March 2004

Environmental Technology Verification Report

ABRAXIS LLC
ATRAZINE ELISA KIT

Prepared by
Battelle

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The Business of Innovation

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ETV ETV ETV

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Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

Abraxis LLC
Atrazine ELISA Kit

by

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Notice

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technologies across all media and to report this objective information to permitters, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of seven environmental technology centers. Information about each of these centers can be found on the Internet at http://www.epa.gov/etv/.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. Under a competitive cooperative agreement, Battelle has received EPA funding to plan, coordinate, and conduct such verification tests for "Advanced Monitoring Systems for Air, Water, and Soil" and report the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at http://www.epa.gov/etv/centers/center1.html.

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List of Abbreviations

AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
DOC	dissolved organic carbon
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
GC/MS	gas chromatography/mass spectrometry
ID	identification
L	liter
LFB	laboratory-fortified blank
MCL	maximum contaminant level
MDL	method detection limit
mL	milliliter
µm	micrometer
ppb	parts per billion
PE	performance evaluation
PT	performance test
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
RB	reagent blank
RPD	relative percent difference
RSD	relative standard deviation
SOP	standard operating procedure
STL	Severn Trent Laboratories
TSA	technical systems audit

Chapter 1
Background

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative 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.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; 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 EPA's National Exposure Research Laboratory and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of the Abraxis LLC Atrazine ELISA (enzyme-linked immunosorbent assay) Kit for measuring atrazine in water.

Chapter 2
Technology Description

The objective of the ETV AMS Center is to verify the performance characteristics of environmental monitoring technologies for air, water, and soil. This report provides results for verification testing of the Atrazine ELISA Kit for measuring atrazine in water (Figure 2-1). Following is a description of the test kit, based on information provided by the vendor. The information provided below was not verified in this test.

The Atrazine ELISA Kit applies the principle of ELISA to determine atrazine in water samples. The Atrazine ELISA Kit uses a colorimetric procedure to detect atrazine. A sample and an enzyme conjugate are added to a disposable test tube, followed by atrazine antibodies attached covalently to paramagnetic particles. Any atrazine that may be in the sample competes with the atrazine enzyme label conjugate for a finite number of antibody binding sites. At the end of a 15-minute incubation period, a magnetic field is applied; and atrazine and labeled-atrazine bind to the antibodies on the paramagnetic particles in proportion to their original concentration. Unbound reagents are decanted. After decanting, the particles are washed with a washing solution. A substrate is added and enzymatically converted from a colorless to a blue solution until terminated by acidification. The atrazine concentration is determined by measuring the absorbance of the sample solution with a photometer and comparing it to the absorbance of the standards.

Figure 2-1. Abraxis LLC Atrazine ELISA Kit

The Atrazine ELISA Kit (Figure 2-1) contains a 65-milliliter (mL) vial of atrazine antibody (rabbit anti-atrazine covalently bound to paramagnetic particles suspended in a buffered soution with preservative and stabilizers), a 35-mL vial of horseradish peroxidase-labeled atrazine analog diluted in a buffered solution with preservative and stabilizers, three 2.0-mL vials of atrazine standard concentrations (0.1, 1.0, 5.0 parts per billion [ppb]) with preservative and stabilizers, a 2.0-mL vial of concentrated atrazine (3 ± 0.6 ppb) with preservative and stabilizers, a 35-mL vial of an atrazine-free solution with preservative and stabilizers for use as a zero standard, a 65-mL vial of a hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine solution in an organic base, a 60-mL vial of diluted acid, a 250-mL vial of preserved deionized water, and five bags of 22 polystyrene tubes.

Other materials that are required but are not provided with the Atrazine ELISA Kit are pipettes (including a repeating pipette for the addition of reagents), a vortex mixer, a magnetic separation system, and a photometer capable of readings at 450 nanometers (nm). These materials can be purchased separately or rented.

The Atrazine ELISA Kit is 14 by 6-¼ by 3-½ inches. Final results and calibration curves are printed out on the photometric analyzer or sent directly to a lab computer. List price is $350 for a 100-test kit.

Chapter 3
Test Design and Procedures

3.1 Introduction

This verification test was conducted according to procedures specified in the *Test/QA Plan for Verification of Test Kits for Detection of Atrazine in Water*[1]. A variety of sample matrices were tested: American Society for Testing and Materials (ASTM) Type I water[2], fresh pond water, brackish pond water, shallow (i.e., alluvial) groundwater, and chlorinated drinking water. These matrices are examples of water types that are typically monitored using the Atrazine ELISA Kit; however, they do not represent all possible water types that could be tested.

Test kits specific for atrazine are typically cross-reactive for a variety of triazine analogues, some of which are degradation products of atrazine. Cross-reactivity information is provided in the Atrazine ELISA Kit instructions. The effect of two cross-reactive atrazine degradation products (hydroxylatrazine and desethyl atrazine) on the performance of the Atrazine ELISA Kit was verified in this test. The Atrazine ELISA Kit was evaluated for the following parameters:

- Accuracy
- Precision
- Linearity
- Method detection limit (MDL)
- Cross-reactivity of hydroxylatrazine and desethyl atrazine
- Matrix interference effects
- Occurrence of false positive and false negative results
- Other factors (ease of use, reliability, and sample throughput).

Quantitative immunoassay test kits such as the Atrazine ELISA Kit typically will provide more consistent and reliable results when operated by an experienced user, and it should be noted that an analyst with less experience may not achieve the same level of performance. An analyst with five years of previous experience using immunoassay test kits performed all analyses to minimize error due to operator inexperience. The analyst was assisted by a second person, as necessary, during the test but largely the analyses can be conducted by a single person. The vendor provided training to the analyst on the use of the Atrazine ELISA Kit prior to the test. All testing was conducted at the Battelle laboratory in Duxbury, MA.

3.2 Test Design

The verification test involved challenging the Atrazine ELISA Kit with samples of fresh pond water, brackish pond water, groundwater, and chlorinated drinking water. Natural and atrazine-

fortified (i.e., unspiked and spiked) samples were analyzed using both the Atrazine ELISA Kit and a laboratory reference method. ASTM Type I water samples fortified with atrazine or an atrazine degradation product also were analyzed. Physico-chemical parameters (pH, temperature, salinity, conductivity, alkalinity, and dissolved organic carbon [DOC]) were measured in the environmental samples to provide supporting matrix characterization data.

All samples were analyzed by the Atrazine ELISA kit and by gas chromatography/mass spectrometry (GC/MS) according to modified EPA Method 525.2 [(3)]. Each sample was analyzed in triplicate using the test kit. Samples were given to the analyst blind and in random order.

The Atrazine ELISA 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 Atrazine ELISA Kit results for samples that contained one of the degradation compounds, but not atrazine. Potential matrix effects were assessed by comparing accuracy and precision results for environmental samples (i.e., fresh surface water, brackish surface water, groundwater, and chlorinated drinking water) 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. Data analysis procedures are described further in Section 5 of this report.

3.3 Test Samples

Test samples included quality control (QC) samples, performance test (PT) samples, and environmental water samples. Table 3-1 lists the number and type of each sample analyzed. Each type of test sample is described further below.

3.3.1 QC Samples

QC samples included reagent blank (RB) and control samples. The RB samples were prepared from ASTM Type I water and were exposed to identical sample preparation and analysis procedures as the test samples, including the addition of all reagents. These samples were used to help ensure that no sources of contamination were introduced in the sample handling and analysis procedures. At least 10% of the test samples were RB samples. The RB sample results were also used to test for false positives (Section 5.7).

