Battelle
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Environmental Technology Verification Program
Advanced Monitoring Systems Center

Quality Assurance Project Plan For Verification of ANDalyze Lead100 Test Kit and AND1000 Fluorimeter

FINAL

Verification of
ANDalyze Lead100 Test Kit and AND1000 Fluorimeter

July 3, 2012

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SECTION A

PROJECT MANAGEMENT

A1 VENDOR APPROVAL PAGE

ETV Advanced Monitoring Systems Center

Quality Assurance Project Plan
for Verification of
ANDalyze Lead 100 Test Kit and AND1000 Fluorimeter
DRAFT

Name ______________________________________

Company ___________________________________

Date _______________________________________

A2 TABLE OF CONTENTS

Section Page

List of Figures Page

List of Tables

Appendices

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A4 LIST OF ACRONYMS AND ABBREVIATIONS

ADQ	Audit of Data Quality
AMS	Advanced Monitoring Systems
$CaSO_4$	calcium Sulfate
COC	chain of custody
CV	coefficient of variation
DEI	determination of the effects of interferences
DI	deionized
DLOD	determination of the limit of detection
DLR	determination of linear range
DQO	data quality objective
EPA	Environmental Protection Agency
ETL	Environmental Treatability Laboratory
ETV	Environmental Technology Verification
FCU	failure cause unknown
Fe	iron
HDPE	high density polyethylene
HNO_3	nitric Acid
ICC	Initial Calibration Check
ICP-MS	inductively-coupled plasma mass spectroscopy
IDC	initial demonstration of capability
IFE	instrument failure electrical
IFM	instrument failure mechanical
IOFM	instrument operator failure to follow method
JHA	job hazard analysis
KCl	potassium Chloride
LFM	laboratory-fortified matrix
LOD	limit of detection
LRB	laboratory record book
MCL	maximum contaminant level
MGD	million gallons per day
$MgSO_4$	magnesium sulfate
MLC	minimum water level change

NaCl	sodium Chloride
$NaHCO_3$	sodium Bicarbonate
Pb	lead
PEA	performance evaluation audit
PO	Project Officer
ppb	parts per billion
ppm	parts per million
PT	performance testing
QA	quality assurance
QAPP	quality assurance project plan
QC	quality control
QCS	quality control standard
QM	Quality Manager
QMP	Quality Management Plan
RB	reagent blank
RMO	Records Management Office
SD	standard deviation
SFC	sensor failure chemical
SFM	sensor failure mechanical
SOP	standard operating procedure
TDS	total dissolved solid
TL	tolerance limit
TPC	three point calibration
TSA	technical systems audit
VTC	Verification Test Coordinator
WHO	World Health Organization

A5 VERIFICATION TEST ORGANIZATION

The verification test will be conducted under the U.S. Environmental Protection Agency's (EPA's) Environmental Technology Verification (ETV) Program. It will be performed by Battelle, which manages the ETV Advanced Monitoring Systems (AMS) Center through a cooperative agreement with EPA. The scope of the AMS Center covers verification of monitoring technologies for contaminants and natural species in air, water, and soil.

The day-to-day operations of this verification test will be coordinated and supervised by Battelle, with the participation of ANDalyze, Inc. (Vendor), the Vendor who will be supplying the Lead100 test kit and the AND1000 fluorimeter (Lead100/AND1000) for performance verification. The verification test will be performed using laboratory facilities (i.e., Battelle's Environmental Treatability Laboratory [ETL]) under highly-controlled conditions and selected field sites (e.g., river reaches and reservoirs) under partially-controlled conditions. Verification tests will require the participation of the Vendor and Battelle staff. Vendor representatives, expert peer reviewers and EPA AMS Center management will review this Quality Assurance Project Plan (QAPP), verification reports, and verification statements. The verification statements are then approved by the EPA AMS Center management.

The organization chart in Figure 1 identifies the responsibilities of the organizations and individuals associated with the verification test. Roles and responsibilities are defined further below. Quality assurance (QA) oversight will be provided by the Battelle Quality Manager (QM) and also by the EPA AMS Center QM, at his/her discretion. This verification test is Quality Category III, which requires a QA review of 10% of the test data (see Section C1).

A5.1 Battelle

Mr. Brian Yates is the AMS Center's Verification Test Coordinator (VTC) for this test. In this role, Mr. Yates will have overall responsibility for ensuring that the technical, schedule, and cost goals established for the verification test are met. Specifically, Mr. Yates will:

- Prepare the draft QAPP, verification report, and verification statement;
- Establish a budget for the verification test and manage staff to ensure the budget is not exceeded;

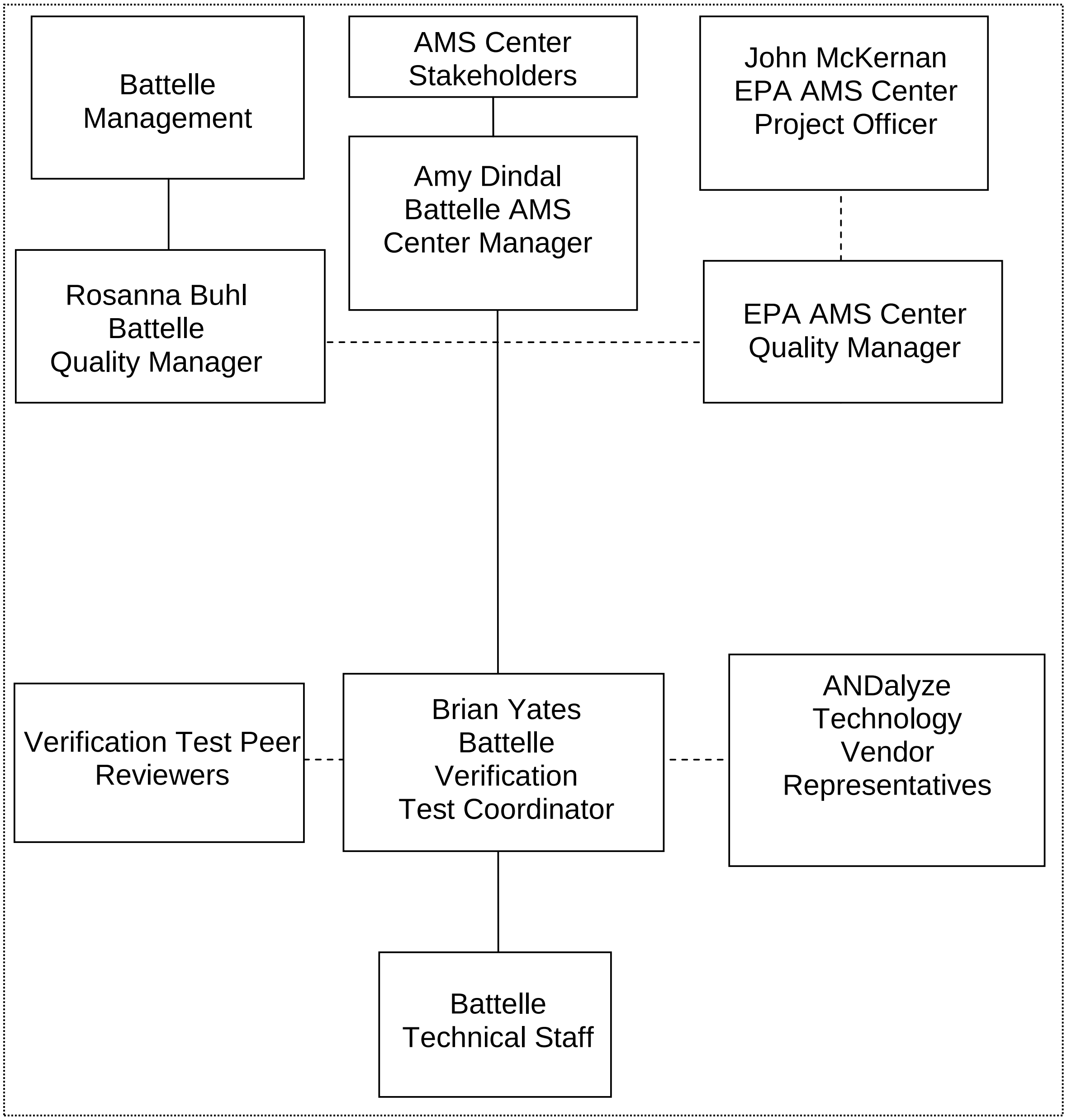


Figure 1. Organization Chart for the Verification Test

- Revise the draft QAPP, verification report, and verification statement in response to reviewer comments;
- Conduct verification testing using the vendor's technology;
- Collect the data and samples during verification testing;
- Direct the team in performing the verification test in accordance with this QAPP;
- Organize and lead a kick-off meeting approximately one (1) week prior to the start of the verification test to review the critical logistical, technical, and administrative aspects of the verification test. Responsibility for each aspect of the verification test will be reviewed to ensure each participant understands his/her role;
- Ensure that all quality procedures specified in this QAPP and in the AMS Center Quality Management Plan[1] (QMP) are followed;
- Serve as the primary point of contact for Vendor representatives;
- Ensure that confidentiality of sensitive Vendor information is maintained;
- Assist Vendor as needed during verification testing;
- Familiarize himself with the operation and maintenance of the technologies through instruction by the Vendor;
- Respond to any issues raised in assessment reports, audits, or from test staff observations, and institute corrective action as necessary;
- Coordinate distribution of the final QAPP, verification report, and verification statement; and
- Respond to QAPP deviations and any issues raised in assessment reports, audits, or from test staff observations, and institute corrective action as necessary.

Ms. Amy Dindal is Battelle's Manager for the AMS Center. As such, Ms. Dindal will oversee the various stages of verification testing. Specifically, Ms. Dindal will:

- Review the draft and final QAPP;
- Attend the verification test kick-off meeting;
- 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 confidentiality of sensitive Vendor information is maintained;
- Support Mr. Yates in responding to any issues raised in assessment reports and audits;
- Maintain communication with EPA's technical and quality managers; and
- Issue a stop work order if Battelle or EPA QA staff discover any situation that will compromise test results.

Battelle Technical Staff will support Mr. Yates in planning and conducting the verification test. Specifically, the technical staff will:

- Assist in planning for the test and making arrangements for the receipt of and training on the technologies;
- Attend the verification test kick-off meeting;
- Assist Vendor staff as needed during technology receipt and training;
- Arrange for and/or acquire adequate supplies, equipment, and facilities/locations for performing verification tests and disposing of generated wastes;
- Perform statistical calculations specified in this QAPP on the technology data as needed;
- Provide results of statistical calculations and associated discussion for the verification reports as needed;
- Support Mr. Yates in responding to any issues raised in assessment reports and audits related to statistics and data reduction as needed; and
- Conduct and observe verification testing on-site, as appropriate. Immediately report deviations to this QAPP to the VTC.

Ms. Rosanna Buhl is the Battelle QM for the AMS Center. Ms. Buhl will:

- Review the draft and final QAPP;
- Attend the verification test kick-off meeting and lead the discussion of the QA elements of the kick-off meeting checklist;
- Review and approve the QAPP, QAPP amendments, deviations and audit reports;
- Work with the VTC and Battelle's AMS Center Manager to resolve data quality concerns and disputes; and

- Recommend a stop work order if audits indicate that data quality or safety is being compromised.
- Assign a Quality Assurance Officer (QAO). Ms. Betsy Cutié is assigned as QAO for this test. She will perform the following functions:
    - Prior to the start of verification testing, verify the presence of applicable training records, including any vendor training on test equipment;
    - Conduct a technical systems audit (TSA) at least once during the verification test;
    - Conduct audits to verify data quality;
    - Prepare and distribute an audit report for each audit;
    - Verify that audit responses for each audit finding and observation are appropriate and that corrective action has been implemented effectively;
    - Provide a summary of the QA/quality control (QC) activities and results for the verification report;
    - Review the draft and final verification report and verification statement;
    - Communicate to the VTC and/or technical staff the need for immediate corrective action if an audit identifies QAPP deviations or practices that threaten data quality;

A5.2 Lead100/AND1000 Vendor

The responsibilities of the Vendor are as follows:

- Review and provide comments on the draft QAPP;
- Accept (by signature of a company representative) the final QAPP prior to test initiation to confirm that the conditions of the test are understood. Provide technology for evaluation during the verification test;
- Supply training on the use of the technology and provide written consent and instructions for test staff to carry out verification testing, including written instructions for routine operation of their technology;
- Provide maintenance and repair support for the technology, onsite if necessary, throughout the duration of the verification test; and
- Review and provide comments on the draft verification report and statement.

A5.3 EPA AMS Center

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

For the verification test, the EPA's AMS Center QM will:

- Review and provide comments on the draft QAPP;
- Perform at his/her option one external technical systems audit (TSA) during the verification test;
- Notify the EPA AMS Center Project Officer (PO) of the need for a stop work order if the external audit indicates that data quality is being compromised;
- Prepare and distribute an assessment report summarizing results of the external audit;
- Perform audits of data quality;
- Notify the EPA AMS Center PO of the need for a stop or modify work order if the audit of data quality indicates that data quality is being compromised; and
- Review draft verification reports and verification statements.

Dr. John McKernan is EPA's PO for the AMS Center. Dr. McKernan, or designee will:

- Review and provide comments on the draft QAPP;
- Approve the final QAPP;
- Review the draft verification reports and verification statements;
- Oversee the EPA review process for the QAPP, verification reports, and verification statements;
- Coordinate the submission of verification reports and verification statements for final EPA approval;
- Post the QAPP, verification reports, and verification statements on the ETV Web site; and
- Review and authorize any QAPP deviations by phone and provide the name of a delegate to the Battelle AMS Center Manager should he not be available during the testing period.

A5.4 Verification Test Stakeholders

This QAPP and the verification report and verification statement based on testing described in this document will be reviewed by experts in the fields related to lead detection technology performance and statistics. The following stakeholders have agreed to provide review:

- Randy Gottler, City of Phoenix, Arizona
- Dr. Edward F. Askew, Askew Scientific, Iowa

A6 BACKGROUND

The ETV Program's AMS Center conducts third-party performance testing of commercially available technologies that monitor, sample, detect, and characterize contaminants or naturally occurring species across all matrices. The purpose of ETV is to provide objective and quality assured performance data on environmental technologies so that users, developers, regulators, and consultants can make informed decisions about purchasing and applying these technologies. Stakeholder committees of buyers and users of such technologies recommend technology categories, and technologies within those categories, as priorities for testing.

