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Environmental Technology Verification Program

Advanced Monitoring Systems Center

Generic Verification Protocol for Test Kits for Detection of Atrazine in Water

GENERIC VERIFICATION PROTOCOL

FOR

TEST KITS FOR DETECTION OF ATRAZINE IN WATER

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FOREWORD

This generic verification protocol is based upon a peer-reviewed specific test/quality assurance (QA) plan entitled "Test/QA Plan for Verification of Test Kits for Detection of Atrazine in Water," Version 1.0 (dated September 15, 2003). The test/QA plan was developed with vendor and stakeholder input by the ETV Advanced Monitoring Systems (AMS) Center. Peer reviewers for the test/QA plan were AMS Center stakeholders Kenneth Wood and Marty Link, and Elin Ulrich of EPA's National Exposure Research Laboratory. In preparing this generic verification protocol, specific names of individuals involved, technology vendors and technologies, test dates, and similar details in the test/QA plan were revised to be generic. The experimental design in the protocol is the same as that in the peer-reviewed test/QA plan.

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ACRONYMS

AMS	Advanced Monitoring Systems
DOC	dissolved organic carbon
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
ETV	Environmental Technology Verification
ID	identification
LFB	laboratory fortified blank
MCL	maximum contaminant level
MDL	method detection limit
NIST	National Institute of Standards and Technology
PE	performance evaluation
ppb	parts per billion
PT	performance test
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
RB	reagent blank
RPD	relative percent difference
RSD	relative standard deviation
TFE	polytetrafluoroethylene

1.0 INTRODUCTION

1.1 Test Description

This protocol provides generic procedures for a performance verification test of test kits for the analysis of atrazine in water. Verification tests are conducted under the auspices of the U.S. Environmental Protection Agency's (EPA) Environmental Technology Verification (ETV) program. The purpose of ETV is to provide objective and quality-assured (QA) performance data on environmental technologies so that users, developers, regulators, and consultants can make informed decisions about purchasing and applying these technologies.

Verification tests of monitoring technologies are coordinated by Battelle, of Columbus, Ohio, which is EPA's verification partner for the ETV Advanced Monitoring Systems (AMS) Center. As such, Battelle will be referred to as the testing laboratory in this document. The scope of the AMS Center covers verification of monitoring methods for contaminants and natural species in air, water, and soil. In performing verification tests, Battelle follows the procedures specified in this protocol and complies with quality requirements in the "Quality Management Plan for the ETV Advanced Monitoring Systems Center" (QMP).[1]

1.2 Test Objective

The purpose of a verification test generated from this protocol is to provide quantitative verification of the performance of test kits for the detection of atrazine in water. These technologies are typically based on immunoassay methods, where specific antibodies may be used to detect and measure the analytes of interest. Immunoassay-based test kits may utilize a competitive enzyme-linked immunosorbent assay (ELISA), where atrazine in the sample competes with enzyme-labeled atrazine for a limited number of antibody binding sites specific to atrazine. After an incubation period, a magnetic field may be used to separate the bound and unbound atrazine. A simple wash step is typically followed by a color development step. The sample concentration is inversely proportional to color development, with a darker color indicating a lower sample concentration and a lighter color indicating a higher concentration.

A variety of quality control (QC), performance test, and environmental water samples shall be analyzed to assess the capabilities of the test kits relative to an accepted reference laboratory method.

1.3 Scope of Work

This generic protocol intends to provide information related to verification of immunoassay test kits that provide qualitative, semi-quantitative, or quantitative measurements of atrazine in water. Test kits that provide qualitative results may only indicate the presence or absence of the target analyte relative to a regulatory or health-based concentration level. Test kit methods that provide semi-quantitative results determine the presence or absence of the analyte within a specified concentration interval. Quantitative results may be obtained using test kits that generate a calibration curve using a range of analyte concentrations. The vendor shall specify the type of the measurement provided by the test kit to be verified.

Qualitative test kits are typically designed to be operated by non-technical users. Semi-quantitative and quantitative immunoassay test kits will yield more consistent and reliable results when operated by an experienced user. In order to minimize the error introduced by operator inexperience, an analyst with previous experience in using immunoassay test kits may analyze all test samples.

Test kits that are specific for atrazine may be cross-reactive for a variety of triazine analogues, some of which are degradation products of atrazine. Cross-reactivity information is typically provided in the test kit documentation. It is assumed that most users of these kits will perform laboratory analysis of samples that provide a positive immunoassay response to confirm the presence and concentration of atrazine in the sample (i.e., the test kits will be used primarily as a screen for the possible presence of atrazine). As such, this test will not verify all vendor-provided information on cross-reactivity; however, the effect of two cross-reactive atrazine degradation products (such as hydroxyatrazine and desethyl atrazine) on test kit performance shall be verified.

Natural and atrazine-fortified water samples shall be analyzed using both the immunoassay test kits and an appropriate reference method. A variety of sample matrices shall be tested: This generic protocol assumes that ASTM Type I water, fresh pond water, brackish pond water, shallow (i.e., alluvial) groundwater, and chlorinated drinking water will be tested. These matrices are examples of water types that are typically monitored using immunoassay test kits to give a sense of the performance using a variety of matrices. However, this is not intended to be an exhaustive study nor to represent all possible water types that could be tested.