Control samples were used to verify that the Atrazine ELISA Kit was calibrated properly and reading within defined control limits. Control samples were analyzed in accordance with the vendor instructions using a sample supplied by the vendor.

3.3.2 PT Samples

PT sample types are listed in Table 3-1. All PT samples were prepared at Battelle using certified, commercially available standards. PT sample results were used to assess accuracy,

Table 3-1. Test Samples

Type of Sample	Description	Replicates	Reference Laboratory Analyses	Performance Factor[a]
Quality Control				
Reagent blanks (10%)	minimum 10% frequency	20	1	QC, false positive
Control samples	As required by the test kit protocol (one per run)	2	-	QC
Performance Test				
Performance test #1	0.1 ppb atrazine	3	1	Accuracy, precision, linearity, false positive/negative
Performance test #2	0.5 ppb atrazine	3	1	
Performance test #3	1 ppb atrazine	3	1	
Performance test #4	3 ppb atrazine	3	1	
Performance test #5	5 ppb atrazine	3	1	
Method detection limit	0.1 ppb atrazine	7	1 [b]	Method detection limit
Cross-reactivity test #1	3 ppb hydroxyatrazine	3	1	Cross-reactivity, false positive
Cross-reactivity test #2	3 ppb desethyl atrazine	3	1	
Environmental				
Fresh water	Fresh surface water, unspiked	3	1	Accuracy, precision, matrix effects, false positive/negative
Fresh water spike #1	Fresh surface water with 1 ppb atrazine spike	3	1	
Fresh water spike #2	Fresh surface water with 3 ppb atrazine spike	3	1	
Brackish water	Brackish water, unspiked	3	1	
Brackish water spike #1	Brackish water with 1 ppb atrazine spike	3	1	
Brackish water spike #2	Brackish water with 3 ppb atrazine spike	3	1	
Groundwater	Groundwater, unspiked	3	1	
Groundwater spike #1	Groundwater with 1 ppb atrazine spike	3	1	
Groundwater spike #2	Groundwater with 3 ppb atrazine spike	3	1	
Chlorinated drinking water	Chlorinated drinking water	3	1	
Chlorinated drinking water spike #1	Chlorinated drinking water with 1 ppb spike	3	1	
Chlorinated drinking water spike #2	Chlorinated drinking water with 3 ppb atrazine spike	3	1	
Performance Evaluation (PE) Sample		-	1	PE audit
Total		86	21	-

[a] Other performance factors that were evaluated qualitatively include ease of use and reliability.
[b] This sample was the same sample as the 0.1 ppb atrazine performance test sample; it was analyzed once by the reference method.

precision, linearity, method detection limit, cross-reactivity, and occurrence of false positive and false negative results using the data analysis methods described in Section 5.

The first type of PT sample consisted of ASTM Type I water spiked at five different atrazine concentration levels. The PT sample concentrations spanned the calibration range of the Atrazine ELISA Kit. This range included the EPA maximum contaminant level (MCL) for atrazine in drinking water, which is 3 parts per billion (ppb)[4]. Three replicates of each PT sample were analyzed using the Atrazine ELISA Kit. One replicate of each PT sample was analyzed by the reference method to confirm the nominal spike concentration.

The second type of PT sample was for the MDL determination. Seven replicates of a low-level atrazine-fortified ASTM Type I water sample were analyzed. This sample was spiked at a level of 0.1 ppb, which is two times the vendor-stated detection limit of 0.05 ppb.

The third type of PT sample was a cross-reactivity check sample. Two samples consisted of ASTM Type I water spiked with two different cross-reactive atrazine degradation products (hydroxyatrazine and desethyl atrazine) at a level of 3 ppb. Three replicates of each cross-reactivity check sample were analyzed using the Atrazine ELISA Kit. One replicate was analyzed by the reference method to confirm the absence of atrazine in the samples.

3.3.3 Environmental Samples

Environmental samples were collected from a variety of sources to evaluate the performance of the Atrazine ELISA Kit with various sample matrices. Samples were collected from the following sources:

- Fresh surface water from a South Carolina pond
- Brackish surface water from a South Carolina pond
- Groundwater from an alluvial aquifer on the Missouri River
- Chlorinated drinking water from the Battelle Duxbury, MA laboratory.

As shown in Table 3-1, each environmental water sample also was fortified with atrazine at two spike levels. The fortified samples were prepared at Battelle to increase the analyte concentration by the amount shown in Table 3-1. The spike solution was prepared in the laboratory from a certified, commercially available atrazine standard. Three replicates of each sample were analyzed. The data for the environmental samples were used to assess accuracy, precision, potential matrix effects, and occurrence of false positives and false negatives following the statistical analysis procedures described in Section 5.

3.4 Sample Collection

Environmental samples were collected within 14 days of the preparation of atrazine-fortified samples. The chlorinated drinking water from Battelle was collected directly from the tap into certified clean amber glass bottles. Fresh and brackish pond water samples were collected directly into certified clean amber glass bottles. The samples were collected near the shoreline by

submerging the containers no more than one inch below the surface of the water. The groundwater sample was collected directly from a tap at the well head.

The sample identification (ID) information, date, name of person collecting the sample, sample location, time of collection, and sample temperature at the time of collection were recorded on a chain-of-custody form for all field samples. All environmental samples collected in the field were stored at 4°C and shipped to Battelle on the day of collection, following chain-of-custody procedures. Samples were stored in the dark at 4°C until test sample preparation (see Section 3.5).

3.5 Sample Preparation

All samples were assigned a unique sample ID at the time of preparation. The sample ID did not contain information about the nature of the sample. Prior to sample preparation, the fresh and brackish pond water samples were filtered with a 0.45-micrometer (µm) filter in the laboratory to remove gross particulate matter, as recommended by the vendor protocol. After filtration, the following physico-chemical parameters were measured in each environmental water sample to characterize the sample matrix: pH, temperature, salinity, conductivity, and alkalinity. The physico-chemical parameters were measured in the laboratory instead of in the field to provide information about the sample matrix immediately prior to analysis using the Atrazine ELISA Kit. All instruments used to measure physico-chemical parameters were calibrated prior to use according to the applicable Battelle standard operating procedures (SOPs)[5]. All measurements were recorded manually on data sheets designed specifically for this verification test. Instrument model, serial number, and calibration information were recorded on data sheets, and calibration records are maintained in the verification test files. An aliquot of each environmental sample was collected and shipped to Severn Trent Laboratories (STL) in Burlington, VT for DOC analysis according to Method 9060.[6] STL filtered all samples using a 0.45-micrometer (µm) filter immediately upon receipt and prior to DOC analysis.

The PT and fortified environmental samples were prepared from certified, commercially available standard solutions. The purchased standards were diluted to the appropriate concentration using pesticide-grade or equivalent solvent. All samples were stored in the dark at 4°C until use. No other preservatives were added to the samples because atrazine is stable in water for up to two years when samples are refrigerated[7]. The PT and fortified environmental samples were analyzed 4 days after sample preparation.

Each sample was split into 1-liter (L) and 40-milliliter (mL) aliquots. The 40-mL aliquot was retained for Atrazine ELISA Kit analysis and stored in the dark at 4°C until use. Two 1-L aliquots were sent to the EPA's Office of Pesticide Programs Environmental Chemistry Laboratory at the John C. Stennis Space Center for analysis by modified EPA Method 525.2.