Lead (Pb) is a naturally occurring metal in the aquatic environment; however, most Pb contamination of concern arises from anthropogenic sources (such as deposition of Pb dust from combustion processes in natural waterways or due to its use in plumbing materials). Although Pb is not commonly found in natural waters, high concentrations of Pb in the natural environment can compromise the health of ecosystems and lead to their degradation. Humans are exposed to Pb through ingestion of Pb-laden foods or waters, or inhalation of Pb-containing dust which can lead to numerous health effects. Specifically, Pb in drinking water can cause a variety of adverse health effects including delays in physical and mental development, along with deficits in attention span and learning disabilities in children. In adults, consumption of Pb-contaminated drinking water over the EPA maximum contaminant level (MCL) can lead to acute high blood pressure or, if ingested over long periods of time, can lead to chronic kidney problems including necrosis or hepatic cancers[3].

Due to the importance of Pb in both the natural environment and in finished drinking waters, there is an immediate need for the determination of soluble, bioavailable aqueous Pb at relevant toxicological levels (µg/L [ppb] range). A number of instrumental methods exist for the determination of Pb in environmental aqueous samples such as graphite furnace atomic absorption spectroscopy, inductively-coupled plasma mass spectroscopy (ICP-MS) and electrochemical methods such as cyclic voltametry and chronoamperometric methods. While well established, these methods are time consuming and, in general, expensive to carry out on a routine basis. Other shortcomings associated with traditional methods for the determination of Pb in the aquatic environment is that these methods are not portable and analysis time (time-to-result) are too long for the determination of Pb at relevant time-scales. In addition, an accurate and rapid method of Pb detection is also necessary to ensure that municipal wastewater and industrial wastewater effluents discharged to natural waterways will not exceed the total maximum daily load permissible for Pb to said waterways.

The purpose of this QAPP is to specify procedures for a verification test applicable to Pb sensing in environmental water samples, finished drinking water samples, and municipal wastewater and industrial wastewater effluent water samples. The purpose of the verification test is to evaluate the performance of Lead100/AND1000 for Pb measurement by challenging it under a variety of combinations of Pb concentrations and water compositions.

A6.1 Technology Need

The World Health Organization (WHO) has recommended the upper limit for Pb in natural waterways at less than 10 µg/L[4] while the EPA has set an MCL for Pb in drinking water of 15 µg/L[3]. In addition, EPA has also set discharge limits for industrial wastewater and municipal wastewater effluents to natural waterways depending on the sensitivity of the receiving water body and the probability of Pb contamination from the specified source. It is therefore essential that accurate and precise methods for detection of aqueous Pb at relevant Pb concentrations be developed and verified in a systematic and scientific fashion so that citizens, regulators and operators of facilities from which Pb discharges are probable can have the access to and confidence in Pb measurements in natural waterways, drinking water and municipal wastewater and industrial wastewater effluents. Specifically, these technologies should:

- be able to precisely and accurately detect aqueous Pb at levels relevant to regulations and adverse health effects (1 to 100 µg/L [ppb]);
- be able to detect aqueous Pb in a variety of aquatic matrices such as finished drinking water, groundwater, freshwater, wastewater effluents and seawater;
- have high sample throughput;
- be easy to operate with little chemistry or engineering background;
- be rugged and portable.

A6.2 Technology Description

The Lead100/AND1000 is designed to test for Pb in drinking water and environmental waters. Testing is intended to take place onsite at the source of collection or in a temperature controlled facility a minimal distance from the collection site without sample preservation. The test makes use of two primary components: a handheld fluorimeter (AND1000) and a consumable test kit (Lead100) specific to each metal or target; in the present case, Pb.

The AND1000 fluorimeter is specifically coded to provide an interactive experience and allow testing, data storage, and signal output without the use of a separate computational device. The fluorimeter has the capability to analyze multiple targets with the appropriate test kit, though the sole target discussed in this method is aqueous Pb in drinking water, wastewater effluent and environmental waters. The AND1000 fluorimeter enables field testing to be done in two steps. The fluorimeter uses a sensitive measurement technique to determine the levels of Pb contaminants. ANDalyze’s catalytic DNA sensors use a metal-specific DNAzyme reaction that leads to an increase in fluorescence in the presence of a target contaminant substance such as Pb. The fluorescence of the reaction is measured by a fluorimeter to determine the concentration of the target heavy metal and is reported in ppb. Using the product to test for metals is a simple, quantitative test that allows for detection in a linear range of 2 to 100 ppb — at and below EPA standards in drinking water. The test is performed by taking a buffered 1 mL water sample, injecting it through the sensor, and into the AND1000 fluorimeter. This sample is then automatically analyzed and reports results in less than two minutes.

The second component is the Lead100 test kit specific to Pb, which provides all necessary materials for in-field instrument calibration and sample testing with minimal sample pretreatment. This kit contains the DNA sensors specific to a given analyte. The kit is color coded for ease of use and a product manual is provided with detailed step-by-step instructions including photographs. It should be noted that laboratory evaluation may require additional supplies and standard laboratory glassware.

A7 VERIFICATION TEST DESCRIPTION AND SCHEDULE

This verification test will assess the performance of the Lead100/AND1000 relative to key verification parameters including accuracy, precision, sample throughput and ease of use. These performance parameters will be evaluated using multiple variables that will challenge the Lead100/AND1000's ability to detect Pb in a variety of aqueous matrices. In performing the verification test, Battelle will follow the technical and QA procedures specified in this QAPP and will comply with the data quality requirements in the AMS Center QMP[1] and the ETV QMP[2] . This verification test is an EPA designated QA Level III verification.

A7.1 Verification Test Description

Specific procedures described herein are based on Battelle's experience and expertise in testing of heavy metals in aqueous solution and peer reviewer input. The Lead100/AND1000 technology evaluation is organized as four main tests. Each test evaluates the performance of Lead100/AND1000 to operate under different laboratory and field conditions. The four tests are:

1. Initial demonstration of capability and performance testing (PT) including determination of the limit of detection (DLOD), determination of linear range (DLR) and determination of the effects of interferences (DEI)
2. Testing accuracy and precision of the instrument for the analysis of finished drinking water samples
3. Testing accuracy and precision of the instrument for the analysis of environmental water samples

4. Testing accuracy and precision of the instrument for the analysis of wastewater effluent samples.

The initial demonstration of capability, PT (including DLOD and DLR) as well as the DEI will be performed in a highly-controlled environment within Battelle's ETL and involve the detection of a Pb spike (25 ppb) in reagent grade water. The DLOD will be performed by measuring seven replicates of Pb spiked at five times the purported limit of detection (10 ppb). The DLR will be carried out by measuring the Lead100/AND1000's ability to precisely and accurately measure five samples with Pb concentrations of 0 ppb, 5 ppb, 15 ppb, 25 ppb, 50 ppb, 75 ppb and 100 ppb. The samples will be analyzed in triplicate and the coefficient of determination will be used to assess the linearity of the response of the instrument within this range. Finally, DEI will be determined using three synthetic water samples. The first sample will have low total dissolved solids concentration (Low TDS Water), the second high total dissolved solids concentration (High TDS Water) and the third will contain high iron (Fe) concentration and other dissolved solids (High Fe Water). Each of these synthetic water samples will be split into required 100 mL subsamples and receive a Pb spike of 25 ppb and 50 ppb before Pb is measured in triplicate from each subsample by Lead100/AND1000. Normal sample preparation procedures will be followed for the Low TDS Water and High TDS Water, and a special sample preparation procedure for the removal of Fe interference will be used to prepare the High Fe Water sample for Pb analysis. The accuracy and precision of the instrument will be evaluated for DEI.

The next set of tests will determine the accuracy and precision of the Lead100/AND1000 in recovery of Pb spikes in finished drinking water. Three sets of samples will be prepared with Pb spikes of 25 ppb: finished drinking water samples collected from a water fountain (Water Fountain), bottled mineral water purchased from a local supermarket (Bottled Water) and finished drinking water collected from the effluent of a local water treatment facility treating groundwater (Finished Well Water). All waters will be analyzed in triplicate both with and without a Pb spike of 25 ppb.

The third series of tests aims at determining the accuracy and precision of the Lead100/AND1000 in recovering Pb spikes in environmental water samples. The environmental water samples proposed for this study include samples collected from both freshwater and saltwater sources. Three freshwater sources will be sampled including water collected from the reach of a freshwater river (River Water), samples collected from a freshwater reservoir (Reservoir Water) and raw groundwater collected at the source of a drinking water treatment facility (Raw Well Water). For consistency and logistical simplicity, the Raw Well Water will be collected from the source that feeds the facility from which the Finished Well Water will be collected. In addition, one seawater sample will be collected for testing (Seawater) to determine the accuracy and precision of the Lead100/AND1000 in testing natural waters with high salinity. All four environmental samples will be analyzed in triplicate after the addition of a Pb spike to 25 ppb and one sample with no spike in the field. Performance of tests on Seawater differ from the performance of tests on freshwater in that seawater will be diluted tenfold before being subjected to LEAD100/AND1000 testing and results will be analyzed qualitatively, not quantitatively as with freshwater samples.

The final series of tests aims at determining the accuracy and precision of the Lead100/AND1000 in recovering Pb spikes in wastewater effluent samples. Three samples will be analyzed during this series of tests: two effluent samples collected from two separate traditional activated sludge treatment facilities treating domestic wastewater (Municipal Wastewater Effluent #1 and Municipal Wastewater Effluent #2) and a sample collected from the effluent of a metal finishing works (Metal Finishing Wastewater Effluent). The Metal Finishing Wastewater Effluent will be collected from a facility conforming to 40 CFR 433 and/or 40 CFR 413 after all on-site pretreatment.

All tests will be performed with the Lead100/AND1000 operating according to the vendor's recommended procedures as described in the user's instructions/manual (see Appendices A and B) and/or during training provided to the operator. Similarly, calibration and maintenance of the technologies will be performed as specified by the vendor. Results from the technologies being verified will be recorded in laboratory record books (LRBs) and transferred to an appropriate electronic format (i.e., Microsoft® Excel).

A verification report describing the results obtained during the evaluation will be drafted. In addition, a verification statement summarizing the results will be drafted. The report and verification statement will be reviewed by the Vendor, EPA, and the peer reviewers. In performing the verification tests, Battelle will follow the technical and QA procedures specified in this QAPP and comply with the data quality requirements in the AMS Center QMP[1]. Quality procedures include a TSA and audits of data quality (ADQs). The Battelle QAO will perform the TSA.

A7.2 Verification Test Schedule

Table 1 shows a general schedule of testing and data analysis/reporting activities to be conducted in this verification test.

A7.3 Test Site Descriptions

PT (including DLOD, DLR and DEI) will be completed at Battelle's ETL according to this QAPP.

Finished drinking water samples will be collected from three separate sources. Water Fountain will be collected from a water fountain located within Battelle headquarters in Columbus, OH. Battelle receives its finished drinking water from the Dublin Road Water Purification Plant in Columbus, OH, which treats raw water from Scioto River within the city limits. Bottled Water will be purchased from a local supermarket (e.g., Kroger, Giant Eagle) in 1-gal quantities and transferred to ETL for analysis. Finished Well Water will be collected from a small water treatment facility at the Plainview Christian School located in Plain City, OH. An on-site measurement will be made immediately after sample collection and a 1 L sample of the Finished Well Water will be transferred to ETL for analysis under highly-controlled laboratory conditions.

Environmental samples include River Water, Reservoir Water, Raw Well Water and Seawater. River Water will be collected from the east bank of the Scioto River in Columbus, OH, no more than 5 miles north of Griggs Reservoir (see Figure 2). The Scioto River is one of the longest rivers in Ohio as it runs over 230 miles from Auglaize County, in the western part of the state, through Columbus to Portsmouth where it empties into the Ohio River. Two dams

Table 1. General Verification Test Schedule

Approximate Month of Completion	Testing Activities	Data Analysis and Reporting
July	• Training of Battelle VTC by vendor • Prepare sampling and analysis equipment for upcoming testing • Organize shipment of Seawater and Metal Finishing Wastewater Effluent	• Prepare LRB for data recording
	• Completion of performance evaluation audit (PEA) • Completion of initial demonstration of capability including on-site calibration and three point calibration • Completion of PT including DLOD, DLR and DEI	• Record testing data in LRB and/or data recording forms • Transfer testing data to electronic format as convenient
	• Collection and testing on Water Fountain, Bottled Water and Finished Well Water • Completion of TSA	• Record testing data in LRB • Transfer testing data to electronic format as convenient
	• Collection and testing on River Water, Reservoir Water and Raw Well Water • Receipt and testing on Seawater	• Record testing data in LRB • Transfer testing data to electronic format as convenient
	• Collection and testing on Municipal Wastewater Effluent #1 and Municipal Wastewater Effluent #2 • Receipt and testing on Metal Finishing Wastewater Effluent	• Record testing data in LRB • Transfer testing data to electronic format as convenient
	• None	• Compilation and transfer of all data to electronic format • Statistical analysis of data
	• None	• Draft Verification Report completed • Draft Verification Statement completed
August	• None	• Audits of data quality by Battelle QAO • Draft Verification Report and Verification Statement sent to Vendor
	• None	• Address reviewers comments • Revise Verification Report and Verification Statement • Draft Verification Report and Verification Statement sent to Peer Reviewers
September	• None	• Address reviewers comments • Revise Verification Report and Verification Statement • Draft Verification Report and Verification Statement sent to EPA Project Office for QA review
	• None	• Submission of final of Verification Report and Verification Statement to EPA for approval

have been built on the Scioto River, both in Columbus, for drinking water and recreation purposes[5]. River Water will be collected from the surface of the river no more than 1 ft below the water surface and no more than 20 ft from the eastern shoreline. Reservoir water will be collected from Griggs Reservoir on the Scioto River in Columbus, OH. Griggs Dam was the first dam to be built on the Scioto River in Columbus, Ohio in 1908, which forms the Griggs Reservoir. Griggs Reservoir is a long, narrow body of water at almost 6 miles long and 500 feet wide with a 1.2 million gallon capacity. The Reservoir is a major drinking water source for Columbus. Reservoir Water will be sampled from within the boundaries of Griggs Reservoir, and, similarly to the River Water, will be sampled from the surface, no more than 1 ft below the water surface and no more than 20 ft from the eastern shore of the reservoir (see Figure 2). Raw Well Water will be collected from a well located in Plain City, OH. Note that this is the raw groundwater that serves as the water source for the Plainview Christian School, the same treatment facility that is providing Finished Well Water. The treatment system operating at the Plainview Christian School is an on-demand coagulation/filtration system treating approximately 10 gallons per minute. Seawater will be collected from the Atlantic Ocean off West Palm Beach, FL (see Figure 3). Seawater will be collected from the surface of the ocean no more than 1 ft below the water surface and no more than 20 ft from the shoreline.