The performance of each test kit shall be evaluated quantitatively by comparing the test kit and reference method results. Each test kit also shall be qualitatively evaluated for ease of use, cost, and sample throughput. The test kits shall be evaluated for the following parameters:

- accuracy
- precision
- linearity
- method detection limit
- cross reactivity of cross-reactive atrazine degradation products (such as hydroxyatrazine and desethyl atrazine)
- matrix interference effects, and
- occurrence of false positive and false negative results.

Qualitative technologies should not be evaluated for linearity or method detection limit because they only provide a positive or negative result relative to a specified concentration level. The experimental design is described further in Section 2.0.

1.4 Organization and Responsibility

The organizational chart provided in Figure 1 identifies the responsibilities of the organizations and individuals associated with the verification test. Roles and responsibilities are defined further below.

1.4.1 Battelle

Battelle staff will direct and support atrazine test kit verification in the following capacities: Verification Test Coordinator, Verification Testing Leader, AMS Center Manager, Quality Manager, and technical staff. The activities and responsibilities of each of these positions are described below.

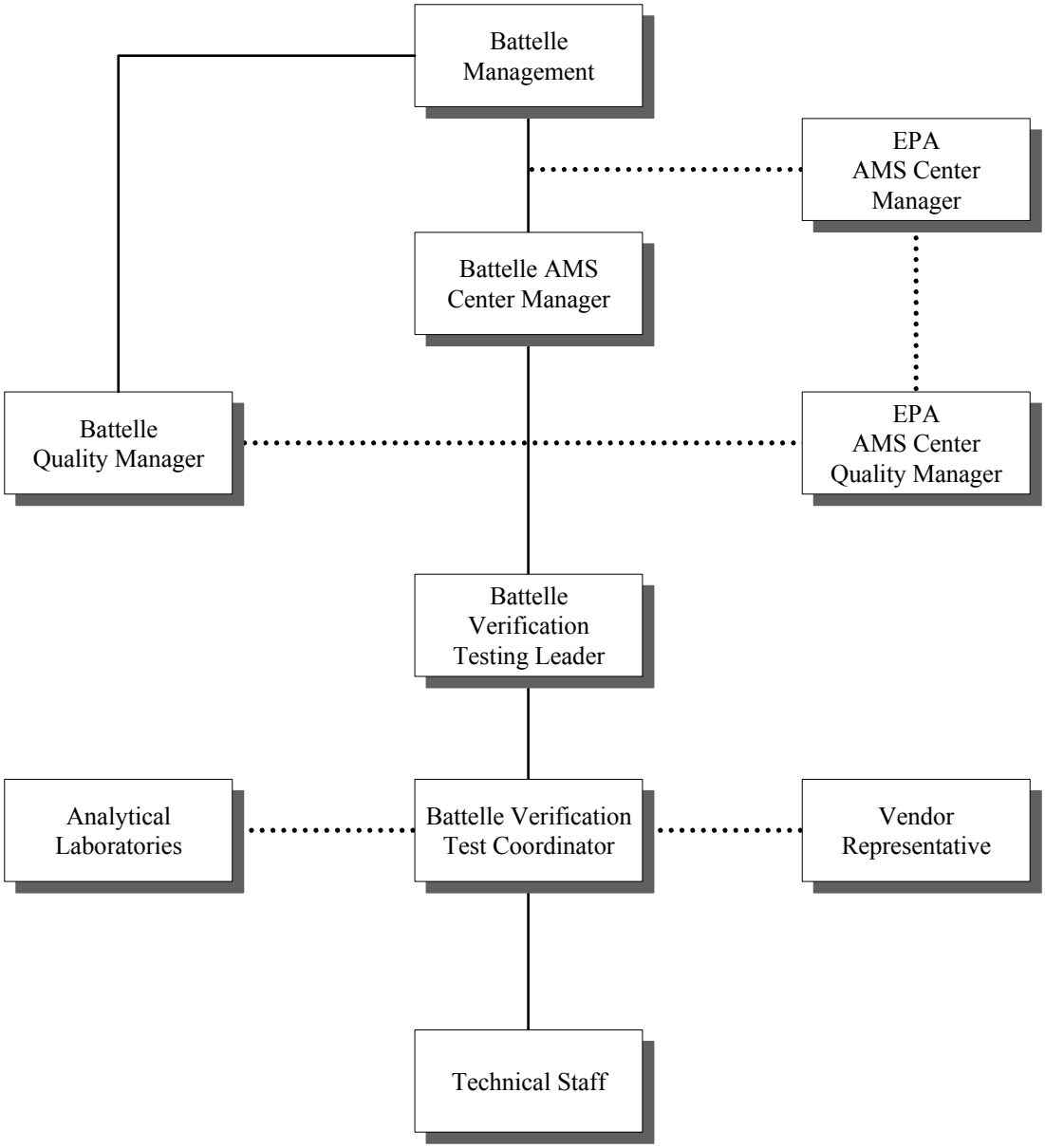

Figure 1. Generic Organization Chart for Verification Test

The Verification Test Coordinator will have overall responsibility for ensuring that the technical, schedule, and cost goals established for the verification test are met, and will:

- Assemble a team of qualified technical staff to conduct the verification test.

