or in the laboratory, and the RaPID Assay[®] provides quantitative, semi-quantitative, or qualitative data. The RaPID Assay[®] test kit consists of antibody-coated magnetic particles; enzyme conjugate; color development, stop, and wash reagents for analysis of either 100 or 30 test tubes; standards (atrazine at 0.1, 1.0, and 5.0 parts per billion [ppb]); control (atrazine at 3 ± 0.6 ppb); dilute/zero standard (an atrazine-free solution with preservative and stabilizers); disposable test tubes; and instructions. The prepared sample, enzyme conjugate, and antibody-coupled magnetic particles are added to test tubes and incubated. The tubes are decanted and washed using a magnetic separation rack (which is not part of the kit). Color reagents are added to the tubes, and they are incubated again; after which stopping solution is added, and the quantitative results are calculated and printed using a photometer. The vendor-stated detection limit of the test kit is 0.05 ppb atrazine.

The 30-tube RaPID Assay[®] Kit costs \$230, and the 100-tube kit costs \$510. The 30-tube kit analyzes up to 21 samples, and the 100-tube kit up to 80 samples. Other materials that are required but are not provided with the RaPID Assay[®] Kit are pipettes (including a repeating pipette for the addition of reagents), an electric timer, a vortex mixer, a magnetic separation rack, and a photometer capable of readings at 450 nanometers (nm). These materials can be purchased separately or rented.

VERIFICATION OF PERFORMANCE

Quantitative performance results for all parameters except ease of use, reliability, and sample throughput are summarized in the table below.

Parameter	Performance Results	Comments
Accuracy (percent recovery) PT samples, 0.1 – 5 ppb atrazine Environmental samples: 1 ppb and 3 ppb atrazine-fortified, respectively: Fresh pond water Brackish pond water Groundwater Chlorinated drinking water	96% - 151%; average 124% 156% and 102% 145% and 102% 137% and 118% 177% and 133%	Background atrazine concentrations in all environmental samples were <0.062 ppb.
Precision (relative standard deviation) PT samples, 0.1 – 5 ppb atrazine and cross-reactivity samples Environmental samples: 1 ppb and 3 ppb atrazine-fortified, respectively: Fresh pond water Brackish pond water Groundwater Chlorinated drinking water	0.9% - 51.1%; average 14.7% 10.6% and 16.7% 6.8% and 8.6% 4.2% and 3.1% 2.6% and 6.3%	
Linearity Slope of regression equation y-intercept Correlation coefficient (r)	0.93 0.26 0.9950	Results for PT samples from 0.1 ppb to 5 ppb atrazine used to assess linearity.
MDL	0.10 ppb atrazine	Based on analysis of 0.1 ppb atrazine spiked into ASTM Type I water sample.
Cross-reactivity 3 ppb hydroxyatrazine 3 ppb desethyl atrazine	Average result 0.07 ppb atrazine Average result 0.34 ppb atrazine	Cross-reactivity samples did not contain atrazine.
Matrix interference effects	Possible minor interference from brackish pond water matrix; atrazine was detected in the background sample at 0.09 ppb (reference analysis showed <0.062 ppb atrazine in this sample).	1 ppb and 3 ppb atrazine-fortified brackish pond water samples had similar recoveries as 1 ppb and 3 ppb atrazine-fortified PT samples.
False positive results	4 out of 38 results	Evaluated relative to 0.1 ppb atrazine (lowest calibration standard). Three of the four false positive results associated with a sample containing an atrazine degradation product.
False negative results	None	Evaluated relative to 0.1 ppb atrazine (lowest calibration standard). Three of these results associated with a sample containing an atrazine degradation product.

During the test, the analyst recorded observations regarding ease of use, reliability, and sample throughput. The RaPID Assay[®] Kit was easy to use for an analyst with previous experience in performing immunoassay analyses. An analyst with less experience may not achieve the same level of performance. Consistency in analytical technique was a critical parameter, particularly during the addition of reagents. Although a single analyst can analyze samples with the RaPID Assay[®] Kit, the process was more efficient and less prone to error with a second person available to assist. A one-page summary diagram of the analytical sequence facilitated the analysis and reduced the chance for analyst error. The RaPID Assay[®] Kit is readily transportable and can be used in a mobile laboratory or indoor work space.