Wastewater effluent samples will be collected from three separate sources. Municipal Wastewater Effluent #1 will be collected from the final effluent sampling location within the Southerly Wastewater Treatment Plant in Columbus, OH (Southerly). Southerly, built in 1967, is one of two treatment plants servicing Columbus, OH, located south of the city in Lockbourne, Ohio (see Figure 2). Southerly receives and treats influent from the northeast and eastern half of Franklin County through a series of physical and biological processes. Southerly has the capacity to treat 114 million gallons per day (MGD) and discharges into the Scioto River. Municipal Wastewater Effluent #2 will be collected from the final effluent sampling location within the Jackson Pike Wastewater Treatment Plant in Columbus, OH (Jackson Pike). Jackson Pike, built in 1935, is the original treatment plant servicing Columbus, OH and is located on the southern limit of the city (see Figure 2). Jackson Pike receives and treats influent from the northwestern and western half of Franklin County through a series of physical and biological processes. Jackson Pike has the capacity to treat 68 MGD with a peak treatment capacity of 102 MGD and,

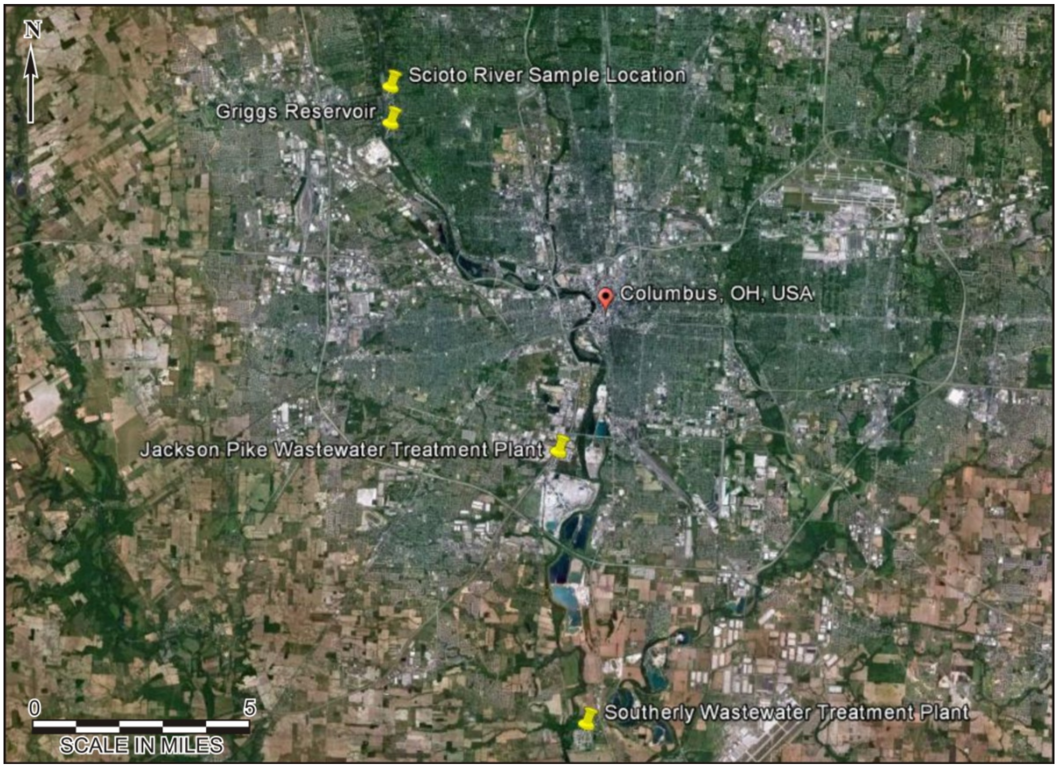


Figure 2. Site Map Showing the Locations of Two of the Environmental Samples (River Water and Reservoir Water) and Municipal Wastewater Effluent #1 (Jackson Pike Wastewater Treatment Plant) and Municipal Wastewater Effluent #2 (Southerly Wastewater Treatment Plant)

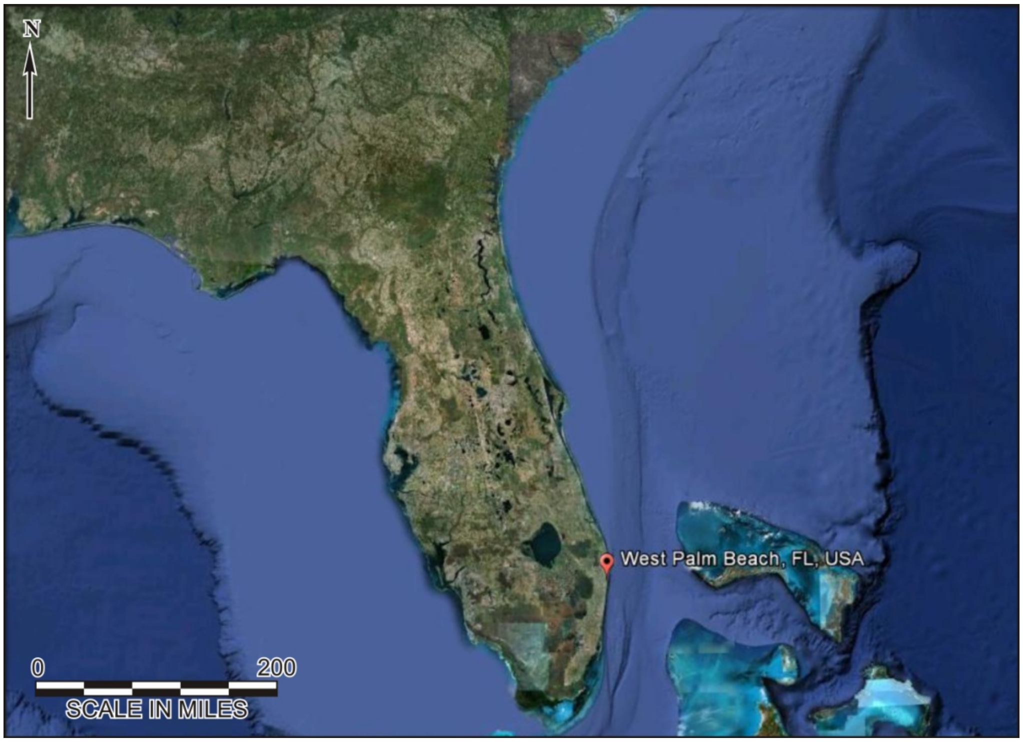


Figure 3. Site Location Map Showing the Location of the Seawater Sample

like Southerly, discharges into the Scioto River. The third effluent sample, Metal Finishing Wastewater Effluent, will be collected by the vendor from a facility conforming to 40 CFR 433 and /or 40 CFR 413 (electroplating, electroless plating, anodizing, coating [chromating, phospating and coloring], chemical etching and milling, and printed circuit board manufacturing). Table 2 indicates the sampling locations and brief test site descriptions for all samples considered in this QAPP.

A7.4 Health and Safety

Battelle will conduct all sample collection and verification testing following the safety and health protocols in place for the locations used for testing. In addition, a job hazard analysis (JHA) will be prepared to describe the specific hazards associated with sampling efforts and sample handling, as well as the use of engineering controls and other procedures required to reduce the possibility of adverse health and safety issues. These include not only the use of personal protective equipment (e.g., lab coat, gloves, protective eyewear) but also the implementation of engineering controls (e.g., chemical hood). Promoting awareness of safe chemical and waste handling methods will also be emphasized. Standard Battelle JHA forms will be completed once any potential hazardous activities are defined. The JHA forms will be physically present at the location where verification testing is being conducted (both in the field and at ETL). All test participants will be required to review and understand the JHA forms prior to initiating laboratory or field work and adhere to its procedures during all verification tests. In addition, all recommended safety procedures for the use of Vendor equipment will be discussed with an authorized Vendor representative during the training session and diligently followed for all activities involving the use of the Vendor equipment.

Table 2. Sampling Locations and Descriptions

Sample Name	Description
Water Fountain	Water Fountain sample to be collected from a water fountain within Battelle
Bottled Water	Bottled spring water sample to be purchased in 1-gal quantities from a local supermarket (e.g., Kroger, Giant Eagle)
Finished Well Water[(a)]	Finished well water sample to be collected from the Plainview Christian School in Plain City, Ohio
River Water[(a)]	River water sample to be collected from the surface of the Scioto River in Columbus, OH. Sample will be collected no more than 1 ft below the river surface and no more than 20 ft from the eastern shoreline no more than 5 miles north of Griggs Reservoir (see Figure 2)
Reservoir Water[(a)]	Reservoir water sample to be collected from the surface of Griggs Reservoir in Columbus, OH. Sample will be collected no more than 1 ft below the reservoir surface and no more than 20 ft from the eastern shoreline (see Figure 2)
Raw Well Water[(a)]	Raw Well Water sample to be collected from the raw water intake at the Plainview Christian School in Plain City, Ohio
Seawater	Seawater to be collected from the surface of the Atlantic Ocean in West Palm Beach, FL. Sample will be collected no more than 1 ft below the ocean surface and no more than 20 ft from the shoreline (see Figure 3)
Municipal Wastewater Effluent #1[(a)]	Municipal Wastewater Effluent #1 to be collected from the effluent sampling location at the Jackson Pike Wastewater Treatment Facility in Columbus, OH (see Figure 2)
Municipal Wastewater Effluent #2[(a)]	Municipal Wastewater Effluent #2 to be collected from the effluent sampling location at the Southerly Wastewater Treatment Facility in Columbus, OH (see Figure 2)
Industrial Effluent	Industrial Effluent to be collected by the vendor from a facility that has been classified as a 40 CFR 413 or 40 CFR 433. The sample will shipped to ETL in Columbus, OH

(a) Samples will be analyzed both on-site and at ETL.

A8 QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA

In performing the verification test, Battelle will follow the technical and QA/QC procedures specified in this QAPP and comply with the data quality requirements in the AMS Center QMP [1] and ETV QMP[2]. Data quality objectives (DQOs) have been established to ensure that this verification test provides suitable data for a robust evaluation of performance. The DQOs are used to develop the testing variable tolerances required to meet the objectives of the verification test. The DQOs for this verification test were established to assess the performance of the Lead100/AND1000 and its ability to detect Pb in laboratory, environmental, waste and drinking water effluent samples. For this verification test, the DQOs are evaluated by the acceptance criteria determined in Section B5 to detect Pb in laboratory, environmental, waste and drinking water effluent samples. The DQOs of this verification test are to:

- Evaluate the ability of the Lead100/AND1000 to accurately and precisely detect Pb in laboratory, environmental, waste and drinking water effluent samples
- Determine the limit of detection and linear range of the Lead100/AND1000 for the detection of Pb in laboratory samples
- Evaluate the effect of matrix and interfering species on the ability of the Lead100/AND1000 to accurately and precisely measure Pb in laboratory, environmental, waste and drinking water effluent samples
- Evaluate the effect of environmental control (i.e., partially-controlled field environments vs. highly-controlled laboratory environments) on the ability of the Lead100/AND1000 to accurately and precisely measure Pb in environmental and wastewater effluent samples

Assessing the DQOs is also a key component of the ETV PEA process. The PEA will be used to independently confirm the accuracy of the reference analytical measurements (e.g., EPA Method 200.8).

The Battelle QAO will perform a TSA at least once during this verification test to verify that testing and analysis were performed according to the QAPP. The EPA QM also may conduct an independent TSA, at his/her discretion.

A9 SPECIAL TRAINING/CERTIFICATION

Documentation of training related to sample collection and handling, technology testing, analytical method analysis, operation of ancillary equipment used to collect supporting data, routine laboratory procedures, and reporting is maintained for all Battelle technical staff in training files at their respective Battelle location. The Battelle QAO will verify the presence of appropriate training records prior to the start of testing. The Vendor will train the Battelle technical staff prior to the start of testing. Battelle will document this training with a consent form, signed by the Vendor or designated representative, which identifies Battelle technical staff who have been trained to use their technologies and can train other staff. In the event that other staff members are required to use the technologies, they will be trained by staff trained by the Vendor. All technical staff will have a minimum of a bachelor's degree in science/engineering.

Battelle will conduct all verification testing using the engineering controls and safety procedures described in the JHA forms and will document that all testing staff have read, understood, and agreed to adhere to the procedures described in the JHA prior to and during work on the test.

A10 DOCUMENTATION AND RECORDS

The documents for this verification test will include the QAPP, Vendor instructions, verification report, verification statement, and audit report. Project records will include: LRBs and/or data collection forms, supporting laboratory records, training records, electronic files (both raw data and spreadsheets), JHA forms, and QA audit files. Table 3 summarizes the types of data to be recorded. The raw and final results from the test measurements will be collected by Battelle, and technology data will be copied from the AND1000 fluorimeter result screen. Section B10 details the data recording practices and responsibilities. Documentation of Battelle staff training by the Vendor and copies of other project specific training will also be included in the project files. All of these records will be maintained at the test site, ETL or in the VTC's office during the test and then transferred to permanent storage at Battelle's Records Management Office (RMO) at the conclusion of the verification test.

All data generated during the conduct of this project will be recorded directly, promptly, and legibly in permanent ink in the LRB and/or data collection forms. All data entries will be dated on the date of entry and signed or initialed by the person entering the data. Any changes will be made so as not to obscure the original entry, dated and signed or initialed at the time of the change, and indicate the reason for the change.