3.6 Sample Analysis

A technical staff member from the Texas Commission on Environmental Quality with five years of previous experience in performing immunoassay analyses analyzed the complete set of samples using the Atrazine ELISA Kit. The analyses were performed according to the instructions provided with the test kit. The photometer was provided and maintained by the vendor. Calibration curves were automatically calculated and stored by the photometer. Calibration parameter settings for the photometer are provided in the Atrazine ELISA Kit protocol.

Test kit results were recorded manually on data sheets designed specifically for this verification test. In addition to the test kit results, the data sheets included records of the time required for sample analysis and operator observations concerning the use of the test kit (e.g., ease of use, reliability). Test kit results were also stored electronically by the photometer and provided as an instrument print-out.

3.7 Reference Analysis

The EPA reference method for atrazine was performed on a Hewlett-Packard 5971 GC/MS by EPA's Office of Pesticide Programs Environmental Chemistry Laboratory. The reference instrument was operated according to the recommended procedures in the instrument operating manual, and samples were analyzed according to modified EPA Method 525.2[3]. The modifications to the reference method were as follows: 1) hydrochloric acid was not used to preserve the samples, because atrazine is stable without acid preservation, and 2) the extraction solvents were changed from a mixture of ethyl acetate and methylene chloride to methylene chloride only. These modifications were adopted to improve the quantification of atrazine.

Samples were submitted to the reference laboratory blind, with the exception of the unspiked environmental samples, which were identified so that they could be used as laboratory matrix spike (MS) samples. Prior to reference analysis, the chlorinated water sample was treated with sodium sulfite according to Method 525.2[3] at the reference laboratory to remove the chlorine. The samples were stored in the dark in amber glass bottles at 4°C until extraction. The reference method sample extraction was performed from September 25 through October 2, 2003, and analysis was performed from September 25 through October 3, 2003. Results from the reference analysis were recorded electronically and compiled by the laboratory into a report format, including the sample ID and the analyte concentration for each sample.

3.8 Verification Schedule

The verification test took place over a four-week period. Table 3-2 shows the activities that were conducted, the corresponding dates, and the location.

Table 3-2. Verification Test Schedule

Date	Location	Activity
9/9/03	South Carolina	Collection of fresh and brackish pond water and shipment to Battelle laboratory
9/17/03	Missouri River	Collection of groundwater sample and shipment to Battelle laboratory
9/19/03	Battelle Laboratory	Environmental sample filtration
9/22/03	Battelle Laboratory	Collection of chlorinated drinking water sample
9/22/03	Battelle Laboratory	Environmental sample physico-chemical characterization, test sample preparation, shipment of reference samples and DOC samples to appropriate laboratories
9/26/03	Battelle Laboratory	Analysis of all samples using Atrazine ELISA Kit
9/25/03 – 10/03/03	EPA Environmental Chemistry Laboratory	Analysis of test samples using reference method
10/8/03	STL Burlington	Analysis of environmental water samples for DOC

Chapter 4
Quality Assurance/Quality Control

QA/QC procedures were performed in accordance with the quality management plan (QMP) for the AMS Center[8] and the test/QA plan for this verification test[1]. QA/QC procedures and results are described below.

4.1 Laboratory QC for Reference Method

Laboratory QC for the reference method included analysis of laboratory RB, MS, analytical duplicate, and laboratory-fortified blank (LFB) samples. The instrument used for reference analyses was calibrated initially according to the procedures specified in the reference method. Instrument calibration was verified using an appropriate calibration check sample. All calibration check sample results were within 20% of the value of the standard.

Laboratory RB samples were analyzed to ensure that no sources of contamination were present. Four laboratory RB samples were analyzed with the test samples. Atrazine was not detected in any of the laboratory RB samples.

Laboratory MS samples were analyzed at a frequency of at least 5% to assess whether matrix effects potentially influenced the results of the reference analyses. The percent recovery (R) of the laboratory MS samples was calculated from Equation 1:

$$R = \frac{C_s - C}{s} \times 100 \quad (1)$$

where C_s is the analyzed concentration of the spiked sample, C is the analyzed concentration of the unspiked sample, and s is the concentration equivalent of the atrazine spike. If the percent recovery of a MS sample fell outside the range of 70 to 130%, then a matrix effect was suspected. MS sample results are presented in Table 4-1. All MS recoveries were within the acceptable range.

Duplicates were analyzed to assess analytical precision. The relative percent difference (RPD) between the two duplicates was calculated from Equation 2.

$$RPD = \frac{|\ (C - C_D)\ |}{(C + C_D)/2} \times 100 \quad (2)$$

where C is the concentration of the sample analysis, and C_D is the concentration of the duplicate sample analysis. An LFB sample was analyzed in duplicate for this test. The duplicate concentrations were 0.97 ppb and 0.98 ppb atrazine. The RPD of 1% was within the acceptable limit of 30%.

Table 4-1. Reference Method Matrix Spike Sample Results

Sample ID	Sample Description [(a)]	MS Sample Concentration (ppb)	Background Concentration (ppb)	Spike Concentration (ppb)	Percent Recovery
CAE-9	Fresh pond water	1.13	<0.25	1	113%
CAE-12	Brackish pond water	1.09	<0.25	1	109%
CAE-15	Groundwater	1.06	<0.25	1	106%

[(a)] A MS of the chlorinated drinking water sample was not prepared.

LFB samples were analyzed to determine whether the accuracy of the method was in control. The recovery of the LFB was calculated using Equation 1. LFB sample results are presented in Table 4-2. All atrazine recoveries were within the acceptable range of 70% to 130%.

Table 4-2. Reference Method Laboratory-Fortified Blank Sample Results

Sample ID	Analysis Date	LFB Sample Concentration (ppb)	Spike Concentration (ppb)	Percent Recovery
LFB A [(a)]	9/25/03	0.98	1	98%
LFB B	9/25/03	0.97	1	97%
LFB	9/29/03	0.95	1	95%
LFB	10/03/03	1.02	1	102%
LFB	10/03/03	0.99	1	99%

[(a)] LFB A and LFB B were analyzed in the same batch.

4.2 Audits

Three types of audits were performed during the verification test: a performance evaluation (PE) audit of the reference method, a technical systems audit (TSA) of the verification test performance, and a data quality audit. Audit procedures are described further below.

4.2.1 Performance Evaluation Audit

A PE audit was conducted to assess the quality of the reference measurements performed for the verification test. The PE audit involved challenging the reference instrument with an independent atrazine standard. For the PE audit, an independent, certified standard was obtained from a

commercial supplier. The PE sample result had to be within the certified range to be considered acceptable. As shown in Table 4-3, the PE sample result was within the certified range.

Table 4-3. Reference Method Performance Evaluation Audit Results

Sample ID	Date of Analysis	Atrazine Concentration (ppb)	Certified Range (ppb)
PE sample Rep 1	9/24/03	10.49	5.5 - 14.5
PE sample Rep 2	9/24/03	11.66	5.5 - 14.5

4.2.2 Technical Systems Audit

Battelle Quality staff conducted a TSA from September 19 through 26, 2003 to ensure that the verification test was being conducted in accordance with the test/QA plan[(1)] and the AMS Center QMP[(8)]. As part of the TSA, test procedures were compared to those specified in the test/QA plan, data acquisition and handling procedures were reviewed, and the reference standards and method were reviewed. Observations and findings from the TSA were documented and submitted to the Battelle Verification Test Coordinator for response. None of the findings of the TSA required corrective action. TSA records are permanently stored with the Battelle Quality Manager.