- Direct the team in performing the verification test in accordance with the procedures in this protocol.

- Ensure that all quality procedures specified in this protocol and in the AMS Center QMP are followed. Prepare the draft and final verification reports and verification statements.

- Revise the draft verification reports and verification statements in response to reviewers' comments.

- Respond to any issues raised in assessment reports and audits, including instituting corrective action as necessary.

- Serve as the primary point of contact for vendor representatives.

- Coordinate distribution of the final verification reports and statements.

- Establish a budget for the verification test and monitor staff effort to ensure that budget is not exceeded.

- Ensure that confidentiality of vendor information is maintained.

The Verification Testing Leader will provide technical guidance and oversee the various stages of verification testing, and will:

- Support the Verification Test Coordinator in organizing the test.

- Review the draft and final verification reports and verification statements.

The Battelle AMS Center Manager will:

- Review the draft and final verification reports and verification statements.

- Ensure that necessary Battelle resources, including staff and facilities, are committed to the verification test.

- Ensure that vendor confidentiality is maintained.

- Support the Verification Test Coordinator in responding to any issues raised in assessment reports and audits.

- Maintain communication with EPA's technical and quality managers.

Technical staff will conduct sample collection and the testing of the atrazine test kits during the verification test. The responsibilities of the technical staff include:

- Assist in the collection of environmental water samples and ship them to the testing laboratory.
- Assist in the receipt, and storage of environmental samples.
- Filter environmental water samples, as required.
- Measure physicochemical parameters on environmental water samples prior to preparation of atrazine-fortified samples.
- Prepare the atrazine spiking solution and fortify the test samples as required.
- Analyze test samples using the atrazine test kits.
- Split and ship water samples to the analytical laboratory for measurement of atrazine and dissolved organic carbon (DOC) as part of the physicochemical characterization.

The Battelle Quality Manager will:

- Conduct a technical systems audit once during the verification test, or designate another QA Manager to conduct the audit.
- Audit at least 10% of the verification data.
- Prepare and distribute an assessment report for each audit.
- Verify implementation of any necessary corrective action.
- Notify Battelle's AMS Center Manager about the need for a stop work order if self audits indicate that data quality is being compromised.
- Provide a summary of the QA/QC activities and results for the verification reports.
- Review the draft and final verification reports and verification statements.
- Assume overall responsibility for ensuring that the protocol is followed.

1.4.2 Vendors

The responsibilities of the vendor representatives are as follows:

- Provide off-the-shelf test kits for analysis of all verification test samples.
- Provide all other equipment needed to complete the immunoassay analyses, including a spectrophotometer.
- If desired, provide training to Battelle on operating the test kits and associated equipment prior to testing.
- Review the draft verification statement and report.

1.4.3 EPA

EPA's responsibilities in the AMS Center are based on the requirements stated in the "Environmental Technology Verification Program Quality Management Plan" (EPA QMP).[2] The roles of the specific EPA staff are as follows:

The EPA AMS Center Quality Manager will:

- Perform at his or her option one external technical system audit during the verification test.
- Notify the EPA AMS Center Manager of the need for a stop work order if external audit indicates that data quality is being compromised.
- Prepare and distribute an assessment report summarizing results of external audit.
- Review draft verification reports and statements.

The EPA AMS Center Manager will:

- Review the draft verification reports and statements.
- Oversee the EPA review process of the verification reports and statements.
- Coordinate the submission of verification reports and statements for final EPA approval.

1.4.4 Analytical Laboratories

The analytical laboratories at Battelle, a subcontractor, and/or a partnering organization will analyze DOC in environmental samples as part of the physicochemical characterization. The analytical laboratory will also conduct reference analysis of atrazine in water. All DOC and atrazine results will be QA-reviewed by the generating analytical laboratory before used in verification test data analysis.

2.0 VERIFICATION APPROACH

2.1 Experimental Design

The verification shall involve challenging each test kit with a variety of water samples that are intended to represent those typically monitored using the test kits. Sample matrices may include fresh pond water, brackish pond water, alluvial groundwater, and chlorinated drinking water. Natural and atrazine-fortified (i.e., unspiked and spiked) samples of each matrix shall be analyzed. ASTM Type I (or equivalent) water samples fortified with atrazine or an atrazine degradation product also shall be analyzed. Physicochemical parameters (pH, temperature, salinity, conductivity, alkalinity, and DOC) shall be measured in environmental samples to provide supporting characterization data.

All samples shall be analyzed by each test kit and by the reference method. Each sample shall be analyzed in triplicate with each test kit. Samples shall be given to the analyst blind and in random order.

Test kit and reference method results shall be used to assess test kit accuracy and linearity. Replicate sample results shall be used to assess test kit precision. Results for replicates of a low-level spiked sample shall be used to evaluate the method detection limit. Cross-reactivity of hydroxyatrazine and desethyl atrazine shall be assessed by evaluating the test kit results for samples that contain only the degradation compound, but not atrazine. Potential matrix effects shall be 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, shall be based on documented observations of the analyst. Sample throughput shall be estimated based on the time required to analyze a sample set.

2.2 Test Samples

Test samples to be used in this verification test shall include QC samples, performance test (PT) samples, and environmental water samples. Table 1 lists the number and type of each sample to be analyzed in the verification test. Each type of test sample is described further below.