Table 3. Summary of Records to be Collected and Maintained[(a)]

Record/Data	Where Recorded	How often recorded	Disposition of Data
Dates, sampling locations, times of sample collection and test events	LRBs and/or data collection sheets	Start/end of each collection event and test run	Use to organize/check test results; manually incorporate in data spreadsheets
Test variables	LRBs and/or data collection sheets	When set or changed, or as needed to document notable details during testing	Use to organize/check test results; manually incorporate in data spreadsheets, or visually record test
Technology data	LRBs and/or data collection sheets	During each test run	Use to document and interpret performance of the technology
Field data	LRBs and/or data collection sheets	During each collection event and field test run	Use to interpret performance of the technology under specified field conditions
PEA records	LRBs and/or data collection sheets	Before testing begins	Use to verify the accuracy of the reference analytical equipment
Analytical method, sample preparation, sample analysis, chain of custody, and results	LRBs and/or data collection forms, chain of custody forms, or data acquisition system, as appropriate	When test samples are aliquoted for the analysis and throughout sample handling and analysis process	Use to organize/check test results; manually incorporate in data spreadsheets; transfer to spreadsheets/agreed upon report; project files; retain for documentation of analytical method performance

(a) Battelle is responsible for collecting and maintaining all specified records.

SECTION B

MEASUREMENT AND DATA ACQUISITION

B1 EXPERIMENTAL DESIGN OVERVIEW

This verification test is designed to evaluate the accuracy, precision, functionality and ease-of-use of the Lead100/AND1000 in detecting Pb in laboratory, environmental, waste and drinking water effluent samples including deionized (DI) water with and without interfering species. The characteristics of independent variables have been selected and will be established during the runs to determine the response of the dependent variables. Performance parameters will be evaluated based on the responses of the dependent variables (i.e., comparison of the Lead100/AND1000 performance to reference method performance) and used to characterize the Lead100/AND1000 performance.

Dependent Variable Responses--The Lead100/AND1000 will be evaluated with respect to its ability to accurately and precisely determine aqueous Pb concentrations in a variety of water samples. Detection of aqueous Pb concentration thus represents the only quantitative dependent variable included in the test. In addition, functionality and ease of use will be evaluated on a subjective basis.

Independent Variable Levels--The levels of the independent variables with respect to water samples to be tested will be the following: (1) prevailing water quality characteristics dictated by environmental conditions (e.g., pH, major anions, major cations); and/or (2) water quality characteristics artificially imparted on synthetic environmental or laboratory samples including synthetic matrices and Pb spikes. Additionally, other qualitative independent variables include operator ability and prevailing field conditions.

The verification test will consist of four portions (in addition to QA testing): (1) performance testing (including DLOD, DLR and DEI); (2) finished drinking water sample testing; (3) environmental water sample testing; and (4) wastewater effluent water sample testing. The four tests are described in further detail in the following:

1. Performance Testing

PT is focused on initial demonstration of capability (IDC) and determination of the inherent features and limitations of Lead100/AND1000. In addition to the IDC and three point calibration (TPC), PT tests also include DLR, DLOD, and DEI. In all cases, the tests are designed to determine the instrument response to a known concentration of aqueous Pb contamination in controlled laboratory samples (both DI and DI with added interferences). Instrument accuracy, precision and ease of use during aqueous Pb detection in the laboratory samples will be determined during these tests. Before a new sample matrix is analyzed, the Lead100/AND1000 will be subjected to on-site calibration as outlined in the Vendor instrument manuals. An experimental matrix for performance testing is presented as Table 4.

IDC aims at demonstrating the technology with clean samples spiked with a known concentration of Pb. DI will be spiked with 25 ppb Pb before being analyzed with the Lead100/AND1000. Further, these analyses will be carried out in triplicate to assess the precision of the Lead100/AND1000.

TPC is carried out to provide a baseline calibration in DI water. Subsequent calibrations (on-site calibrations) augment this TPC and correct measurements for matrix effects. The TPC is stored in the Lead100/AND1000 and is augmented each time Pb is measured in a new matrix (on-site calibration). The TPC consists of analyzing one sample each of DI spiked with Pb at 25 ppb, 50 ppb and 75 ppb. Initial Calibration Check (ICC) serves to confirm calibration accuracy.

DLR of the instrument will be accomplished through a series of triplicate tests on DI spiked with Pb at six different concentrations within the purported linear range of the Lead100/AND1000 (2 to 100 ppb). The six Pb concentrations specified for the DLR experiments are 0 ppb, 5 ppb, 15 ppb, 25 ppb, 50 ppb, 75 ppb and 100 ppb. The accuracy and precision of the instrument as well as the linearity of the concentration curve will be the performance metrics for the DLR tests.

Limit of detection (LOD) of the Lead100/AND1000 has been reported by the Vendor as 2 ppb Pb and will be confirmed through a series of seven replicate tests of DI samples spiked with one concentration of Pb at five times the purported detection limit (10 ppb).

Table 4. Experimental Matrix and Sample Summary for PT

Test Name	Matrix	Pb Spike (ppb)	Number of analyses
Initial Demonstration of Capability IDC	DI	25	3
Three-Point Calibration TPC	DI	25	1
		50	1
		75	1
Initial Calibration Check ICC	DI	25	3
Determination of Linear Range DLR	DI	0	3
		5	3
		15	3
		25	3
		50	3
		75	3
		100	3
Determination of Limit of Detection DLOD	DI	10	7
Determination of the Effect of Interferences DEI	Low TDS(a)	25	3
		50	3
	High TDS(b)	25	3
		50	3
	High Iron(c)	25	6(d)
		50	6(d)

(a) Low TDS water has the following makeup: 95 ppm $NaHCO_3$, 50 ppm $CaSO_4$, 60 ppm $MgS0_4$, 4 ppm KCl and 10 ppm glucose.

(b) High TDS water has the following makeup: 380 ppm $NaHCO_3$, 200 ppm $CaSO_4$, 240 ppm $MgS0_4$, 16 ppm KCl and 100 ppm glucose.

(c) High Iron water has the following make-up: 1 ppm Fe, 3 ppm NaCl and 142 ppb $CaSO_4$.

(d) Three samples analyzed by Lead100/AND1000 with special pretreatment procedure for the removal of the effects of Fe interference and three additional samples analyzed by Lead100/AND1000 without the special pretreatment procedure.

The final aspect of PT is the DEI on the instrument's ability to accurately and precisely measure Pb in aqueous samples with added interferences. Triplicate interference tests will be carried out each on three samples of DI not only with added interferences (referred to as Low

TDS Water, High TDS Water or High Fe Water) but also spiked with Pb in the amount of 50 ppb. The ability of the Lead100/AND1000 to accurately and precisely measure Pb at the specified concentration will be the performance metric for the interference tests. The first two samples will be analyzed after pretreatment with only the vendor-recommended buffer (required for all samples; see Appendices A and B), while the third will be pretreated with both the vendor-recommended buffer and a special vendor-provided pretreatment method for the removal of effects of Fe interference. Note that an on-site calibration must be performed separately for each of the two samples (i.e., one on-site calibration for the un-treated sample and one on-site calibration for the treated sample). A series of three measurements will also be made on the High Fe Water with only the vendor-provided buffer to assess the utility of the additional pretreatment method for removal of the effects of Fe interference. The specific water makeup of the three samples is as follows (see Section B1.1.7 for detailed sample preparation methods):

- Low TDS Water:
  - 95 ppm $NaHCO_3$
  - 50 ppm $CaSO_4$
  - 60 ppm $MgS0_4$
  - 4 ppm KCl
  - 10 ppm glucose
- High TDS Water:
  - 380 ppm $NaHCO_3$
  - 200 ppm $CaSO_4$
  - 240 ppm $MgS0_4$
  - 16 ppm KCl
  - 100 ppm glucose
- High Iron Water:
  - 1 ppm Fe
  - 3 ppm NaCl
  - 142 ppb $CaSO_4$

The tests outlined in this section do not explicitly include the QA samples required (see Section B5). Data collected during the PT include concentration of Pb reported by the Lead100/AND1000 and the reference method and qualitative data collected regarding ease of operation.

2. Analysis of Finished Drinking Water Samples

Analyses of finished drinking water samples aims at determining the accuracy and precision of the Lead100/AND1000 in measuring soluble Pb in water matrices other than highly controlled, “clean” laboratory samples, but not as complex as environmental samples. Three samples will be analyzed in triplicate each spiked with 25 ppb Pb: Water Fountain, Bottled Water and Finished Well Water. In addition, Finished Well Water will not only be analyzed in triplicate ETL with a Pb spike of 25 ppb but also once in the field with no spike. Samples collected from the treatment facility in Plain City, OH will be transferred to the ETL and analyzed with Lead100/AND1000 under highly-controlled laboratory conditions to determine the differences (if any) between the Lead100/AND1000 performance in the field and in the laboratory. The tests outlined in this section are in addition to QA samples required (see Section B5). Data collected during this phase of the test include concentration of Pb reported by Lead100/AND1000 and the reference method and qualitative data collected regarding ease of operation especially differences in ease of use between field and laboratory analyses and difficulties encountered in field analyses (if any).

3. Analysis of Environmental Water Samples

The analysis of environmental water samples aims at determining the accuracy and precision of the Lead100/AND1000 in measuring soluble Pb in water matrices naturally occurring in the environment. Three samples will be analyzed in triplicate each spiked with 25 ppb Pb: River Water, Reservoir Water and Raw Well Water. River Water will be collected from a reach of the Scioto River and Reservoir Water will be collected from south of the River Water sampling location in Grigg’s Reservoir (see Figure 2 for a map of sampling locations). Both samples will be collected from the shore of the water bodies (less than 20 ft from shore). The samples will also be collected from the surface of the water bodies (less than 1 ft depth). The

Raw Well Water will be collected from the raw water intake tap at a small water treatment facility at the Plainview Christian School located in Plain City, OH; note that this is the same facility from which the Finished Well Water will be collected. The freshwater environmental samples will be analyzed in the field without a Pb spike to determine the ability of the Lead100/AND1000 to detect background Pb levels in the samples. In addition to field analyses, both freshwater environmental samples will be transferred to the ETL with a 25 ppb Pb spike and analyzed with Lead100/AND1000 identically as in the field under highly-controlled laboratory conditions to determine differences (if any) between Lead100/AND1000 performance in the field and in the laboratory. The tests outlined in this section are in addition to QA samples required (see Section B5). Data collected during this phase of the test include concentration of Pb reported by Lead100/AND1000 and the reference method and qualitative data collected regarding ease of operation; especially differences in ease of use between field and laboratory analyses and difficulties encountered in field analyses (if any).

In addition to the two freshwater samples, one additional sample (Seawater) will be collected from a location off West Palm Beach, FL (see Figure 3 for the Seawater sampling location). The Seawater sample will be collected in the same manner as the two freshwater samples (i.e., less than 20 ft from the shoreline and less than 1 ft depth). The Seawater sample will be shipped by overnight services to ETL where two samples will be separately spiked with 25 ppb Pb and 50 ppb Pb and analyzed by the Lead100/AND1000 in triplicate each to determine the accuracy of the Lead100/AND1000 in recovering the Pb spike as well as the precision of the instrument. In addition to spiked samples, Seawater will also be analyzed without a Pb spike to determine the ability of the Lead100/AND1000 to detect background Pb levels in the samples. Due to the high dissolved solids anticipated in Seawater, the results of the analysis of Seawater (both spiked and un-spiked) will be qualitative in nature indicating whether or not the Seawater samples have low, medium or high concentrations of Pb.

4. Analysis of Wastewater Effluent Samples

The final series of tests for the verification will be the analysis of three effluent water samples collected from wastewater treatment operations. Two samples (Municipal Wastewater Effluent #1 and Municipal Wastewater Effluent #2) will be collected from two separate domestic wastewater treatment facilities in Columbus, OH (see Figure 2 for the locations of the treatment facilities). Municipal Wastewater Effluent #1 will be collected from the final effluent sampling location at Jackson Pike and Municipal Wastewater Effluent #2 will be collected from the final effluent sampling location at Southerly (see Figure 2 for a map of the facilities' locations). Due to the nature of the Lead100/AND1000 and the high levels of interferences in the municipal wastewater effluent samples, all samples will be diluted ten-fold with DI before any sample preparation (i.e., Pb spike) or analysis. Note that dilution of Municipal Wastewater Effluent #1 raises the limit of detection from 2 ppb Pb to 20 ppb Pb for these samples. After dilution, samples will be analyzed in the field without a Pb spike. Samples will then be transferred to the ETL where they will be analyzed in triplicate after spiking to 25 ppb Pb.

In addition to the two municipal wastewater effluent samples (i.e., Municipal Wastewater Effluent #1 and Municipal Wastewater Effluent #2), one industrial wastewater effluent sample (Metal Finishing Wastewater Effluent) will be supplied by the vendor. The industrial wastewater effluent sample will be collected from a metal finishing operation conforming to 40 CFR 433 and/or 40 CFR 413. Due to the nature of the Lead100/AND1000 and the high levels of interferences in the industrial wastewater effluent samples, Metal Finishing Wastewater Effluent will be diluted ten-fold with DI before any sample preparation (i.e., Pb spike) or analysis Samples will be collected by the vendor after all on-site pretreatment and shipped to ETL where they will be spiked with 25 ppb Pb and analyzed by the Lead100/AND1000 in triplicate to determine the accuracy of the Lead100/AND1000 in recovering the Pb spike as well as the precision of the instrument. One additional aliquot of unspiked effluent from each facility will be analyzed to determine the ability of the Lead100/AND1000 to detect background Pb levels in each of the samples. Note that dilution of the Metal Finishing Wastewater Effluent raises the limit of detection from 2 ppb Pb to 20 ppb Pb for these samples.

Table 5 summarizes the water samples to be tested.

Other Monitoring Data--Other variables may influence the operability of the Lead100/AND1000 and information on these other variables will be collected during the tests but not controlled. Monitoring data that will be recorded include field and laboratory temperature, field and laboratory barometric pressure and general field conditions (e.g., weather).