4.2.3 Data Quality Audit

At least 10% of the data acquired during the verification test were audited. Battelle's Quality Manager traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

4.3 QA/QC Reporting

Each audit was documented in accordance with Sections 3.3.4 and 3.3.5 of the QMP for the ETV AMS Center[(8)]. Once the audit reports were prepared, the Battelle Verification Test Coordinator ensured that a response was provided for each adverse finding or potential problem. Minor deviations related to equipment calibration, use of Class A glassware for sample preparation, and chain-of-custody procedures were documented. These deviations did not negatively impact the quality of the test data. The results of the TSA were submitted to EPA.

4.4 Data Review

Records generated in the verification test were reviewed before these records were used to calculate, evaluate, or report verification results. Table 4-4 summarizes the types of data that

were recorded and reviewed. All data were recorded by Battelle or partner organization staff. Data were reviewed by a Battelle technical staff member involved in the verification test, but not the staff member that originally generated the record. The person performing the review added his/her initials and the date to a hard copy of the record being reviewed. Review of the data sheets was conducted throughout testing and not later than two weeks after data generation.

Table 4-4. Summary of Data Recording Process

Data Recorded	Responsible Party	Where Recorded	How often Recorded	Disposition of Data[(a)]
Dates and times of test events	Battelle and partner organization staff	ETV data sheets	Start/end of test	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Calibration information and results for physico-chemical parameters (temperature, salinity, etc.)	Battelle	ETV data sheets	Prior to sample preparation	Manually incorporated in data spreadsheets as necessary
Sample collection and preparation information, including chain-of-custody	Battelle and partner organization staff	ETV data sheets and chain-of-custody forms	At time of sample collection and preparation	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Test kit procedures and sample results	Battelle and partner organization staff	ETV data sheets	Throughout test duration	Manually incorporated in data spreadsheets
Reference method procedures and sample results	Partner organization staff	Data sheets or data acquisition system, as appropriate	Throughout sample analysis process	Transferred to spreadsheets
DOC analysis procedures and results	STL laboratory staff	Data sheets or data acquisition system, as appropriate	Throughout sample analysis process	Transferred to spreadsheets

[(a)] All activities subsequent to data recording were carried out by Battelle or partner organization staff.

Chapter 5
Statistical Methods

The statistical methods used to evaluate the performance factors listed in Section 3.1 are presented in this chapter. Qualitative observations were also used to evaluate verification test data.

5.1 Accuracy

PT sample accuracy was assessed relative to the nominal spike level, and environmental sample accuracy was assessed relative to the reference method results. The triplicate test kit results for each set of analyses were averaged, and the accuracy was expressed in terms of a percent recovery (*R*) as calculated from Equation 3:

$$R = \overline{C} \; / \; C_R \times 100 \qquad (3)$$

where $\overline{C}$ is the average concentration measured by the test kit, and C_R is the nominal spike level for the PT samples, or the reference measurement for the environmental samples.

5.2 Precision

The standard deviation (*S*) of the results for the three replicate samples was calculated for each sample using Equation 4:

$$S = \left[\frac{1}{n-1} \sum_{k=1}^{n} \left(C_k - \overline{C} \right)^2 \right]^{1/2} \qquad (4)$$

where *n* is the number of replicate samples, C_k is the concentration measured for the k^{th} sample, and $\overline{C}$ is the average concentration of the replicate samples. The precision for each sample was reported in terms of the relative standard deviation (*RSD*) as calculated using Equation 5:

$$RSD = \left| \frac{S}{\overline{C}} \right| \times 100 \qquad (5)$$

5.3 Linearity

Linearity was assessed by performing a linear regression with the nominal spike concentration as the independent variable and the Atrazine ELISA Kit result as the dependent variable. Individual replicate results for the five PT samples were used in the linear regression. Linearity was expressed in terms of the slope, intercept, and correlation coefficient (r).

5.4 Method Detection Limit

An MDL was determined following standard EPA methodology[9]. The MDL was calculated using results from seven replicate analyses of an ASTM Type I water sample spiked at a level of 0.1 ppb atrazine, which is two times the vendor-stated detection limit of 0.05 ppb. The standard deviation of the seven replicate samples was calculated using Equation 4. The MDL was calculated using Equation 6:

$$MDL = t \times S \tag{6}$$

where t is the Student's t value for a 99% confidence level and S is the standard deviation of the seven replicate samples.

5.5 Cross-Reactivity

The cross-reactivity of the Atrazine ELISA Kit to two atrazine degradation products hydroxyatrazine and desethyl atrazine was assessed qualitatively by evaluating the test kit results for samples that contained only one of the degradation compounds, and no atrazine. The reference analysis results were used to confirm the absence of atrazine in the samples.

5.6 Matrix Interferences

The potential effect of the sample matrix on Atrazine ELISA Kit performance was evaluated qualitatively by comparing the accuracy and precision results for the natural and atrazine-fortified environmental samples to those for the PT samples.

5.7 False Positive/False Negative Results

False positive and false negative results were assessed relative to the Atrazine ELISA Kit's lowest calibration standard (0.1 ppb). A false positive result was defined as a test kit result above 0.1 ppb, when reference method analysis indicated that the atrazine concentration in the sample was less than 0.1 ppb. A false negative result was defined as a test kit result below 0.1 ppb, when the reference method analysis indicated that the atrazine concentration in the sample was above 0.1 ppb. Reagent blanks, PT samples, and environmental samples were included in the analysis. Samples with a nominal spike concentration of 0.1 ppb were not included in the analysis.

Chapter 6
Test Results

The results of the verification test of the Atrazine ELISA Kit are presented in this section. Tables 6-1a and 6-1b present the sample results for the PT and environmental samples, respectively, including the test kit results and the reference method results. Sample results that were obtained from a diluted sample are noted as such. Some test kit results were below the vendor-stated detection limit and were reported as <0.05 ppb atrazine. The MDL for the reference analyses was 0.062 ppb. Test kit QC results are presented first, followed by the results for each performance factor.

6.1 QC Results

The test samples were analyzed with the Atrazine ELISA Kit in two batches. Each batch included its own calibration standards consisting of four concentration levels (0, 0.1, 1.0, and 5.0 ppb atrazine), with two replicates of each standard. Eight RB samples were analyzed with the first batch, and twelve were analyzed with the second batch. Two control samples also were analyzed with each batch of samples. The analytical order for each batch was as follows:

- Calibration standards;
- Control sample;
- Detection limit samples (first batch only);
- Test samples in random order, including RB samples;
- Control sample.

Although the test kit protocol specified the analysis of one control sample per batch, a second control sample was analyzed at the end of each run as an additional calibration check. The total number of samples in each batch, including calibration standards and test samples, were 52 and 55, respectively.

Calibration acceptance criteria specified in the test kit protocol ($r > 0.99$, coefficient of variation between 2 replicate standards < 10%) were met for both batches. Atrazine was not detected in any of the RB samples. Control sample results are presented in Table 6-2. The control sample at the end of the first batch was above the acceptable range specified in the test kit protocol; however, no action was taken because the control sample required by the protocol was within the acceptable range.