2.2.1 QC Samples

QC samples will include reagent blank (RB) and calibration check samples. The RB samples will be prepared from ASTM Type I water and will be exposed to identical sample preparation and analysis procedures as the test samples, including the addition of all reagents. These samples will be used to help ensure that no sources of contamination are introduced in the sample handling and analysis procedures. At least 10% of the test samples will be RB samples.

Calibration check samples may be used to verify that the test kits are properly calibrated and reading within defined control limits. Calibration check samples will be analyzed in accordance with the vendor-provided test kit protocol using standards or calibration check samples supplied by the vendor.

2.2.2 PT Samples

PT sample types are listed in Table 1. The first type of PT sample consists of ASTM Type I water spiked at five different atrazine concentration levels. The PT sample concentrations will span the calibration range of the immunoassay test kits. This range includes the EPA maximum contaminant level (MCL) for atrazine in drinking water, which is 3 parts per billion [ppb][3]. Three replicates of each PT sample will be analyzed using the test kits.

Table 1. Sample Summary for Verification of Test Kits for Atrazine in Water

Type of sample	Description	Replicates for test kit analysis	Total No. laboratory reference analyses	Performance Factor[a]
Quality Control				
Reagent blanks (10%)	minimum 10% frequency	20	1	False positive/negative
Calibration check samples	As required by the test kit protocol	TBD	0	-
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	Atrazine concentration 2X vendor-stated detection limit	7	-	Method detection limit
Cross-reactivity test #1	3 ppb hydroxyatrazine	3	1	Cross-reactivity, false positive/negative
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 Sample		-	1	-
Total		84	21	-

[a] Other performance factors to be evaluated qualitatively include ease of use and reliability.

The second type of PT sample is a low-level atrazine fortified sample. Seven replicates of this sample will be analyzed for the method detection limit determination. This sample will be spiked at a level of two times the vendor-stated test kit detection limit.

The third type of PT sample is a cross-reactivity check sample. Two samples will consist 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 will be analyzed using the test kits. One replicate will be analyzed by the reference method to confirm the absence of atrazine in the samples.

All PT samples will be prepared by Battelle using certified, commercially-available standards. PT sample results will be used to assess test kit accuracy, precision, linearity, method detection limit, cross-reactivity, and occurrence of false positive and false negative results using the data analysis methods described in Section 5.2.

2.2.3 Environmental Samples

Environmental samples will be collected from a variety of sources to evaluate the performance of the test kits with various sample matrices. For this generic protocol, it is assumed that samples will be collected from the following sources:

- Fresh surface water
- Brackish surface water
- Groundwater
- Chlorinated drinking water.

It is recognized that the composition of surface water, groundwater, and chlorinated drinking water will vary considerably depending upon the sampling location, source, and/or chlorination method. Use of surface water from two sources (fresh and brackish), groundwater from one source, and chlorinated drinking water from one source will provide a single example (not a comprehensive overview) of possible matrix effects associated with these environmental samples.

Some test kit protocols recommend filtration for removal of gross particulate matter prior to sample analysis. Therefore, the fresh and brackish pond water samples will be filtered with a 0.45 µm filter prior to sample spiking/preparation and analysis. After filtration, the following physicochemical parameters will be measured in each environmental water sample to characterize the sample matrix: pH, temperature, salinity, conductivity, and alkalinity. An aliquot of each environmental sample will be collected and sent to the analytical laboratory for DOC analysis. The physicochemical parameters will be measured in the testing laboratory instead of in the field to provide information about the sample matrix immediately prior to test kit analysis.

As shown in Table 1, each environmental water sample also will be fortified with atrazine at two spike levels. The fortified samples will be spiked to increase the analyte concentration by the amount shown in Table 1. The spike solution will be prepared from a certified, commercially-available atrazine standard. Three replicates of each sample will be analyzed. The data for the environmental samples will be used to assess accuracy, precision, potential matrix effects, and occurrence of false positives and false negatives following the data analysis procedures provided in Section 5.2.

2.3 Reference Method

The laboratory reference method to be used for this verification test is an EPA standard method for the analysis of drinking water. Each sample will be analyzed using gas chromatography / mass spectrometry (GC/MS) according to EPA Method 525.2[4].

3.0 MATERIALS AND EQUIPMENT

In general, this verification test will rely on test kit materials and equipment provided by the vendors. Battelle will provide the following equipment and materials for the collection, preparation, storage, and shipment of test samples.

3.1 Field Supplies

The following supplies will be needed for the collection of field samples:

- 1-L or 2-L certified clean amber glass bottles fitted with polytetrafluoroethylene (TFE)-lined screw caps
- Solvent-rinsed foil to keep bottle caps contaminant-free during sampling
- Disposable latex gloves
- Sample labels
- Clear packing tape (to affix the sample label to bottle and seal the cooler for sample shipment)
- Chain-of-custody forms
- Bubble wrap to prevent sample breakage
- Coolers and blue ice packs for sample storage and shipping
- Address labels for the coolers.

3.2 Laboratory Supplies

The following laboratory supplies will be needed for the preparation of the PT and laboratory-fortified environmental samples and sample shipment. Vendors will provide all equipment needed to perform the immunoassay test kit analyses.