B1.1 Detailed Test Procedures

B1.1.1 Glassware

All glassware, whether used at ETL or in the field, will be purchased and cleaned before first use and between uses with the following procedure:

1. Scrub with 1% Alconox solution in tap water
2. Triple rinse with DI
3. Soak overnight in 10% HNO_3
4. Triple rinse with DI
5. Air dry for at least 8 hours

All glassware used to measure volume will be Class A and all stoppered glassware will be equipped with ground-glass stoppers.

B1.1.2 Reagents

All reagents not provided by the vendor will be purchased from reputable sources (e.g., Fisher Scientific, Sigma-Aldrich) and will be accompanied with a certificate of analysis. Table 6 provides the details of reagents to be used in this verification. DI water will be obtained from ETL and will have a resistance of >18.2 MΩ.

Table 5. Sample Summary for Water Sample Testing

Test	Test Sample	Performance Parameter	Independent Variables	# of Analyses
Analysis of Finished Drinking Water Samples	Water Fountain	▪ Percent recovery of 25 ppb Pb spike ▪ Standard deviation and coefficient of variation of triplicate analyses	▪ Water composition (metals and anions) • Prevailing field conditions (e.g., temperature)	4 (three 25 ppb spikes; 1 unspiked)
	Bottled Water	▪ Percent recovery of 25 ppb Pb spike ▪ Standard deviation and coefficient of variation of triplicate analyses	▪ Water composition (metals and anions) ▪ Prevailing field conditions (e.g., temperature)	4 (three 25 ppb spikes; 1 unspiked)

Test	Test Sample	Performance Parameter	Independent Variables	# of Analyses
	Finished Well Water	▪ Percent recovery of 25 ppb Pb spike ▪ Standard deviation and coefficient of variation of triplicate analyses	▪ Water composition (metals and anions) ▪ Prevailing field conditions (e.g., temperature)	4 (three 25 ppb spikes; 1 without a Pb spike)
Analysis of Environmental Water Samples	River Water	▪ Percent recovery of 25 ppb Pb spike ▪ Standard deviation and coefficient of variation of triplicate analyses	▪ Water composition (metals and anions) ▪ Prevailing field conditions (e.g., temperature)	4 (three 25 ppb spikes; 1 without a Pb spike)
	Reservoir Water	▪ Percent recovery of 25 ppb Pb spike ▪ Standard deviation and coefficient of variation of triplicate analyses	▪ Water composition (metals and anions) ▪ Prevailing field conditions (e.g., temperature)	4 (three 25 ppb spikes; 1 without a Pb spike)
	Seawater(a)	▪ Percent recovery of 25 ppb Pb spike ▪ Standard deviation and coefficient of variation of triplicate analyses	▪ Water composition (metals and anions) ▪ Prevailing field conditions (e.g., temperature)	7 (three 25 ppb spikes; three 50 ppb spikes; 1 unspiked at ETL)
Analysis of Wastewater Effluent Samples(a)	Municipal Wastewater Effluent #1	▪ Percent recovery of 25 ppb Pb spike ▪ Standard deviation and coefficient of variation of triplicate analyses	▪ Water composition (metals and anions) ▪ Prevailing field conditions (e.g., temperature)	4 (three 25 ppb spikes; 1 without a Pb spike)
	Municipal Wastewater Effluent #2	▪ Percent recovery of 25 ppb Pb spike ▪ Standard deviation and coefficient of variation of triplicate analyses	▪ Water composition (metals and anions) ▪ Prevailing field conditions (e.g., temperature)	4 (three 25 ppb spikes; 1 without a Pb spike)
	Metal Finishing Wastewater Effluent	▪ Percent recovery of 25 ppb Pb spike ▪ Standard deviation and coefficient of variation of triplicate analyses	▪ Water composition (metals and anions) ▪ Prevailing field conditions (e.g., temperature)	4 (three 25 ppb spikes; 1 unspiked at ETL)

(a) Samples will be diluted tenfold before any sample preparation or analysis

Table 6. Reagents Used During Verification Testing

Reagent	CAS Number	Description of use	Purity/ Concentration	Mass/Volume	Vendor	Catalogue Number
NIST-Traceable Lead Solution	10099-74-8	Preparing lead spikes; preparing calibration standards; preparing PEA standards	1,000 ppm	100 mL	Fisher Scientific	SL21-100
Sodium Bicarbonate Anhydrous	144-55-8	Preparing Low TDS and High TDS synthetic Waters	>99.7%	500 g	Fisher Scientific	S233500
Calcium Sulfate Hemihydrate	10034-76-1	Preparing Low TDS and High TDS and High Fe synthetic Waters	97%	100 g	Fisher Scientific	AC38535-1000
Magnesium Sulfate Anhydrous	7487-88-9	Preparing Low TDS and High TDS synthetic Waters	>97%	500 g	Fisher Scientific	AC41348-5000
Potassium Chloride Anhydrous	7447-40-7	Preparing Low TDS and High TDS synthetic Waters	>99%	500 g	Fisher Scientific	P217-500
Sodium Chloride Anhydrous	7647-14-5	Preparing Low TDS, High TDS and High Fe synthetic Waters	>99%	500 g	Fisher Scientific	S271500
Glucose aqueous solution	50-99-7	Preparing Low TDS and High TDS synthetic waters	20% w/v	100 mL	Ricca Chemical	R3254000100
Iron Solution	7437-89-6	Preparing High Fe Water	1,000 ppm	100 mL	Acros Organics	AC19605-1000

B1.1.3 Laboratory Equipment

Table 7 summarizes the specifications of general laboratory equipment that will be used throughout the verification test.

Table 7. Laboratory Equipment Used During Verification Testing

Equipment	Make/Model	Notes
Analytical Balance	Sartorius Cubis	±0.00001g
Drying Oven	VWR 1330 FSM	275°C Max
Pipette	Eppendorf Research	1-5 mL (±0.1 mL)
Stir Bars	VWR	1/8" diameter; 1/2" length
DI Water System	Barnstead Nanopure® Diamond	>18.2 MΩ

B1.1.4 Analytical Procedures

All samples analyzed by Lead100/AND1000 will also be analyzed by a reference method to determine the accuracy of the Lead100/AND1000 in recovering Pb spikes. Pb will be measured by ICP-MS by EPA Method 200.8 (Pb Reference Method). In addition, each of the finished drinking water, environmental water and wastewater effluent samples will be analyzed once for metals and cations by EPA Method 200.8 (Cation Reference Method), major anions by EPA Method 300.1 (Anion Reference Method) and alkalinity (including total, carbonate, bicarbonate and hydroxide alkalinity) by Standard Method 2320B (Alkalinity Reference Method). Cation Reference Method reports the concentrations of the following species: aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, lead, manganese, mercury, molybdenum, nickel, selenium, silver, thallium, thorium, uranium, vanadium and zinc. Anion Reference Method reports the concentrations of the following species: bromide, chloride, fluoride, nitrate, nitrite, ortho-phosphate and sulfate. Samples will be preserved as per the methods indicated and sent at 4±2°C to DHL Analytical (Round Rock, TX) for analysis within the hold times specified by the reference methods.

The Lead100/AND1000 will be operated exactly as specified in the vendor-provided "AND1000 Fluorimeter for Water Testing User Manual" (AND-prod-1000-2-2012) and the "Lead Testing and On-Site Calibration for Water Testing" (AND-Lead-100-02-2012) (see

Appendices A and B). Any deviation in procedure from these documents as suggested by the vendor will be explicitly noted in the LRB for this verification test. In addition, environmental water testing, use of the iron interference pretreatment and three-point calibration will be carried out as described in the appropriate solution notes (see Appendices C, D and E, respectively).

B1.1.5 Preparation of Pb Stock Solution

A single standard solution will be used for Lead100/AND1000 three-point calibration, spiking of samples and PEA and will be prepared from a NIST-traceable 1,000 ppm Pb standard solution in 3% HNO_3. The 1 ppm standard solution will be prepared by filling a 1,000 mL volumetric flask halfway with DI water. Next, 1 mL of the 1,000 ppm Pb standard solution will be pipetted to the volumetric flask. The volumetric flask will then be filled to the line with DI water washing down the insides of the volumetric flask to ensure that a 1:1000 dilution is made. The resulting solution is a 1 ppm Pb standard solution in DI water (Pb Stock Solution). All other Pb standards will be prepared from the Pb Stock Solution. Pb Stock Solutions will be prepared at least daily and each will be analyzed by the Pb Reference Method to ensure the ongoing accuracy of the Pb Stock Solution.

B1.1.6 Preparation of Pb-spiked Samples

Throughout the verification study, several spikes to 100 mL samples will be necessary in order to prepare samples with specific Pb concentrations (depending on the test). As the required volume for each Lead100/AND1000 analysis is only 5 mL, all spiked samples will be prepared in 100 mL volumetric flasks which will be sufficient for each test. In order to determine the volume of Pb Stock Solution to be spiked to samples, a mass balance equation was used (see below):

$$mass\ Pb\ in\ standard\ additon\ (\mu g\ Pb) = mass\ Pb\ in\ 100\ mL\ spiked\ sample\ (\mu g\ Pb)$$

$$1{,}000\ \mu g/L\ Pb * x\ L\ Pb\ Stock\ Solution = Target\ Pb\ Concentration\ (\mu g/L\ Pb) * 0.100L$$

$$x\ L\ Pb\ Stock\ Solution = Target\ Pb\ Concentration\ \mu g/L\ Pb * \frac{0.100\ L}{1{,}000\frac{\mu g}{L}Pb}$$

$$x\ mL\ Pb\ Stock\ Solution = Target\ Pb\ Concentration \frac{\mu g}{L} Pb * \frac{0.100\ L}{1{,}000 \frac{\mu g}{L} Pb} * \frac{1{,}000\ mL}{1\ L}$$

$$x\ mL\ Pb\ Stock\ Solution = Target\ Pb\ Concentration \frac{\mu g}{L} Pb * 0.100 \frac{L - mL}{\mu g}$$

By simply multiplying the target Pb concentration (in ppb) of the 100 mL sample by 0.100 L-mL/µg, the volume of Pb Stock Solution (in mL) to be added to the 100 mL sample is obtained. Table 8 presents the amount of Pb Stock Solution to be spiked to each 100 mL sample required for analysis.

Table 8. Volume of Pb Standard Solution Additions to 100 mL of Sample to Obtain Required Pb Spike

Sample Volume (mL)	Target Pb Concentration (ppb)	Volume of Pb Stock Solution spike (mL)
100	5[a]	0.5
100	10[b]	1.0
100	15[c]	1.5
100	25[d]	2.5
100	50[e]	5.0
100	75[f]	7.5

(a) For DLR
(b) For DLOD
(c) For DLR
(d) For DLR, DEI, Water Fountain, Bottled Water, Finished Well Water, River Water, Reservoir Water, Raw Well Water, Seawater, Municipal Wastewater Effluent #1, Municipal Wastewater Effluent #2 and Metal Finishing Wastewater Effluent
(e) For DLR, DEI and Seawater
(f) For DLR

To prepare Pb-spiked samples, a 100 mL volumetric flask will be filled halfway with the appropriate matrix (i.e., DI, finished drinking water sample, environmental sample or wastewater effluent sample) and spiked with Pb by pipetting the appropriate amount of Pb Stock Solution to the 100 mL flask (see Table 8). The 100 mL volumetric flask will then be filled to the line with DI water by washing down the insides of the flask using a squirt bottle. The 100 mL volumetric flask will be stoppered and inverted three times to ensure proper mixing. All spiked samples will be prepared in this manner.

B1.1.7 Preparation of Synthetic Waters for DEI Testing

B1.1.7.1 Preparation of High TDS Water

High TDS Water will be prepared in a 1 L volumetric flask. A 1 L volumetric flask will be filled halfway with DI water. Approximately 10 g each of $NaHCO_3$, $CaSO_4•0.5H_2O$, $MgSO_4$ and KCl will be placed in separate glass weigh dishes and dried at 105°C in the drying oven for 30 minutes. After drying, the samples will be individually removed from the drying oven. A stock solution of 8,400 ppm $NaHCO_3$ will be prepared by weighing 8,400 mg ± 10 mg of $NaHCO_3$ in a plastic weigh dish in the analytical balance and washed into a 1 L volumetric flask with DO water by means of a squirt bottle. The plastic weigh dish will then be triple rinsed with DI water by means of a squirt bottle and the rinsate also added to the 1 L volumetric flask. A stock solution of 7,455 ppm KCl will be prepared by weighing 7,455 mg ± 10 mg of KCl in a plastic weigh dish in the analytical balance and washed into a 1 L volumetric flask with DO water by means of a squirt bottle. The plastic weigh dish will then be triple rinsed with DI water by means of a squirt bottle and the rinsate also added to the 1 L volumetric flask. The following will be weighed separately on individual plastic weigh dishes 202 mg ± 10 mg $CaSO_4•0.5H_2O$, 240 mg ± 10 mg $MgSO_4$. All measured weights will be recorded in the LRB. After weighing, the $CaSO_4•0.5H_2O$ and $MgSO_4$ samples will be washed into the 1 L volumetric flask with DI water by means of a squirt bottle. The plastic weigh dish will then be triple rinsed with DI water by means of a squirt bottle and the rinsate also added to the 1 L volumetric flask. After the salts have been added to the 1 L volumetric flask, 5 mL of 20% (w/v) glucose solution will be pipetted to the 1 L volumetric flask. Finally, 45.700 mL of the $NaHCO_3$ stock solution and 2.146 mL of the KCl stock solution will be added to the volumetric flask. The 1 L volumetric flask will be filled to the line with DI water before the addition of a stir bar. The High TDS Water will then be allowed to mix by means of the magnetic stir bar for 30 minutes at 120 rpm to ensure that the salts have dissolved. Table 9 presents the composition of the High TDS Water.