Table 6-1a. Test Kit and Reference Method Results for PT Samples

Sample Description	Sample ID	Replicate	Test Kit Result (ppb atrazine)	Reference Result (ppb atrazine)
0.1 ppb atrazine	CAE-2	1	0.13	0.09 [a]
0.1 ppb atrazine	CAE-2	2	0.14	
0.1 ppb atrazine	CAE-2	3	0.11	
0.5 ppb atrazine	CAE-3	1	0.50	0.54
0.5 ppb atrazine	CAE-3	2	0.57	
0.5 ppb atrazine	CAE-3	3	0.46	
1 ppb atrazine	CAE-4	1	1.20	1.20
1 ppb atrazine	CAE-4	2	1.03	
1 ppb atrazine	CAE-4	3	1.43	
3 ppb atrazine	CAE-5	1	3.76	3.71
3 ppb atrazine	CAE-5	2	4.08	
3 ppb atrazine	CAE-5	3	3.43	
5 ppb atrazine	CAE-6D[b]	1	5.86	5.61
5 ppb atrazine	CAE-6D[b]	2	6.72	
5 ppb atrazine	CAE-6D[b]	3	5.68	
Method detection limit	CAE-2	1	0.11	0.09 [a]
Method detection limit	CAE-2	2	0.08	
Method detection limit	CAE-2	3	0.10	
Method detection limit	CAE-2	4	0.11	
Method detection limit	CAE-2	5	0.09	
Method detection limit	CAE-2	6	0.10	
Method detection limit	CAE-2	7	0.14	
3 ppb hydroxyatrazine	CAE-7	1	0.08	<0.062
3 ppb hydroxyatrazine	CAE-7	2	0.06	
3 ppb hydroxyatrazine	CAE-7	3	0.05 [c]	
3 ppb desethyl atrazine	CAE-8	1	0.23	<0.062
3 ppb desethyl atrazine	CAE-8	2	0.26	
3 ppb desethyl atrazine	CAE-8	3	0.26	

[a] Concentration was above the reference method MDL of 0.062 ppb but below the 0.25 ppb limit of quantitation. One reference method analysis was performed for the 0.1 ppb atrazine PT sample and the method detection limit sample.

[b] Sample diluted by a factor of two using zero standard provided with the test kit.

[c] Concentration detected at the detection limit.

Table 6-1b. Test Kit and Reference Method Results for Environmental Samples

Sample Description	Sample ID	Replicate	Test Kit Result (ppb atrazine)	Reference Result (ppb atrazine)
Fresh pond water	CAE-9	1	<0.05	<0.062
Fresh pond water	CAE-9	2	<0.05	
Fresh pond water	CAE-9	3	<0.05	
Fresh pond water + 1 ppb atrazine	CAE-10	1	1.43	1.15
Fresh pond water + 1 ppb atrazine	CAE-10	2	1.52	
Fresh pond water + 1 ppb atrazine	CAE-10	3	1.52	
Fresh pond water + 3 ppb atrazine	CAE-11	1	3.57	3.53
Fresh pond water + 3 ppb atrazine	CAE-11	2	3.25	
Fresh pond water + 3 ppb atrazine	CAE-11	3	4.01	
Brackish pond water	CAE-12	1	0.08	<0.062
Brackish pond water	CAE-12	2	<0.05	
Brackish pond water	CAE-12	3	<0.05	
Brackish pond water + 1 ppb atrazine	CAE-13	1	1.18	1.18
Brackish pond water + 1 ppb atrazine	CAE-13	2	1.18	
Brackish pond water + 1 ppb atrazine	CAE-13	3	1.52	
Brackish pond water + 3 ppb atrazine	CAE-14	1	3.6	3.58
Brackish pond water + 3 ppb atrazine	CAE-14	2	3.77	
Brackish pond water + 3 ppb atrazine	CAE-14	3	4.13	
Groundwater	CAE-15	1	0.12	<0.062
Groundwater	CAE-15	2	<0.05	
Groundwater	CAE-15	3	<0.05	
Groundwater + 1 ppb atrazine	CAE-16	1	1.36	1.13
Groundwater + 1 ppb atrazine	CAE-16	2	1.21	
Groundwater + 1 ppb atrazine	CAE-16	3	1.18	
Groundwater + 3 ppb atrazine	CAE-17	1	3.02	3.3
Groundwater + 3 ppb atrazine	CAE-17	2	3.57	
Groundwater + 3 ppb atrazine	CAE-17	3	3.31	
Chlorinated drinking water	CAE-18	1	<0.05	<0.062
Chlorinated drinking water	CAE-18	2	<0.05	
Chlorinated drinking water	CAE-18	3	<0.05	
Chlorinated drinking water + 1 ppb atrazine	CAE-19	1	1.07	0.79
Chlorinated drinking water + 1 ppb atrazine	CAE-19	2	1.10	
Chlorinated drinking water + 1 ppb atrazine	CAE-19	3	1.15	
Chlorinated drinking water + 3 ppb atrazine	CAE-20	1	3.73	2.73
Chlorinated drinking water + 3 ppb atrazine	CAE-20	2	3.26	
Chlorinated drinking water + 3 ppb atrazine	CAE-20	3	3.00	

Table 6-2. Control Sample Results

Control Sample	Test Result (ppb)	Acceptable Range (ppb)
Run 1 beginning	3.41	3.0 ± 0.6
Run 1 end (not required by protocol)	4.31	3.0 ± 0.6
Run 2 beginning	3.42	3.0 ± 0.6
Run 2 end (not required by protocol)	3.06	3.0 ± 0.6

6.2 Accuracy

Accuracy results for the PT and environmental samples are presented in Tables 6-3a and 6-3b, respectively. Percent recoveries ranged from 102% to 127% for the PT samples, and from 100% to 140% for the environmental samples.

6.3 Precision

Precision results for PT and environmental samples are presented in Tables 6-3a and 6-3b, respectively. RSDs were calculated if atrazine was detected in all three replicates. RSDs ranged from 6.9% to 24.1% for the PT samples, and from 3.5% to 15.2% for the environmental samples.

6.4 Linearity

The linearity of the Atrazine ELISA Kit results was assessed by performing a linear regression of the test kit results versus the nominal spike concentration for the five PT samples ranging in concentration from 0.1 ppb to 5 ppb atrazine. Figure 6-1 presents the results of the linear regression. The slope, intercept and correlation coefficient for the regression were 1.23, -0.025, and 0.9937, respectively.

6.5 Method Detection Limit

The MDL for the Atrazine ELISA Kit was assessed by analyzing seven replicates of an ASTM Type I water sample spiked with 0.1 ppb atrazine, which is two times the vendor-stated detection limit of 0.05 ppb. Table 6-4 presents the method detection limit replicate sample results, the standard deviation, and the calculated MDL. The MDL based on this analysis was 0.06 ppb.

Table 6-3a. Accuracy and Precision Results for PT Samples

Sample Description	Sample ID	Replicate	Test Result (ppb atrazine)	Average (ppb atrazine)	Spike level (ppb atrazine)	Percent Recovery	Precision (RSD)
0.1 ppb atrazine	CAE-2	1	0.13	0.13	0.1	127%	12.1%
0.1 ppb atrazine	CAE-2	2	0.14				
0.1 ppb atrazine	CAE-2	3	0.11				
0.5 ppb atrazine	CAE-3	1	0.50	0.51	0.5	102%	10.9%
0.5 ppb atrazine	CAE-3	2	0.57				
0.5 ppb atrazine	CAE-3	3	0.46				
1 ppb atrazine	CAE-4	1	1.20	1.22	1	122%	16.5%
1 ppb atrazine	CAE-4	2	1.03				
1 ppb atrazine	CAE-4	3	1.43				
3 ppb atrazine	CAE-5	1	3.76	3.76	3	125%	8.7%
3 ppb atrazine	CAE-5	2	4.08				
3 ppb atrazine	CAE-5	3	3.43				
5 ppb atrazine	CAE-6D	1	5.86	6.09	5	122%	9.1%
5 ppb atrazine	CAE-6D	2	6.72				
5 ppb atrazine	CAE-6D	3	5.68				
3 ppb hydroxyatrazine	CAE-7	1	0.08	0.06	0	N/A	24.1%
3 ppb hydroxyatrazine	CAE-7	2	0.06				
3 ppb hydroxyatrazine	CAE-7	3	0.05				
3 ppb desethyl atrazine	CAE-8	1	0.23	0.25	0	N/A	6.9%
3 ppb desethyl atrazine	CAE-8	2	0.26				
3 ppb desethyl atrazine	CAE-8	3	0.26				

N/A = not applicable.