- ASTM Type I water
- Class A volumetric flask
- 10-µL to 500-µL adjustable pipette and disposable tips
- Repeating pipette and disposable tips (volume appropriate for each test kit)
- Certified, commercially-available atrazine, hydroxyatrazine and desethyl atrazine standards

- Performance evaluation (PE) atrazine standard
- Dichloromethane, acetone, and/or methanol, as applicable (pesticide-grade or equivalent)
- Glass vials fitted with TFE-lined screw caps for test kit aliquots
- Pre-cleaned glass container of sufficient size to prepare atrazine-fortified samples
- 1-L certified clean amber glass bottles fitted with TFE-lined screw caps for reference sample aliquots
- Pre-cleaned glass container fitted with TFE-lined screw caps for DOC samples (provided by the laboratory subcontractor)
- Sample filtration equipment; 0.45 μm cellulose filters
- Conductivity meter
- Salinity refractometer
- Alkalinity meter
- pH meter capable of reading pH levels of 1 to 14
- Thermometers to measure air and water temperature
- Sample labels
- Clear packing tape (to affix sample label to bottles and seal cooler for sample shipment)
- Chain-of-custody forms
- Bubble wrap to prevent sample breakage
- Coolers and blue ice packs for sample shipping and address labels for coolers.

4.0 PROCEDURES

4.1 Environmental Sample Collection and Storage

Before environmental sample collection, the total volume of sample material needed to complete all required analyses for both the test kit and reference methods at all required spike

levels will be determined. Environmental samples will be collected within 21 days of the preparation of atrazine-fortified samples for the verification test.

The chlorinated drinking water sample will be collected directly from a tap into certified clean amber glass bottles. The drinking water source for the tap (city, state, chlorination method) will be documented. These samples will be stored in the dark at 4°C until test sample preparation (see Section 4.3). Fresh and brackish pond water samples will be collected directly into certified clean amber glass bottles. The samples will be collected at the surface of the water near the shoreline by submerging the containers no more than one inch below the surface of the water. The groundwater sample will be collected directly from a tap prior to any pretreatment.

Each sample will be assigned a unique sample identification (ID) number (see Section 4.4). The sample ID number, date, name of person collecting the sample, sample location, and time of collection will be recorded on a chain-of-custody form for all field samples. All environmental samples collected in the field will be stored at 4°C and shipped to the testing laboratory on the day of collection following chain-of-custody procedures (see Section 6.1).

4.2 Equipment Calibration

Table 2 identifies the laboratory equipment that requires calibration. All equipment will be calibrated according to the criteria provided in Table 2. The spectrophotometer for immunoassay analyses will be provided by the vendor and will be calibrated in accordance with the manufacturer's recommended procedures.

Table 2. Calibration Procedures for Sample Preparation and Physicochemical Characterization

Equipment	Calibration Frequency	Acceptance Criteria
Thermometer	Glass – annually Electronic - quarterly at two temperatures that bracket target temperature(s) against an NIST traceable thermometer	Appropriate correction factors applied
Variable volume pipettes (*i.e.*, Eppendorf)	Monthly	3% of known of true value.
Salinity (refractometer)	In accordance with manufacturer's instructions	In accordance with manufacturer's instructions
Conductivity meter	Daily	In accordance with manufacturer's instructions
pH meter	Prior to the analysis	In accordance with manufacturer's instructions
Alkalinity meter	Prior to the analysis	In accordance with manufacturer's instructions

4.3 Test Sample Preparation and Storage

Prior to test kit analysis, environmental samples will be filtered and characterized, and fortified PT samples will be prepared. These procedures are described below.

4.3.1 Physicochemical Characterization

Prior to the physicochemical characterization, the fresh and brackish pond water will be filtered with a 0.45 μm cellulose filter. All environmental samples will be measured for temperature using a thermometer, for salinity using a refractometer, for conductivity using a conductivity meter, for alkalinity using an alkalinity meter, and for pH using a pH meter. All measurements will be recorded manually by the analyst on data sheets designed specifically for this verification test. All instruments used to measure physicochemical parameters will be calibrated prior to use (Table 2). Instrument model, serial number, and calibration information will be recorded on data sheets, and calibration records will be maintained in the test files. An aliquot of each environmental sample will be collected and sent to the analytical laboratory for DOC analysis according to Method 9060[5].

4.3.2 Sample Preparation

The PT and fortified environmental samples will be prepared from certified, commercially-available standard solutions. The purchased standards will be diluted to the appropriate concentration using pesticide-grade or equivalent solvent in Class A volumetric glassware. All samples will be stored in the dark at 4°C until use. No other preservatives will be added to the samples because atrazine has been shown to be stable in water for up to two years when samples are stored under refrigerated conditions[6]. The PT and fortified environmental samples will be analyzed within 14 days of sample preparation.

Each sample will be split into 1-L and 100-mL aliquots. The 100-mL aliquots will be retained for test kit analysis and stored in the dark at 4°C until use. Two 1-L aliquots will be sent to the reference laboratory for reference analysis. The chlorinated water sample will be treated with sodium sulfite according to Method 525.2 at the analytical laboratory prior to analysis. The samples for reference analysis will be stored in the dark in amber glass bottles at 4°C until

extraction. The reference method sample extraction will be performed within 14 days of sample preparation (i.e., spiking), and analysis will be performed within 30 days of sample extraction.