Table 9. Composition of High TDS Water

Component	Component Volume (mL)	Component Mass (mg)	Final Volume (L)	Component Concentration (ppm)
$NaHCO_3$[(a)]	45.700	380	1	380
$CaSO_4$	NA	200	1	200
$MgSO_4$	NA	240	1	240
KCl[(b)]	2.146	16	1	16
Glucose[(c)]	5	100	1	100

[(a)] $NaHCO_3$ added as a 8,400 ppm stock solution
[(b)] KCl added as a 7,455 ppm stock solution
[(c)] Glucose added as a 20% (w/v) solution

B1.1.7.2 Preparation of Low TDS Water

Low TDS Water will be prepared in a 1 L volumetric flask. A 1 L volumetric flask will be filled halfway with DI water. Approximately 10 g each of $NaHCO_3$, $CaSO_4 \bullet 0.5H_2O$, $MgSO_4$ and KCl will be placed in separate glass weigh dishes and dried at 105°C in the drying oven for 30 minutes. After drying, the samples will be individually removed from the drying oven and weighed on a plastic weigh dish in the analytical balance to the following weights: 50 mg ± 1 mg $CaSO_4 \bullet 0.5H_2O$ and 60 mg ± 0.1 mg $MgSO_4$. The measured weights will be recorded in the LRB. After weighing, each sample will be washed into the 1 L volumetric flask with DI water by means of a squirt bottle. The plastic weigh dish will then be triple rinsed with DI water by means of a squirt bottle and the rinsate also added to the 1 L volumetric flask. After the salts have been added to the 1 L volumetric flask, 0.5 mL of 20% (w/v) glucose solution will be pipetted to the 1 L volumetric flask. Finally, 11.400 mL of the $NaHCO_3$ stock solution and 0.537 mL of the KCl stock solution will be added to the volumetric flask. The 1 L volumetric flask will be filled to the line with DI water before the addition of a stir bar. The Low TDS Water will then be allowed to mix by means of the magnetic stir bar for 30 minutes at 120 rpm to ensure that the salts have dissolved. Table 10 presents the composition of the Low TDS Water.

Table 10. Composition of Low TDS Water

Component	Component Volume (mL)	Component Mass (mg)	Final Volume (L)	Component Concentration (ppm)
$NaHCO_3$[a]	NA	95	1	95
$CaSO_4$	NA	50	1	50
$MgSO_4$	NA	60	1	60
KCl[b]	NA	4	1	4
Glucose[c]	5	10	1	10

[a] $NaHCO_3$ added as a 8,400 ppm stock solution
[b] KCl added as a 7,455 ppm stock solution
[c] Glucose added as a 20% (w/v) solution

B1.1.7.3 Preparation of High Fe Water

The salts of the High Fe Water will be prepared in a 1 L volumetric flask at one hundred times concentration and diluted by pipetting 10 mL of the concentrated solution to a 1 L volumetric flask. Fe will then be added by means of a 1000 ppm solution before filling to the line (1 mL of 1000 ppm Fe solution diluted to 1 L). For preparation of the one hundred-times concentrated salt solution, a 1 L volumetric flask will be filled halfway with DI water. Approximately 1 g each of NaCl and $CaSO_4$ will be placed in separate glass weigh dishes and dried at 105°C in the drying oven for 30 minutes. After drying, the samples will be individually removed from the drying oven and weighed on a plastic weigh dish in the analytical balance to the following weights: 300 mg ± 10 mg NaCl and 14.2 mg ± 1 mg $CaSO_4 \bullet 0.5H_2O$. The measured weights will be recorded in the LRB. After weighing, each sample will be washed into the 1 L volumetric flask with DI water by means of a squirt bottle. The plastic weigh dish will then be triple rinsed with DI water by means of a squirt bottle and the rinsate also added to the 1 L volumetric flask. The volumetric flask will be filled to the line with DI water before the addition of a stir bar and then be allowed to mix by means of the magnetic stir bar for 30 minutes at 120 rpm to ensure that the salts have dissolved. 10 mL of the solution will then be pipetted to a separate 1 L volumetric flask pre-filled halfway with DI water. 1 mL of 1,000 ppm Fe solution will be pipetted to the volumetric flask (1 mg Fe) and the flask will be filled to the line and inverted 16 times to ensure proper mixing. Table 11 presents the composition of the High Fe Water.

The pH of the High Fe Water will be confirmed by the pH Meter and the pH will be recorded in the LRB. Depending on the pH of the High Fe Solution, pH adjustment may be necessary as described in the vendor-provided literature.

Table 11. Composition of High Fe Water

Component	Component Volume (mL)	Component Mass (mg)	Final Volume (L)	Component Concentration (ppm)
NaCl[(a)]	NA	3	1	95
$CaSO_4$[(a)]	NA	0.142	1	0.142[(c)]
Fe[(d)]	1	1	1	1

[(a)] Diluted from a one hundred-times concentrated stock solution; 10 mL concentrated stock to 1000 mL final volume
[(b)] Diluted from a one hundred-times concentrated stock solution; 10 mL concentrated stock to 1000 mL final volume
[(c)] Equivalent to 100 ppb SO_4
[(d)] Fe added from a 1,000 ppm solution in 3% HNO_3

B1.1.8 Sequence of Procedures

The entire verification test will be conducted over a period of four working days; Table 12 indicates the experimental matrix for the verification test.

B1.1.8.1 IDC Testing

IDC testing will be carried out at ETL. IDC-25 will be prepared by spiking DI water to 25 ppb Pb before it is analyzed in triplicate by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the IDC-25 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis.

B1.1.8.2 TPC Testing

TPC testing will be carried out at ETL. TPC-25 will be prepared by spiking DI water to 25 ppb Pb. Similarly, TPC-50 and TPC-75 will be prepared by spiking DI water to 50 ppb Pb and 75 ppb Pb, respectively. Each of the TPC samples will be analyzed once by the Lead100/AND1000 and results recorded in LRB. If TPC samples have a recovery within 85 – 115 %, then a TPC will not be stored in the AND1000 and factory calibration will be used for

Table 12. Verification Schedule and Test Matrix[a]

Test Day	Test Name	Sample Name	Matrix	Pb Spike (ppb)	AND1000 Analyses	Pb Reference Analyses	Cations Reference Analyses	Anions Reference Analyses	Alkalinity Reference Analyses
1	IDC	IDC-25	DI	25	3	1	0	0	0
	TPC	TPC-25	DI	25	1	1	0	0	0
		TPC-50		50	1	1	0	0	0
		TPC-75		75	1	1	0	0	0
	ICC	ICC-25	DI	25	3	1	0	0	0
	DLOD	DLOD-10	DI	10	7	1	0	0	0
	DLR	DLR-5	DI	5	3	1	0	0	0
		DLR-15		15	3	1	0	0	0
		DLR-25		25	3	1	0	0	0
		DLR-50		50	3	1	0	0	0
		DLR-75		75	3	1	0	0	0
		DLR-100		100	3	1	0	0	0
	DEI	HTDS-0	High TDS Water	0	1	1	1	1	1
		HTDS-25		25	3	1	0	0	0
		HTDS-50		50	3	1	0	0	0
		LTDS-0	Low TDS Water	0	1	1	1	1	1
		LTDS-25		25	3	1	0	0	0
		LTDS-50		50	3	1	0	0	0
		HFe-0	High Fe Water	0	2[b]	1	1	1	1
		HFe-25		25	6[b]	1	0	0	0
		HFe-50		50	6[b]	1	0	0	0
2	Finished Drinking Water Samples	WF-0	Water Fountain	0	1	1	1	1	1
		WF-25		25	3	1	0	0	0
		BW-0	Bottled Water	0	1	1	1	1	1
		BW-25		25	3	1	0	0	0
		FWW-0	Finished Well Water	0	2[c]	1	1	1	1
		FWW-25		25	3	1	0	0	0
	Environmental Water Samples	RWW-0	Raw Well Water	0	2[c]	1	1	1	1
		RWW-25		25	3	1	0	0	0
3		ReW-0	Reservoir Water	0	2[c]	1	1	1	1
		ReW-25		25	3[c]	1	0	0	0
		RiW-0	River Water	0	2[c]	1	1	1	1
		RiW-25		25	3[c]	1	0	0	0
		SW-0	Seawater	0	1	1	1	1	1
		SW-25		25	3	1	0	0	0
		SW-50		50	3	1	0	0	0
4	Wastewater Effluent Water Samples[d]	MWWE#1-0	Municipal Wastewater Effluent #1[e]	0	2[c]	1	1	1	1
		MWWE#1-25		25	3	1	0	0	0
		MWWE#2-0	Municipal Wastewater Effluent #2[f]	0	2[c]	1	1	1	1
		MWWE#2-25		25	3	1	0	0	0
		MFWWE-0	Metal Finishing Wastewater Effluent[g]	0	1	1	1	1	1
		MFWWE-25		25	3	1	0	0	0

a. Does not include all required QA samples. Required QA samples are discussed in Section B1.
b. Three samples analyzed by Lead100/AND1000 with special pretreatment procedure for the removal of the effects of Fe interference and three additional samples analyzed by Lead100/AND1000 without the special pretreatment procedure.
c. Two sets of samples analyzed by Lead100/AND1000 each in the field and at ETL.

d. All Wastewater Effluent Samples diluted 1:10 in DI water and filtered through a 0.20 µm Nylon filter before addition of Pb spike.
e. Obtained from Jackson Pike Wastewater Treatment Plant (Columbus, OH).
f. Obtained from Southerly Wastewater Treatment Plant (Columbus, OH).
g. Provided by vendor from 40 CFR 433/413 facility after all on-site treatment, properly labeled and preserved sample bottle provided by DHL Analytical and properly stored until they are sent for Pb Reference Analysis.

ICC, DLOD, DLR tests. If recovery is outside this range, then TPC will be stored in AND1000 as per vendor provided literature. In any case, results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the TPC samples will be filtered through a 0.20 µm Nylon syringe filter into a properly labeled and preserved sample bottles provided by DHL Analytical and properly stored until they are sent for Pb Reference Analysis.

B1.1.8.3 ICC Testing

ICC testing will be carried out at ETL. ICC-25 will be prepared by spiking DI water to 25 ppb Pb before it is analyzed in triplicate by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the ICC-25 sample will be filtered through a 0.20 µm Nylon syringe filter into a properly labeled and preserved sample bottle provided by DHL Analytical and properly stored until they are sent for Pb Reference Analysis.

B1.1.8.4 DLOD Testing

DLOD testing will be carried out at ETL. DLOD-10 will be prepared by spiking DI water to 10 ppb Pb before it is analyzed in septuplet by the Lead100/AND1000 per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the DLOD-10 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and properly stored until it is sent for Pb Reference Analysis. Note that the reported LOD is applicable only for the matrix under investigation and does not apply to the other matrices evaluated in this QAPP.

B1.1.8.5 DLR Testing

DLR testing will be carried out at ETL. DLR-5 will be prepared by spiking DI water to 5 ppb Pb. Similarly, DLR-15, DLR-25, DLR-50, DLR-75 and DLR-100 will be prepared by spiking DI water to 15 ppb Pb, 25 ppb Pb, 50 ppb Pb, 75 ppb and 100 ppb Pb, respectively. Each of the DLR samples will be analyzed in triplicate by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the DLR samples will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and properly stored until they are sent for Pb Reference Analysis.

B1.1.8.6 DEI Testing

DEI testing includes testing the accuracy and precision of Lead100/AND1000 on three different synthetic waters (High TDS Water, Low TDS Water and High Fe Water). The methods for preparing these waters can be found in Section B1.1.7.

DEI testing on High TDS Water will be carried out at ETL. HTDS-25 and HTDS-50 will be prepared by spiking High TDS Water to 25 ppb Pb and 50 ppb Pb, respectively, before they are analyzed in triplicate by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the HTDS-25 and HTDS-50 samples will be filtered through a 0.20 µm Nylon filter into two separate properly labeled and preserved sample bottles provided by DHL Analytical and stored at 4±2°C until they are sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity Reference Analysis.

DEI testing on Low TDS Water will be carried out at ETL. LTDS-25 and LTDS-50 will be prepared by spiking Low TDS Water to 25 ppb Pb and 50 ppb Pb, respectively before they are analyzed in triplicate by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the LTDS-25 and LTDS-50 samples will be filtered through a 0.20 µm Nylon filter into two separate properly labeled and preserved sample bottles provided by DHL Analytical and stored at 4±2°C until they are sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity Reference Analysis.

DEI testing on High Fe Water will be carried out at ETL. HFe-25 and HFe-50 will be prepared by spiking High Fe Water to 25 ppb Pb and 50 ppb, respectively, before they are analyzed in triplicate each by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Two sets of triplicate experiments will be carried out: one using a special vendor-recommended pretreatment method for removal of Fe interference and one using the standard pretreatment method. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the HFe-25 and HFe-50 samples will be filtered through a 0.20 µm Nylon filter into two separate properly labeled and preserved sample bottles provided by DHL Analytical and stored at 4±2°C until they are sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity Reference Analysis.

B1.1.8.7 Finished Drinking Water Testing

Finished drinking water testing includes testing the accuracy and precision of the Lead100/AND1000 on three different finished drinking waters (Water Fountain, Bottled Water and Finished Well Water).

Water Fountain will be collected from a water fountain within Battelle. The water fountain will be activated and water will be allowed to flow through the tap for 60 seconds (1-2 L throughput) before samples are collected in a 1 L high-density polyethylene (HDPE) container. Water Fountain will be collected in a manner to avoid sample agitation and entrainment of air. The 1 L sample collection container will be sealed and transported to ETL where it will be subsampled to two 100 mL volumetric flasks. WF-25 will be prepared by spiking Water Fountain to 25 ppb Pb before it is analyzed in triplicate by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the WF-

25 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis. In addition the 100 mL sample without the Pb spike (WF-0) will be analyzed once by the Lead100/AND1000 and the remainder of the sample will be filtered through a 0.20 µm Nylon filter into two separate properly labeled and preserved sample bottles provided by DHL Analytical and stored at 4±2°C until they are sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity Reference Analysis.

Bottled Water is a 1 gal sample of spring water obtained from a local supermarket in Columbus, OH (e.g., Kroger, Giant Eagle). The 1 gal Bottled Water will be transported to ETL where it will be subsampled to two 100 mL volumetric flasks. BW-25 will be prepared by spiking one of the 100 mL samples to 25 ppb Pb before it is analyzed in triplicate by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the BW-25 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis. In addition, the 100 mL sample without the Pb spike (BW-0) will be analyzed once by the Lead100/AND1000 and the remainder of the sample will be filtered through a 0.20 µm Nylon filter into two separate properly labeled and preserved sample bottles provided by DHL Analytical and stored at 4±2°C until they are sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity Reference Analysis.