Table 6-3b. Accuracy and Precision Results for Environmental Samples

Sample Description	Sample ID	Replicate	Test Result (ppb atrazine)	Average (ppb atrazine)	Reference Result (ppb atrazine)	Percent Recovery	Precision (RSD)
Fresh pond water	CAE-9	1	<0.05	NC	<0.062	N/A	N/A
Fresh pond water	CAE-9	2	<0.05				
Fresh pond water	CAE-9	3	<0.05				
Fresh pond water + 1 ppb atrazine	CAE-10	1	1.43	1.49	1.15	130%	3.5%
Fresh pond water + 1 ppb atrazine	CAE-10	2	1.52				
Fresh pond water + 1 ppb atrazine	CAE-10	3	1.52				
Fresh pond water + 3 ppb atrazine	CAE-11	1	3.57	3.61	3.53	102%	10.6%
Fresh pond water + 3 ppb atrazine	CAE-11	2	3.25				
Fresh pond water + 3 ppb atrazine	CAE-11	3	4.01				
Brackish pond water	CAE-12	1	0.08	NC	<0.062	N/A	N/A
Brackish pond water	CAE-12	2	<0.05				
Brackish pond water	CAE-12	3	<0.05				
Brackish pond water + 1 ppb atrazine	CAE-13	1	1.18	1.29	1.18	110%	15.2%
Brackish pond water + 1 ppb atrazine	CAE-13	2	1.18				
Brackish pond water + 1 ppb atrazine	CAE-13	3	1.52				
Brackish pond water + 3 ppb atrazine	CAE-14	1	3.60	3.83	3.58	107%	7.1%
Brackish pond water + 3 ppb atrazine	CAE-14	2	3.77				
Brackish pond water + 3 ppb atrazine	CAE-14	3	4.13				

NC = Not calculated if at least one replicate had a non-detect result.
N/A = not applicable.

Table 6-3b. Accuracy and Precision Results for Environmental Samples, continued

Sample Description	Sample ID	Replicate	Test Result (ppb atrazine)	Average (ppb atrazine)	Reference Result (ppb atrazine)	Percent Recovery	Precision (RSD)
Groundwater	CAE-15	1	0.12	NC	<0.062	N/A	N/A
Groundwater	CAE-15	2	<0.05				
Groundwater	CAE-15	3	<0.05				
Groundwater + 1 ppb atrazine	CAE-16	1	1.36	1.25	1.13	111%	7.7%
Groundwater + 1 ppb atrazine	CAE-16	2	1.21				
Groundwater + 1 ppb atrazine	CAE-16	3	1.18				
Groundwater + 3 ppb atrazine	CAE-17	1	3.02	3.30	3.3	100%	8.3%
Groundwater + 3 ppb atrazine	CAE-17	2	3.57				
Groundwater + 3 ppb atrazine	CAE-17	3	3.31				
Chlorinated drinking water	CAE-18	1	<0.05	NC	<0.062	N/A	N/A
Chlorinated drinking water	CAE-18	2	<0.05				
Chlorinated drinking water	CAE-18	3	<0.05				
Chlorinated drinking water + 1 ppb atrazine	CAE-19	1	1.07	1.11	0.79	140%	3.7%
Chlorinated drinking water + 1 ppb atrazine	CAE-19	2	1.10				
Chlorinated drinking water + 1 ppb atrazine	CAE-19	3	1.15				
Chlorinated drinking water + 3 ppb atrazine	CAE-20	1	3.73	3.33	2.73	122%	11.1%
Chlorinated drinking water + 3 ppb atrazine	CAE-20	2	3.26				
Chlorinated drinking water + 3 ppb atrazine	CAE-20	3	3.00				

NC = Not calculated if at least one replicate had a non-detect result.
N/A = not applicable.

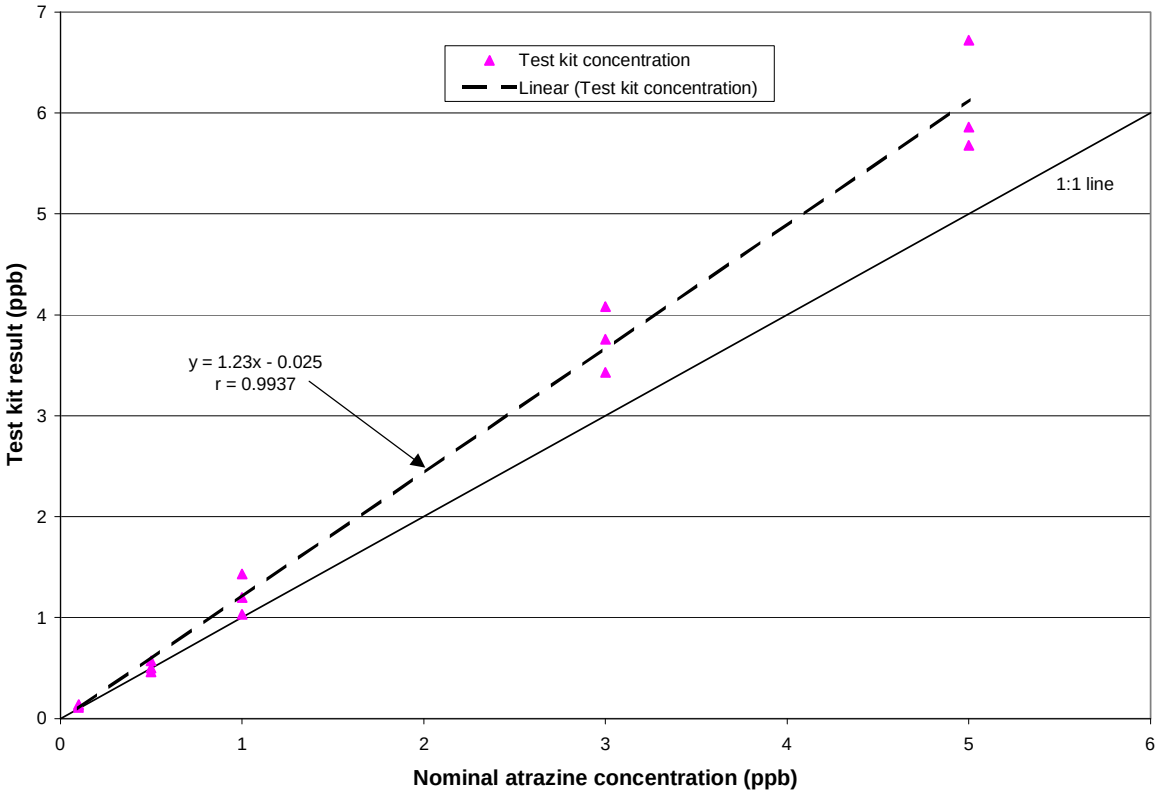

Figure 6-1. Linearity of Atrazine ELISA Kit Results

Table 6-4. Method Detection Limit Results

Sample Description	Sample ID	Replicate	Test Result (ppb atrazine)	Standard Deviation (ppb atrazine)	MDL (ppb atrazine)
Method Detection Limit	CAE-2	1	0.11	0.02	0.06
Method Detection Limit	CAE-2	2	0.08		
Method Detection Limit	CAE-2	3	0.10		
Method Detection Limit	CAE-2	4	0.11		
Method Detection Limit	CAE-2	5	0.09		
Method Detection Limit	CAE-2	6	0.10		
Method Detection Limit	CAE-2	7	0.14		