4.4 Sample Identification

All samples will be assigned a unique sample ID number at the time of preparation. The sample ID will include a survey code and sequential number, and will not contain information about the nature of the sample (i.e., the samples will be blind to the test kit analyst). A master log of the sample description, sample ID number, and preparation date will be maintained by Battelle. Each sample container will be labeled with the sample ID, container number (e.g. 1 of 3), preparation date, and initials of the person preparing the sample.

4.5 Sample Analysis

4.5.1 Test Kits

Each vendor will provide test kits and other necessary equipment (e.g. vortexer, spectrophotometer) for the analysis of all samples. The full set of samples listed in Table 1 will be analyzed by each test kit. The vendors are encouraged to train the analyst prior to test kit analysis. A technical staff member with previous experience in performing immunoassay analyses will analyze the complete set of samples for each technology. The analyses will be performed according to the vendor's recommended procedures as described in the test kit instructions or user manual. Calibration and maintenance of the test kits will be performed as specified by the vendor.

Test kit results will be recorded manually by the analyst on data sheets designed specifically for this verification test. In addition to the test kit results, the data sheets will include records of the time required for sample analysis and operator observations concerning the use of the test kit (e.g. ease of use, maintenance, etc.).

4.5.2 Reference Method

The reference method for analysis of atrazine will be performed on a Hewlett-Packard 5971 GC/MS or equivalent. The reference instrument will be operated according to the recommended procedures in the instrument operating manual, and samples will be analyzed according to EPA Method 525.2, "Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry"[4].

Results from the reference analyses will be recorded electronically and compiled by the analytical laboratory into a report format, including the sample ID and the analyte concentration for each sample.

4.6 Schedule

The sample collection, preparation, and analysis are expected to take place over a four-week period. The time period for each activity should be considered approximate. Table 3 lists the activities to be conducted and a nominal schedule.

All samples will be analyzed by one test kit before proceeding to the next test kit if more than one technology is involved in the verification test. Test conditions will be kept as similar as possible throughout the duration of the test (e.g., air temperature and lighting conditions) to minimize error introduced by variable test conditions.

Participating vendors must provide the test kits and other sample analysis equipment to Battelle one week before the start of sample analysis, so that technical staff may become familiar with the kits and equipment prior to test initiation. This period will also be used to clarify any questions about the test kit's operation. Vendor staff may be present for this familiarization stage and may provide training in the operation of the test kits. Vendors are encouraged to stay and observe during the duration of the tests. Unused test kits and associated equipment will be returned to the vendors at the completion of testing.

Table 3. Approximate Schedule of Verification Test Days

Testing Period	Test Location	Activity
Days 1-21	Field locations	Collection of environmental samples and shipment to testing laboratory
Days 14-21	Testing laboratory	Receive test kits and supplies from vendor; test kit familiarization
Day 18	Testing laboratory	Environmental sample filtration
Day 21	Testing laboratory	Training, environmental sample physicochemical characterization, test sample preparation, shipment of reference samples and DOC samples to appropriate analytical laboratory
Days 22-28	Testing laboratory	Analysis of all samples using test kits
Days 22-52	Analytical laboratory	Analysis of reference samples
Days 22-52	Analytical laboratory	Analysis of environmental water samples for DOC

5.0 DATA HANDLING AND REPORTING

5.1 Data Acquisition and Review

Various types of data will be acquired and recorded electronically or manually by Battelle technical staff during this verification test. Table 4 summarizes the types of data to be recorded. All data and observations will be documented on data sheets or in laboratory record books. Results from the laboratory reference instruments will be compiled in electronic format.

Records received by or generated by Battelle in the verification test will be reviewed by a more senior Battelle staff member within two weeks after receipt or generation, respectively, before the records are used to calculate, evaluate, or report verification results. This review will be performed by a Battelle technical staff member involved in the verification test, but not the staff member that originally received or generated the record. The review will be documented by the person performing the review by adding his/her initials and date to a hard copy of the record being reviewed. This hard copy will then be returned to the Battelle staff member who will be storing the record.

In addition, data calculations performed by Battelle will be spot-checked by Battelle technical staff to ensure that calculations are performed correctly. Calculations to be checked include and statistical calculations described in this protocol.

The data obtained from this verification test will be compiled and reported independently for each test kit. Results for test kits from different vendors will not be compared with each other.

Table 4. Summary of Data Recording Process

Data to be Recorded	Responsible Party	Where Recorded	How often Recorded	Disposition of data[(a)]
Dates and times of test events	Battelle	ETV data sheets	Start/end of test, and at each change of a test parameter	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Calibration information and results for physicochemical parameters (temperature, salinity, pH, conductivity, 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	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	ETV data sheets	Throughout test duration	Manually incorporated in data spreadsheets
Reference method procedures and sample results	Analytical laboratory	ETV data sheets, or data acquisition system, as appropriate	Throughout sample analysis process	Transferred to spreadsheets
DOC analysis procedures and sample results	Analytical laboratory	ETV data sheets, or data acquisition system, as appropriate	Throughout sample analysis process	Transferred to spreadsheets

(a) All activities subsequent to data recording are carried out by Battelle.