Finished Well Water will be collected from the effluent sample tap at a small water treatment facility at the Plainview Christian School located in Plain City, OH. The effluent sample tap will be opened and water will be allowed to flow through the tap for 60 seconds (approximately 40 L throughput) before samples are collected in a 1 L HDPE container. Finished Well Water will be collected in a manner to avoid sample agitation and entrainment of air. FWW-0 (Finished Well Water with no Pb spike) will be analyzed onsite once by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the FWW-0 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity Reference Analysis. The remainder of the 1 L sample will be transported to ETL where FWW-0 will be analyzed once by Lead100/AND1000 and FWW-25 will be prepared and analyzed in triplicate. The remainder of the FWW-25 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis. Any differences in results between field and ETL measurements as well as any subjective data concerning ease of use in the field compared to ETL will be noted in the LRB.

B1.1.8.8 Environmental Water Testing

Environmental water testing includes testing the accuracy and precision of Lead100/AND1000 on four different environmental waters (Raw Well Water, Reservoir Water, River Water and Seawater).

Raw Well Water will be collected from the raw water intake tap at a small water treatment facility at the Plainview Christian School located in Plain City, OH; note that this is the same facility from which the Finished Well Water will be collected. The raw water intake will be activated and water will be allowed to flow through the tap for 60 seconds (approximately 40 L throughput) before samples are collected in a 1 L HDPE container. Raw Well Water will be collected in a manner to avoid sample agitation and entrainment of air. RWW-0 (Raw Well Water with no Pb spike) will be analyzed onsite once by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the RWW-0 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity Reference Analysis. The remainder of the 1 L sample will be transported to ETL where RWW-0 will be analyzed once by Lead100/AND1000 and RWW-25 will be prepared and analyzed in triplicate. The remainder of the RWW-25 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis. Any differences in results between field and ETL measurements as well as any subjective data concerning ease of use in the field compared to ETL will be noted in the LRB.

Reservoir Water will be collected from the surface of Grigg's Reservoir on the Scioto River in Columbus, OH (see Figure 2 for sampling location). The sample will be collected by means of a retractable pole with an attached 1 L HDPE sample collection container. The sample pole will be extended to its full length (~20 ft) and a sample will be collected from the surface (no more than 1 ft below water surface) of the reservoir. The sample pole will be retracted and brought to the reservoir shore. ReW-0 (Reservoir Water with no Pb spike) will be analyzed onsite once by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the ReW-0 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity Reference Analysis. The remainder of the 1 L sample will be transported to ETL where ReW-0 will be analyzed once by Lead100/AND1000 and ReW-25 will be prepared and analyzed in triplicate. The remainder of the ReW-25 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis. Any differences in results between field and ETL measurements as well as any subjective data concerning ease of use in the field compared to ETL will be noted in the LRB.

River Water will be collected from the surface of the Scioto River in Columbus, OH (see Figure 2). The sample will be collected in a manner identical to that for the Reservoir Water sample by means of a retractable pole with an attached 1 L HDPE sample collection container. RiW-0 (River Water with no Pb spike) will be analyzed onsite once by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the RiW-0 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity Reference Analysis. The remainder of the 1 L sample will be transported to ETL where RiW-0 will be analyzed once by Lead100/AND1000 and RiW-25 will be prepared and analyzed in triplicate. The remainder of the RiW-25 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis. Any differences in results between field and ETL measurements as well as any subjective data concerning ease of use in the field compared to ETL will be noted in the LRB.

Seawater will be collected in two separate 1 L HDPE sample bottles from the shore of West Palm Beach, FL (see Figure 3 for sampling location) no more than 20 ft from the shoreline and no more than 1 ft depth. Two Seawater samples will be collected for redundancy, however, only one of the samples will be used for testing. The samples will be sealed and placed in a cooler with ice and transported to ETL. Upon receipt, the Seawater sample will be opened and the temperature measured to ensure that the sample remained at 4±2°C during transit. The sample will then be allowed to warm to ambient temperature before further testing. Seawater samples will be diluted 1:10 in DI water and filtered through a 0.20 µm Nylon filter before analysis.

SW-25 and SW-50 will be prepared by spiking Seawater to 25 ppb Pb and 50 ppb Pb, respectively, before they are analyzed in triplicate each by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of both the SW-25 and SW-50 samples will be separately filtered through a 0.20 µm Nylon filter into separate properly labeled and preserved sample bottles provided by DHL Analytical and stored at 4±2°C until they are sent for Pb Reference Analyses. In addition, a 100 mL sample without the Pb spike (SW-0) will be analyzed once by the Lead100/AND1000 and the remainder of the sample will be filtered through a 0.20 µm Nylon filter into two separate properly labeled and preserved sample bottles provided by DHL Analytical and stored at 4±2°C until they are sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity

Reference Analysis. The Lead100/AND1000 analyses of Seawater samples will be considered a qualitative test and will not be subjected to the rigorous statistical analysis of the other samples (see Section B1.2).

B1.1.8.9 Wastewater Effluent Water Samples

Wastewater effluent testing includes testing the accuracy and precision of Lead100/AND1000 on three different wastewater effluent waters (Municipal Wastewater Effluent #1, Municipal Wastewater Effluent #2 and Metal Finishing Wastewater Effluent). All three of the wastewater effluent water samples will be diluted 1:10 in DI water and filtered through a 0.20 µm Nylon filter before analysis.

Municipal Wastewater Effluent #1 will be collected from the effluent sampling location tap at Jackson Pike (see Figure 2 for facility location). Municipal Wastewater Effluent #1 will be collected in a 1 L HDPE container. A 1 L volumetric flask will be filled halfway with DI water and 100 mL of the Municipal Wastewater Effluent #1 sample will then be pipetted to the volumetric flask; the flask will be filled to the line resulting in a 1:10 dilution of Municipal Wastewater Effluent #1. Note that dilution of Municipal Wastewater Effluent #1 raises the limit of detection from 2 ppb Pb to 20 ppb Pb for these samples. MWWE#1-0 (Municipal Wastewater Effluent with no Pb spike) will be analyzed onsite once by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the MWWE#1-0 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity Reference Analysis. The remainder of the 1 L sample will be transported to ETL where MWWE#1-0 will be analyzed once by Lead100/AND1000 and MWWE#1-25 will be prepared and analyzed in triplicate. The remainder of the MWWE#1-25 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis. Any differences in results between field and ETL measurements as well as any subjective data concerning ease of use in the field compared to ETL will be noted in the LRB.

Municipal Wastewater Effluent #2 will be collected from the effluent sampling location tap at the Southerly Wastewater Treatment Plant in Columbus, OH (see Figure 2). Municipal Wastewater Effluent #2 will be collected in a 1 L HDPE container. A 1 L volumetric flask will be filled halfway with DI water and 100 mL of the Municipal Wastewater Effluent #2 sample will then be pipetted to the volumetric flask; the flask will be filled to the line resulting in a 1:10 dilution of the Municipal Wastewater Effluent #2. Note that dilution of Municipal Wastewater Effluent #2 raises the limit of detection from 2 ppb Pb to 20 ppb Pb for these samples. MWWE#2-0 (Municipal Wastewater Effluent with no Pb spike) will be analyzed onsite once by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the MWWE#2-0 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity Reference Analysis. The remainder of the 1 L sample will be transported to ETL where MWWE#2-0 will be analyzed once by Lead100/AND1000 and MWWE#2-25 will be prepared and analyzed in triplicate. The remainder of the MWWE#2-25 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis. Any differences in results between field and ETL measurements as well as any subjective data concerning ease of use in the field compared to ETL will be noted in the LRB.

Metal Finishing Wastewater Effluent will be collected by the vendor from a metal finishing facility conforming to 40 CFR 433 and/or 40 CFR413 in a 1 L HDPE sample collection bottle and sent on ice to ETL. The method of sampling will be documented by the vendor and provided to Battelle. Upon arrival, the sample bottle will be opened and the temperature of the sample will be confirmed. MFWWE-25 will be prepared by spiking Metal Finishing Wastewater Effluent to 25 ppb Pb before it is analyzed in triplicate by the Lead100/AND1000 as per vendor-provided manuals and guidance given during training. Results obtained from the

Lead100/AND1000 analysis will be promptly recorded in the LRB. The remainder of the MFWWE-25 sample will be filtered through a 0.20 µm Nylon filter into a properly labeled and preserved sample bottle provided by DHL Analytical and stored at 4±2°C until it is sent for Pb Reference Analysis. In addition a sample of Metal Finishing Wastewater Effluent without the Pb spike (MFWWE-0) will be analyzed twice (once with a pretreatment method for the removal of Fe interference and once without) by the Lead100/AND1000 and the remainder of the sample will be filtered through a 0.20 µm Nylon filter into two separate properly labeled and preserved sample bottles provided by DHL Analytical and stored at 4±2°C until they are sent for Pb Reference Analysis, Cation Reference Analysis, Anion Reference Analysis and Alkalinity Reference Analysis.

B1.2 Statistical Analysis

Where appropriate, the following statistical analyses will be carried out on results from both the Lead100/AND1000 and the reference method. In all cases, appropriate statistical analyses will be reported for each analysis and the results of those statistical analyses will be used to quantitatively determine the performance of Lead100/AND1000 under different analysis scenarios. The only exception to this are the Seawater-derived samples (i.e., SW-25 and SW-50), the results of analyses of which are considered qualitative.

B1.2.1 Accuracy

Accuracy of the Lead100/AND1000 will be assessed by comparing Pb values obtained from the Lead100/AND1000 (Pb_{AND}) and those reported by the Pb Reference Analysis (Pb_{REF}) on the same samples. The relative percent difference (RPD) between the two measurements will serve as a quantitative measure of the accuracy of the Lead100/AND1000 as detailed in Equation 1.

Equation 1:

$$RPD = \frac{|Pb_{AND} - Pb_{REF}|}{Pb_{REF}}$$

RPD will be reported for all Lead100/AND1000 and Pb reference measurement pairs of data and will be summarized by an average value, however, results from Seawater will be reported qualitatively as high, medium or low Pb concentrations.

RPD will also be calculated between the LFM and LFMD when determining whether to accept an on-site calibration according to the control charts. RPD as defined by the equation below provides a measure of the agreement between the LFM and LFMD.

$$RPD = \frac{|Pb_{LFM} - Pb_{LFMD}|}{(Pb_{LFM} + Pb_{LFMD})/2} * 100$$

Percent recovery will also be calculated for the LFM and LFMD when determining whether to accept an on-site calibration according to the control charts. Percent recovery is calculated as shown below for the LFM.

$$Percent\ Recovery\ (LFM) = \frac{(Pb_{LFM} - s * Pb_0)}{25} * 100$$

Where s is a dilution correction, Pb_{LFM} is the ANDalyze meter reading for the LFM and Pb_0 is the ANDalyze meter reading for the un-spiked sample (if the reading is “Below Limit” a value of 0 ppb will be used). The LFM spike is 25 ppb Pb,

B1.2.2 Precision

Precision of the Lead100/AND1000 will be assessed by comparing the spread of Pb concentration data obtained by Lead100/AND1000 on triplicate samples. Precision will be expressed quantitatively through the standard deviation (SD) and the coefficient of variation (CV).

The SD of triplicate samples S_1, S_2 and S_3 will be computed as expressed in Equation 2.

Equation 2:

$$SD = \sqrt{\frac{1}{3}\sum_{i=1}^{3}(S_i - \mu)^2}$$

where μ is the mean value of the three samples expressed in Equation 3.

Equation 3:

$$\mu = \frac{1}{3}\sum_{i=1}^{3} S_i$$

The CV of triplicate samples S_1, S_2 and S_3 will be computed as expressed in Equation 4.

Equation 4:

$$CV = \frac{SD}{\mu}$$

The mean, SD and CV will be reported for all triplicate samples analyzed by Lead100/AND1000.

B1.2.3 Linearity of Response

During the DLR testing, a series of samples with known concentrations of Pb will be analyzed in triplicate by Lead100/AND1000 (0 ppb, 5 ppb, 15 ppb, 25 ppb, 50 ppb, 75 ppb and 100 ppb). In addition to the accuracy and precision of the instrument for these tests, linearity shall be assessed by linear regression, with the analyte concentration measured by the reference method as the independent variable and the reading from Lead100/AND1000 as the dependent variable. Linearity is expressed in terms of slope, intercept and the coefficient of determination (r^2) as calculated by Microsoft® Excel standard computation tools.

B1.2.4 Limit of Detection

The LOD for the Lead100/AND1000 shall be assessed from seven replicate analyses of a fortified sample with an analyte concentration of five times the vendor's estimated detection limit. In the case of the Lead100/AND1000, the vendor's estimated detection limit is 2 ppb. Thus, the LOD tests will be carried out on 2 ppb Pb * 5 = 10 ppb Pb samples as described in Section B1.1.8.4. The LOD is calculated from Equation 5.

Equation 5:

$$LOD = t * SD$$

where t is the Student's t-test value for a 99% confidence interval and SD is the standard deviation of the replicate samples.

B1.3 Reporting

The data obtained in the verification test will be reported and the statistical analyses described above will be conducted separately for each analysis. Information on the performance parameters will be compiled, and a verification test report will be prepared. The report will show verification results from the Lead100/AND1000, the test procedures and test data, as well as the results of the statistical evaluation of those data.

All actions taken on the technology (such as maintenance, cleaning, and calibration) will be documented at the time of the test and reported. In addition, descriptions of the data-recording procedures, use of vendor-supplied software, and water samples or other consumables used will be presented in the report. The verification test report will briefly describe the ETV program, the AMS Center, and the procedures used in verification testing. The results of the verification test will then be stated quantitatively (or in the case of Seawater analysis, qualitatively) without comparison to any other technology or comment on the acceptability of the technology performance.