6.6 Cross-Reactivity

Results for PT samples fortified with 3 ppb hydroxyatrazine or 3 ppb desethyl atrazine are provided in Table 6-1a. Atrazine was detected at an average concentration of 0.06 ppb in the sample containing only hydroxyatrazine, which is slightly above the vendor-stated detection limit of the Atrazine ELISA Kit (0.05 ppb). Atrazine was detected at an average concentration of 0.25 ppb in the sample containing only desethyl atrazine, which indicates a greater cross-reactivity for this compound.

6.7 Matrix Interferences

Matrix characteristics for the four environmental water sample types (fresh pond water, brackish pond water, groundwater, and chlorinated drinking water) are provided in Table 6-5. Reference method results indicate that atrazine was not present in any of the natural (unspiked) environmental samples above the MDL of 0.062 ppb (Table 6-1b). The percent recoveries for the 1 ppb and 3 ppb atrazine-fortified environmental samples ranged from 100% to 140% (Table 6-3b). These recoveries are similar to those for the atrazine-fortified PT samples (102% to 127%, Table 6-3a). The 140% recovery in the 1 ppb atrazine-fortified drinking water sample appears to be due to the low reference method concentration measured in this sample (0.79 ppb atrazine) rather than to a matrix interference. Precision results for the atrazine-fortified environmental samples and the PT samples are also similar (RSDs of 3.5%-15.2% and 6.9%-24.1%, respectively). Based on these results, the performance of the Atrazine ELISA Kit for the environmental sample matrices included in this test was similar to the performance of the kit for atrazine-fortified PT samples.

6.8 False Positive/False Negative Results

Table 6-6 presents the analysis of false positive and false negative results obtained from the Atrazine ELISA Kit. RB, PT and environmental samples were included in this evaluation, with the exception of the PT samples that were spiked at a level of 0.1 ppb atrazine.

As shown in Table 6-6, 38 samples had atrazine concentrations below 0.1 ppb as measured by the reference method. Four of the 38 samples had false positive results, with Atrazine ELISA Kit results greater than 0.1 ppb. Three of these four samples contained an atrazine degradation product.

Thirty six samples had atrazine concentrations above 0.1 ppb as measured by the reference method (Table 6-6). All of the test kit measurements for these samples were above 0.1 ppb, with no false negative results.

Table 6-5. Physico-chemical Characterization of Environmental Sample Matrices

Sample Type	Temp. at time of sample collection (°C)	Temp. at time of sample preparation (°C)	pH (pH units)	Conductivity (µS)	Salinity (ppt)	Alkalinity (meq/L)	DOC[(a)] (mg/L)
Fresh pond water	25.6	18.8	7.8	1753	0	4.800	17.9
Brackish pond water	26.2	18.0	7.9	19,250	10	3.147	16.7
Groundwater	18.1	18.5	7.6	755	0	4.041	5.1
Chlorinated drinking water	-	19.2	6.5	163	0	0.6885	2.9

[(a)] Samples were filtered at STL with 0.45 µm filter immediately upon receipt at STL. Filter blank DOC concentration was 2 mg/L

Table 6-6. Occurrence of False Positives and False Negatives

Sample Description	Sample ID	Replicate	Test Result (ppb atrazine)	Reference Result (ppb atrazine)	False Positive	False Negative
Reagent blank	CAE-1	1	<0.05	<0.062	N	
Reagent blank	CAE-1	2	<0.05		N	
Reagent blank	CAE-1	3	<0.05		N	
Reagent blank	CAE-1	4	<0.05		N	
Reagent blank	CAE-1	5	<0.05		N	
Reagent blank	CAE-1	6	<0.05		N	
Reagent blank	CAE-1	7	<0.05		N	
Reagent blank	CAE-1	8	<0.05		N	
Reagent blank	CAE-1	9	<0.05		N	

Table 6-6. Occurrence of False Positives and False Negatives, continued

Sample Description	Sample ID	Replicate	Test Result (ppb atrazine)	Reference Result (ppb atrazine)	False Positive	False Negative
Reagent blank	CAE-1	10	<0.05	<0.062	N	
Reagent blank	CAE-1	11	<0.05		N	
Reagent blank	CAE-1	12	<0.05		N	
Reagent blank	CAE-1	13	<0.05		N	
Reagent blank	CAE-1	14	<0.05		N	
Reagent blank	CAE-1	15	<0.05		N	
Reagent blank	CAE-1	16	<0.05		N	
Reagent blank	CAE-1	17	<0.05		N	
Reagent blank	CAE-1	18	<0.05		N	
Reagent blank	CAE-1	19	<0.05		N	
Reagent blank	CAE-1	20	<0.05		N	
0.5 ppb atrazine	CAE-3	1	0.50	0.54		N
0.5 ppb atrazine	CAE-3	2	0.57			N
0.5 ppb atrazine	CAE-3	3	0.46			N
1 ppb atrazine	CAE-4	1	1.20	1.20		N
1 ppb atrazine	CAE-4	2	1.03			N
1 ppb atrazine	CAE-4	3	1.43			N
3 ppb atrazine	CAE-5	1	3.76	3.71		N
3 ppb atrazine	CAE-5	2	4.08			N
3 ppb atrazine	CAE-5	3	3.43			N
5 ppb atrazine	CAE-6D	1	5.86	5.61		N
5 ppb atrazine	CAE-6D	2	6.72			N
5 ppb atrazine	CAE-6D	3	5.68			N
3 ppb hydroxyatrazine	CAE-7	1	0.08	<0.062	N [(a)]	
3 ppb hydroxyatrazine	CAE-7	2	0.06		N [(a)]	
3 ppb hydroxyatrazine	CAE-7	3	0.05		N [(a)]	

[(a)] These samples contained an atrazine degradation product.