5.2 Data Analysis

Procedures for analyzing the data produced in the verification test are described in this section.

5.2.1 Accuracy

For test kits that provide quantitative results, PT sample accuracy will be assessed relative to the spike level, and environmental sample accuracy will be assessed relative to the

reference method results. The results for each set of analyses will be averaged, and the accuracy will be expressed in terms of a percent recovery (R) as calculated from Equation 1:

$$R = \overline{C} \; / \; C_R \times 100 \qquad (1)$$

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

For qualitative results, accuracy will be assessed by determining whether the test kit result agrees with the reference method result. An overall percent agreement will be determined by dividing the number of correct responses to the overall number of analyses.

5.2.2 Precision

For test kits that provide quantitative results, the standard deviation (S) of the results for the three replicate samples will be calculated for each sample using Equation 2:

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

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 will be reported in terms of the relative standard deviation (RSD) as calculated using Equation 3:

$$RSD = \left| \frac{S}{\overline{C}} \right| \times 100 \qquad (3)$$

For test kits that provide qualitative results, precision will be assessed by calculating the percentage of consistent responses.

5.2.3 Linearity

For test kits that provide quantitative results, linearity will be assessed by performing a linear regression with the spiked analyte concentration as the independent variable, and the individual test kit result as the dependent variable. Individual replicate results for the five PT

samples will be used in the linear regression. Linearity will be expressed in terms of the slope, intercept, and correlation coefficient (r).

5.2.4 Method Detection Limit

The method detection limit (MDL) for each test kit will be assessed using results from seven replicate analyses of a sample spiked at a level of two times the vendor-stated test kit detection limit. The standard deviation of the seven replicate samples will be calculated using Equation 2. The MDL will be calculated using Equation 4:

$$MDL = t \times S \tag{4}$$

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

5.2.5 Cross Reactivity

The cross reactivity of the test kits to the atrazine degradation products hydroxyatrazine and desethyl atrazine will be assessed qualitatively by evaluating the test kit results for samples that contain only the degradation compound, but not atrazine. The reference analysis results will be used to confirm the absence of atrazine in the samples.

5.2.6 Matrix Interferences

The potential effect of the sample matrix on the test kit performance will be evaluated qualitatively by comparing the accuracy and precision results for the natural and atrazine-fortified environmental samples to those for the PT samples.

5.2.7 False Positives/False Negatives

For quantitative and semi-quantitative technologies, the occurrence of false positive and false negative results will be assessed relative to the test kit's lowest calibration standard. A false positive is defined as a positive test kit result when reference method analysis indicates that the atrazine concentration in the sample is below the test kit's lowest calibration standard. A

false negative will be defined as a negative test kit result when the reference method analysis indicates that the atrazine concentration in the sample is above the test kit's lowest calibration standard. For qualitative technologies, a false positive is defined as a positive test kit result when the reference method analysis indicates that the atrazine concentration in the sample is below the threshold value (±10%) of the kit. A false negative is defined as a negative test kit result when the reference method analysis indicates that the atrazine concentration in the sample is above the threshold value (±10%) of the kit. The rate of false positives/false negatives will be expressed as a percentage of total number of samples. Reagent blanks, PT samples, and environmental samples will be included in the analysis.

5.3 Reporting

The data obtained in the verification test will be compiled separately for each test kit, and the data evaluation methods described in Section 5.2 will be applied to each data set without comparison to any other technology. At no time will data for test kits from different vendors be intercompared or ranked. Following completion of the data evaluation, a draft verification report will be prepared for each test kit. The verification report will describe the verification test procedures and document the results. Each draft verification report will be submitted to the corresponding vendor for review and comment. Each draft report will be revised in response to the comments provided by the vendor. The revised reports will be submitted for external peer review. The reports will be revised again to address the peer review comments, and then submitted to EPA for final approval.

A verification statement will also be prepared for each test kit. The verification statement is a 2- to 3-page summary of the technology, test procedures, and results. The verification statement will follow the same review and revision process as the verification reports. Upon final approval by EPA, each verification statement will be signed by a senior Battelle manager and an EPA laboratory director. Final verification reports and statements will be posted on the ETV website (http://www.epa.gov/etv), and original signed verification statements will be provided to the vendor.

6.0 QUALITY ASSURANCE /QUALITY CONTROL

The QA/QC activities associated with this verification test will focus primarily on sample preparation and handling, data recording and analysis, and reference laboratory analysis. An independent audit covering each of these areas will be performed by the Battelle Quality Manager to ensure the quality of the verification test.

6.1 Sample Chain-of-Custody Procedures

Sample custody will be documented throughout collection, shipping, and analysis of the samples following standard chain-of-custody procedures. The chain-of-custody form summarizes the samples collected and analyses requested. The custody form will track sample release from the field to the testing laboratory, and from the testing laboratory to the analytical laboratory. Each sample custody form will be signed by the person relinquishing samples once that person has verified that the custody form is accurate. The original sample custody forms accompany the samples; the shipper will keep a copy. Upon receipt at the sample destination, sample custody forms will be signed by the person receiving the samples once that person has verified that all samples identified on the custody forms are present in the shipping container. Any discrepancies will be noted on the form and the sample receiver will immediately contact the Verification Test Coordinator to report missing, broken, or compromised samples.