Table 6-6. Occurrence of False Positives and False Negatives, continued

Sample Description	Sample ID	Replicate	Test Result (ppb atrazine)	Reference Result (ppb atrazine)	False Positive	False Negative
3 ppb desethyl atrazine	CAE-8	1	0.23	<0.062	Y [a]	
3 ppb desethyl atrazine	CAE-8	2	0.26		Y [a]	
3 ppb desethyl atrazine	CAE-8	3	0.26		Y [a]	
Fresh pond water	CAE-9	1	<0.05	<0.062	N	
Fresh pond water	CAE-9	2	<0.05		N	
Fresh pond water	CAE-9	3	<0.05		N	
Fresh pond water + 1 ppb atrazine	CAE-10	1	1.43	1.15		N
Fresh pond water + 1 ppb atrazine	CAE-10	2	1.52			N
Fresh pond water + 1 ppb atrazine	CAE-10	3	1.52			N
Fresh pond water + 3 ppb atrazine	CAE-11	1	3.57	3.53		N
Fresh pond water + 3 ppb atrazine	CAE-11	2	3.25			N
Fresh pond water + 3 ppb atrazine	CAE-11	3	4.01			N
Brackish pond water	CAE-12	1	0.08	<0.062	N	
Brackish pond water	CAE-12	2	<0.05		N	
Brackish pond water	CAE-12	3	<0.05		N	
Brackish pond water + 1 ppb atrazine	CAE-13	1	1.18	1.18		N
Brackish pond water + 1 ppb atrazine	CAE-13	2	1.18			N
Brackish pond water + 1 ppb atrazine	CAE-13	3	1.52			N
Brackish pond water + 3 ppb atrazine	CAE-14	1	3.60	3.58		N
Brackish pond water + 3 ppb atrazine	CAE-14	2	3.77			N
Brackish pond water + 3 ppb atrazine	CAE-14	3	4.13			N
Groundwater	CAE-15	1	0.12	<0.062	Y	
Groundwater	CAE-15	2	<0.05		N	
Groundwater	CAE-15	3	<0.05		N	
Groundwater + 1 ppb atrazine	CAE-16	1	1.36	1.13		N
Groundwater + 1 ppb atrazine	CAE-16	2	1.21			N
Groundwater + 1 ppb atrazine	CAE-16	3	1.18			N

[a] These samples contained an atrazine degradation product.

Table 6-6. Occurrence of False Positives and False Negatives, continued

Sample Description	Sample ID	Replicate	Test Result (ppb atrazine)	Reference Result (ppb atrazine)	False Positive	False Negative
Groundwater + 3 ppb atrazine	CAE-17	1	3.02	3.3		N
Groundwater + 3 ppb atrazine	CAE-17	2	3.57			N
Groundwater + 3 ppb atrazine	CAE-17	3	3.31			N
Chlorinated drinking water	CAE-18	1	<0.05	<0.062	N	
Chlorinated drinking water	CAE-18	2	<0.05		N	
Chlorinated drinking water	CAE-18	3	<0.05		N	
Chlorinated drinking water + 1 ppb atrazine	CAE-19	1	1.07	0.79		N
Chlorinated drinking water + 1 ppb atrazine	CAE-19	2	1.10			N
Chlorinated drinking water + 1 ppb atrazine	CAE-19	3	1.15			N
Chlorinated drinking water + 3 ppb atrazine	CAE-20	1	3.73	2.73		N
Chlorinated drinking water + 3 ppb atrazine	CAE-20	2	3.26			N
Chlorinated drinking water + 3 ppb atrazine	CAE-20	3	3.00			N
				Total sample number	38	36
				Number false positives or negatives	4	0

6.9 Other Factors

During the test, the analyst recorded observations regarding ease of use, reliability, and sample throughput. The Atrazine ELISA Kit was relatively easy to use provided that the analyst has sufficient prior experience in performing immunoassay analyses. Consistency in analytical technique was the most important parameter, particularly during the addition of reagents. The reagent volume must be consistent for all samples, and the time specified for each step of the analysis must be met. During analysis of the first batch of samples, it was discovered that the analyst was pipetting a larger volume of sample and reagents than called for by the test protocol because of incorrect use of the pipette. However, the test results were unaffected because consistent volumes of all samples and reagents were used throughout the test, although test kit reagents were consumed at a faster rate than anticipated.

A repeating pipette facilitated the rapid and consistent addition of reagents. Care was taken to track progress during the addition of reagents to avoid skipping a tube or adding extra reagents to a tube. Although a single analyst can analyze samples with the Atrazine ELISA Kit, the process was more efficient and less prone to error with a second person available to assist. The Atrazine ELISA Kit operated without failure during the test.

The Atrazine ELISA Kit is readily transportable and can be used in a mobile laboratory or indoor work space. Reagents must be stored at 4°C, and warmed to room temperature prior to use. The test kit would be more difficult to use in an outdoor setting because uniform and stable testing conditions (e.g. temperature) will yield more reliable results. The photometer would require a power supply and would need to be protected from the elements.

Test kit components must be from the same lot to achieve optimal results. Lot numbers should not be mixed. Reagents should not be used beyond their stated shelf life (approximately 1-1½ years).

During the test, each batch was analyzed with the Atrazine ELISA Kit in 1½ hours. The total number of samples in each batch, including calibration standards, QC samples, and test samples, was 52 and 55, respectively. The photometer was programmed to automatically calculate the calibration curve, and sample results in ppb atrazine were provided at the conclusion of the analytical run.

Chapter 7
Performance Summary

The Atrazine ELISA Kit was evaluated for the following parameters:

- Accuracy
- Precision
- Linearity
- Method detection limit (MDL)
- Cross-reactivity of hydroxylatrazine and desethyl atrazine
- Matrix interference effects
- Occurrence of false positive and false negative results
- Other factors (ease of use, reliability, and sample throughput).

performance results are summarized in Table 7-1. During the test, the analyst recorded observations regarding ease of use, reliability, and sample throughput. The Atrazine ELISA Kit was relatively easy to use by an experienced analyst. Consistent analytical technique was the most important parameter, particularly with respect to addition of reagents and time required to accomplish each phase of the analysis. Although a single analyst can analyze samples with the Atrazine ELISA Kit, the process was more efficient and less prone to error with a second person available to assist. The Atrazine ELISA Kit operated without failure during the test.

The Atrazine ELISA Kit can be easily transported. Reagents must be stored at 4°C, and warmed to room temperature prior to use. A batch of about 50 samples was analyzed with the Atrazine ELISA Kit in approximately 1½ hours. The photometer was programmed to automatically calculate the calibration curve, and sample results in ppb atrazine were provided at the conclusion of the analytical run.

Table 7-1. Quantitative Performance Summary for the Atrazine ELISA Kit

Parameter	Performance Results	Comments
Accuracy (percent recovery)		
PT samples, 0.1 – 5 ppb atrazine	102% to 127%; average 120%	
Environmental samples: 1 ppb and 3 ppb atrazine-fortified, respectively:		
Fresh pond water	130% and 102%	Background atrazine concentrations in all environmental samples were <0.062 ppb.
Brackish pond water	110% and 107%	
Groundwater	111% and 100%	
Chlorinated drinking water	140% and 122%	
Precision (relative standard deviation)		
PT samples, 0.1 – 5 ppb atrazine and cross-reactivity samples	6.9% to 24.1%; average 13%	
Environmental samples: 1 ppb and 3 ppb atrazine-fortified, respectively:		
Fresh pond water	3.5% and 10.6%	
Brackish pond water	15.2% and 7.1%	
Groundwater	7.7% and 8.3%	
Chlorinated drinking water	3.7% and 11.1%	
Linearity		
Slope of regression equation	1.23	Results for PT samples from 0.1 ppb to 5 ppb atrazine used to assess linearity.
y-intercept	-0.025	
Correlation coefficient (r)	0.9937	
MDL	0.06 ppb atrazine	Based on analysis of 0.1 ppb atrazine spiked into ASTM Type I water sample (seven replicates).
Cross-reactivity		
3 ppb hydroxyatrazine	Average result 0.06 ppb atrazine	Cross-reactivity samples did not contain atrazine.
3 ppb desethyl atrazine	Average result 0.25 ppb atrazine	
Matrix interference effects	No apparent interferences from matrices tested	
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.

Chapter 8
References

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