6.2 Audits

6.2.1 Technical Systems Audit

The Battelle Quality Manager or designee will conduct a technical systems audit at least once during the course of the verification test. The purpose of this audit is to ensure that the verification test is being performed in accordance with this protocol and the AMS Center QMP[1], and that all procedures described in this protocol are being followed. This audit will review the standards and methods used, compare actual test procedures to those specified in this protocol, and review data acquisition and handling procedures. An independent technical systems audit

may also be performed by EPA Quality Management staff during the verification test at EPA's discretion.

6.2.2 Data Quality Audit

At least 10% percent of the data acquired during the verification test will be audited during the verification test. Battelle's Quality Manager or designee will trace the data from its 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 will be checked.

6.2.3 Performance Evaluation Audit

A performance evaluation (PE) audit will be conducted to assess the quality of the reference measurements made in this verification test. A PE audit involves challenging the reference instrument with a standard that is independent of the one used to calibrate the instrument for the test. For the PE audit, a certified standard will be obtained from a commercial supplier other than the one that supplied the standard for the preparation of PT and fortified environmental samples, or the one used to calibrate the reference instrument. The PE sample result must be within the certified range to be considered acceptable. Failure to achieve this agreement will trigger recalibration of the reference instrument with the original QC standard, and a repeat of the PE comparison. Failure in the second comparison requires obtaining another set of standards, and repeating the performance audit.

6.3 Reference Method QC

The performance of the reference method will be demonstrated through the analysis of QC samples. Laboratory RB samples will be analyzed to ensure that no sources of contamination are present. If the analysis of a laboratory RB sample indicates a concentration above the MDL for the reference instrument, then contamination will be suspected. Any contamination source(s) will be corrected, and proper blank readings will be achieved, before proceeding with the reference analyses.

The accuracy of the reference method will be verified before the beginning and at the conclusion of each testing day. The instrument to be used for reference analyses will be initially calibrated according to the procedures specified in the reference method. The instrument calibration will be verified using an appropriate calibration check sample. If the result for the calibration check sample differs by more than ±20% from the value of the standard, then the instrument will be recalibrated before continuation of the test.

Laboratory matrix spike samples will be analyzed at a frequency of at least 5% to assess whether matrix effects potentially influence the results of the reference analyses. The percent recovery (R) of the laboratory matrix spikes will be calculated from the following Equation 5:

$$R = \frac{C_s - C}{s} \times 100 \quad (5)$$

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 analyte spike. If the percent recovery of a matrix spike sample falls outside the range from 70-130%, then a matrix effect will be suspected.

Analytical duplicates will be analyzed at a frequency of at least 5% to assess analytical precision. The relative percent difference (RPD) between the two duplicates should be within 30%.

A Laboratory Fortified Blank (LFB) sample will be analyzed with each set of samples to determine whether the methodology is in control. If the recovery of the LFB falls out of the range of 70-130% of the actual concentration, then work should be stopped until the source of the problem is identified.

Upon completion of the data package and draft final report, the analytical laboratory QA staff will review all data and results for accuracy, precision, completeness, and representativeness. The QA staff may at any time selectively review any data during tabulation, and will be available for consultation with the analytical laboratory technical staff regarding any deviations and corrective action.

6.4 QA/QC Reporting

Each assessment and audit will be documented in accordance with Section 3.3.4 of the AMS Center QMP[1]. The results of the technical systems audit will be submitted to EPA. Assessment reports will include the following:

- Identification of any adverse findings or potential problems.
- Response to adverse findings or potential problems.
- Recommendations for resolving problems.
- Confirmation that solutions have been implemented and are effective.
- Citation of any noteworthy practices that may be of use to others.

6.5 Corrective Action

During the course of any assessment or audit, the Battelle Quality Manager will inform the technical staff of any immediate corrective action that should be taken. If serious quality problems exist, the Battelle Quality Manager will notify the Battelle AMS Center Manager of the need to consider a stop work order. Once the assessment report has been prepared, the Verification Test Coordinator will ensure that a response is provided for each adverse finding or potential problem, and will implement any necessary follow-up corrective action. The Battelle Quality Manager will ensure that follow-up corrective action has been taken.

7.0 REFERENCES

1. Quality Management Plan (QMP) for the ETV Advanced Monitoring Systems Center, Version 5.0. EPA Environmental Technology Verification Program, prepared by Battelle, Columbus, Ohio, March, 2004.

2. Environmental Technology Verification Program Quality Management Plan, December 2002 (EPA/600/R-03/021).

3. National Primary Drinking Water Standards, 40 CFR Part 141.

4. U.S. EPA Method 525.2, Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry, Revision 2.0, 1995.

5. U.S. EPA Method 9060 Total Organic Carbon, Revision 0, September 1986.

6. Ciba Crop Protection Report ABR-94094, Storage Stability of Atrazine, G-30033, G28279, and G28273 in Water Under Refrigerator Storage Conditions, Greensboro, NC *as cited in* "Interlaboratory Validation of an Atrazine Immunoassay." Journal of the American Water Works Association, September 2001.