B2 SAMPLING METHODS REQUIREMENTS

B2.1 Sample Collection, Storage and Shipment

All samples will be collected in 1 L HDPE sample containers. If samples are to be transported over a period of more than 12 hours, they will be placed in a cooler on ice, or by some other means maintained at 0 to 4±2°C until they are received at their final destination. Before samples are subsampled, analyzed or in any way manipulated (with the exception of confirming the sample temperature upon receipt), they will be allowed to equilibrate with ambient conditions until they are within 17 to 30°C. In all cases, laboratory testing will be carried out at 20-25 °C If ambient conditions do not allow for the samples to reach within this temperature range, the samples will be placed in an artificial environment (e.g., laboratory, vehicle) until equilibration within this temperature range has been achieved. In no case will any samples be analyzed by Lead100/AND1000 if the sample temperature is unknown or known to be outside of the 17 to 30°C range.

B3 SAMPLE HANDLING AND CUSTODY REQUIREMENTS

Each sample will be labeled with a unique sample identifier along with the date/time collected and the name of the sample collector. Sample custody will be documented throughout collection and analysis of the test samples following the Battelle Standard Operating Procedure (SOP) for chain of custody[6]. A chain-of-custody (COC) form will include details about the sample such as the time, date, location, and person collecting the sample. The COC form will track sample release from the sampling location to the analysis laboratory. Each COC form will be signed by the person relinquishing samples once that person has verified that the COC form is accurate. Upon arrival at the analytical laboratory, COC forms will be signed by the person receiving the samples (if different from the sample collector) once that person has verified that all samples identified on the COC forms are present. Copies of all COC forms will be delivered to the VTC and maintained with the test records.

B4 ANALYTICAL METHODS REQUIREMENTS

All samples analyzed by the Lead100/AND1000 will also be analyzed by a reference method to determine the accuracy of the Lead100/AND1000 in recovering Pb spikes. Pb will be measured by ICP-MS according to EPA Method 200.8 (i.e., Pb Reference Method). In addition, each of the laboratory, environmental, waste and drinking water effluent samples will be analyzed once for cations according to EPA Method 200.8 (i.e., Cation Reference Method), major anions according to EPA Method 300.1 (i.e., Anion Reference Method) and alkalinity (including total, carbonate, bicarbonate and hydroxide alkalinity) according to Standard Method 2320B (i.e., Alkalinity Reference Method). Samples to be analyzed by Pb Reference Method and Cation Reference Method will be preserved with (1+1) HNO_3 top pH<2, samples to be analyzed by the Anion Reference Method do not require chemical preservation. Samples will be sent at 4±2°C to DHL Analytical (Round Rock, TX) for analysis within the hold times specified by the reference methods. Data for the reference method will be accompanied by a QC narrative, the results of initial and on-going calibration, quality control results, and a statement that data have been independently reviewed for compliance with the reference methods.

The Lead100/AND1000 will be operated exactly as specified in the vendor-provided “AND1000 Fluorimeter for Water Testing User Manual” (AND-prod-1000-2-2012) and the “Lead Testing and On-Site Calibration for Water Testing” (AND-Lead-100-02-2012). Any deviation in procedure from these documents as recommended by the vendor will be explicitly noted in the LRB for this verification test.

B5 QUALITY CONTROL

B5.1 OVERVIEW AND REQUIREMENTS

Steps will be taken to maintain the quality of data collected during this verification test. QC samples are incorporated into the sampling and analysis design to assess the quality of the method of assessment.

Prepared QC samples include both laboratory reagent blanks (RB) and laboratory-fortified matrix (LFM) samples. The RB samples are prepared from DI water and exposed to identical handling and analysis procedures as other prepared samples, including the addition of all reagents. These samples are used to help ensure that no sources of contamination are introduced in the sample handling and analysis procedures. Acceptance criteria for RB are discussed in the next section.

The LFM and LFMD samples are prepared as aliquots of environmental samples and spiked in the field to increase the Pb concentration of the samples to 25 ppb. The Pb Standard Solution used to prepare the LFM is prepared in the laboratory and brought to the field site. These samples are used to help identify whether matrix effects have any influence on the analytical results. At least 10% of all the prepared samples to be analyzed will be RBs, and at least two samples taken from each sampling site will be LFM and LFMD. The following samples satisfy the LFM requirements for field-collected samples: WF-25, BW-25, FWW-25, RWW-25, ReW-25, RiW-25, SW-25, MWWE#1-25, MWWE#2-25, and MFWWE-25. Acceptance criteria for LFM and LFMD are discussed in the next section.

Quality control standards (QCS) are used as a calibration check to verify that Lead100/AND1000 and the reference instruments are properly calibrated and reading within defined control limits. QCS is defined as 30 ppb Pb. These standards will be purchased from Fisher Scientific and will be subject only to dilution. The calibration of all instruments will be verified using a QCS before and after each testing day, as well as after every tenth sample. In addition, instruments and equipment used for this verification will operate at the expected ranges and calibration records will be verified and kept for all monitoring instruments and equipment used during this verification test.

B5.2 Acceptance Criteria and Root Cause Analyses

Acceptance criteria for QC samples vary depending on the sample(s) being analyzed. For each set of samples a root cause analysis may be required before testing begins (see Appendices F, G and H for root cause analysis flow-charts).

For IDC, an initial on-site calibration must be passed before LFM and LFMD samples are analyzed. If on-site calibration is not passed the first time it will be repeated and if a second failure occurs a root cause analysis will be performed and the Vendor will be contacted with the results of the analysis. If on-site calibration is passed either during the first or (if necessary) the second attempt, LFM and LFMD samples will be analyzed. The acceptance criteria for the LFM and LFMD are recovery of the Pb spike of 75-125% and an RPD of less than 30%. If these criteria are not met on-site calibration will be repeated. Only when LFM and LFMD criteria are met will analysis of the samples be initiated (see Appendix F for root cause analysis flowchart).

For ICC and TPC, an initial on-site calibration must be passed before LFM and LFMD samples are analyzed. If on-site calibration is not passed the first time it will be repeated and if a second failure occurs a root cause analysis will be performed and the Vendor will be contacted with the results of the analysis. If on-site calibration is passed either during the first or (if necessary) the second attempt, LFM and LFMD samples will be analyzed. The acceptance criteria for the LFM and LFMD are recovery of the Pb spike of 85-115% and a standard deviation of ± 15% of the expected Pb value. If these criteria are not met on-site calibration will be repeated. Only when LFM and LFMD criteria are met will analysis of the samples be initiated (see Appendix G for root cause analysis flowchart).

For DEI, finished drinking water, environmental waters and wastewater effluents an initial on-site calibration must be passed before LFM and LFMD samples are analyzed. If on-site calibration is not passed the first time it will be repeated and if a second failure occurs a root cause analysis will be performed and the Vendor will be contacted with the results of the analysis as well as information regarding the samples (e.g., pH, color, turbidity and conductivity). If on-site calibration is passed either during the first or (if necessary) the second attempt, LFM and LFMD samples will be analyzed. The acceptance criteria for the LFM and LFMD are recovery of the Pb spike of 75-125% and RPD of less than 30%. If these criteria are not met on-site calibration will be repeated. Only when LFM and LFMD criteria are met will analysis of the samples be initiated (see Appendix H for root cause analysis flowcharts).

Acceptance criteria for RB are set at less than the vendor-reported method detection limit of 2 ppb Pb making the method detection limit and reporting limit identical. If Lead100/AND1000 reports values of Pb equal to or greater than 2 ppb Pb, a second RB will be analyzed and if Lead100/AND1000 indicates a value equal to or greater than 2 ppb Pb, on-site calibration will be repeated before analysis is continued. A value of less than 2 ppb Pb is indicated by the AND1000 displaying the message “*BELOW LIMIT*” while values measured equal to or greater than 2 ppb Pb is indicated by AND1000 as a quantitative result.

Acceptance criteria for QCS are set at ±25% of the expected Pb concentration (i.e., 30 ppb Pb) in QCS samples for both Lead100/AND1000 and Reference Methods.

In the case that a root cause analysis must be completed, the laboratory analyst(s) will perform a root cause analysis. The analysis will have at a minimum the following areas described in detail:

1. Identify the problem: Identify the QC failure. Include instrument, reagent, sampling, personnel and any other problems.
2. Investigate to identify the root cause: Determine how each problem identified interacted with each other to create the QC problem.
3. Come up with the solution: Develop an encompassing solution to address all problems that created the QC failure. This may require contacting ANDalyze
4. Implement the solution: Develop an implementation plan that includes all components of the developed solution and have laboratory management implement it.
5. Document the solution: Document all corrective action steps taken under laboratory management implementation of the corrective action.
6. Communicate the solution: Develop training and management programs to communicate and evaluate all personnel included in the corrective action solution.
7. Evaluate the effectiveness of the solution: Document QC results in trend charts and laboratory staff performance to validate corrective action solution.

The following root cause analysis QC identifiers will be used:

IFM	Instrument Failure Mechanical
IFE	Instrument Failure Electrical
IOFM	Instrument Operator Failure to Follow Method
SFC	Sensor Failure Chemical
SFM	Sensor Failure Mechanical
FCU	Failure Cause Unknown

B5.3 Control Charts

Control charts will be maintained throughout the entire verification testing process as per Standard Methods Section 1020 B[7]. Control limits will be calculated after the first five samples and after every ten samples thereafter. If analysis of the control charts indicate non-conformance as described in Standard Methods Section 1020 B, the vendor will be contacted to determine the cause of the problem, which may include a root cause analysis.

B6 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

The instruments used during the verification test will be inspected and maintained according to the instrument manuals or the laboratory standard operating procedures of DHL Analytical. Operation of the Lead100/AND1000 during the verification test will be performed by Battelle technical staff as directed by the vendor user manuals and during on-site training.

B7 CALIBRATION/VERIFICATION OF TEST PROCEDURES

The instruments used during the verification test (i.e., Lead100/AND1000 and reference instruments) will be calibrated per the instrument manual, the methods being used to make each measurement, or the SOPs of the analysis laboratory. For each measurement, the equipment calibration will be verified. Calibration procedures, checks, and results will be documented in the project files. Testing will not occur until instrument calibration results meet the acceptance criteria as defined in the root cause analyses (see Appendices F-H).

All calibrations performed will be documented by the verification staff in the project LRB. The Lead100/AND1000 technology vendor will provide the Battelle verification staff with the necessary training/information to properly calibrate and maintain Lead100/AND1000. Calibration of Lead100/AND1000 will be done as often as indicated in the Lead100/AND1000 user manual or as suggested by the vendors. Vendors will be required to describe the necessary calibration procedures specific to Lead100/AND1000.

B8 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

All materials, supplies, and consumables used to establish the test conditions will be ordered by the VTC or designee. Where possible, Battelle will rely on sources of materials and consumables that have been used previously as part of ETV verification testing without problems.

Supplies must meet the following criteria:

- Solvent and reagent grades are based on the intended use. All reagents will be of >96% purity (see Table 6)
- Equipment used to generate data must provide appropriate sensitivity.
- A certificate of analysis must be provided and retained for reagents and standards.
- The quality and purity of expendable materials must be documented and adequate to meet the DQOs of the client.

B9 NON-DIRECT MEASUREMENTS

No non-direct measurements will be used during this verification test.

B10 DATA MANAGEMENT

Various types of data will be acquired and recorded electronically or manually by verification staff during this verification test. All data and observations for the operation of the Lead100/AND1000 will be documented by the vendors or verification staff in LRBs, data forms or captured electronically. Results from the laboratory analytical instruments will be compiled by laboratory staff in electronic format and submitted to the VTC or other verification staff upon obtaining results. Hand-transcribed data must be 100% verified by a second person.

Records received or generated by any of the verification staff during the verification test will be reviewed by the VTC or designee within two weeks of receipt or generation, respectively, before the records are used to calculate, evaluate, or report verification results. The review will be documented as the dated initials of the reviewer. If a Battelle staff member generated the record, 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 the hard copy of the record being reviewed. In addition, data calculations performed by verification staff will be spot-checked by Battelle technical staff to ensure that calculations are performed correctly. Calculations to be checked include any statistical calculations described in this QAPP. The data obtained from this verification test will be compiled and reported.

All electronic testing records and documents will be stored on a test-specific networked ETV SharePoint site and common drive within Battelle's network. Testing data will be uploaded to the SharePoint Site within two days of receiving said data. This site is within the protected Battelle network and is backed up regularly. The goal of this data delivery schedule is prompt identification and resolution of any data collection or recording issues.

In addition, once testing is complete, all testing records and documents are sent to Battelle's RMO for archival within two months of project close-out.

SECTION C

ASSESSMENT AND OVERSIGHT

C1 ASSESSMENTS AND RESPONSE ACTIONS

Every effort will be made in this verification test to anticipate and resolve potential problems before the quality of performance is compromised. One of the major objectives of this QAPP is to establish mechanisms necessary to ensure this. Internal quality control measures described in this QAPP will give information on data quality on a daily basis. The responsibility for interpreting the results of these checks and resolving any potential problems resides with the VTC, who will contact the Battelle AMS Center Manager, Battelle AMS Center QM, EPA AMS Center Project Officer, and EPA AMS Center QM if any deviations from the QAPP are observed. The VTC will describe the deviation in a teleconference or by email, and once a path forward is determined and agreed upon with EPA, the deviation and corrective action will be documented in writing. Technical staff have the responsibility to identify problems that could affect data quality or the ability to use the data. Any problems that are identified will be reported to the VTC. Technical staff and the VTC will work with the Battelle QM to resolve any issues. Action will be taken by the VTC and Battelle testing staff to identify and appropriately address the issue, and minimize losses and correct data, where possible. Independent of any EPA QA activities, Battelle will be responsible for ensuring that the following audits are conducted as part of these verification tests.

C1.1 Performance Evaluation Audit

Performance evaluation (PE) audits for the Lead100/AND1000 will be conducted by having two analysts independently take triplicate measurements for a 25 ppb Pb standard (in DI water). To be considered acceptable, the average of the two analysts’ results should agree within 20% and CV of each analysts triplicate measurements should be no more than 20% .

C1.2 Technical Systems Audits

The Battelle QM or designee will perform a TSA during performance evaluation activities. The purpose of these audits is to ensure that the verification tests are being performed in accordance with the AMS Center QMP[1] and this QAPP. The Battelle QM will compare actual test procedures to those specified or referenced in this plan, and review data acquisition and handling procedures. The Battelle QM or designee will prepare a project-specific checklist based on the QAPP requirements to guide the TSA, which will include a review of the test location and general testing conditions; observe the testing activities; and review test documentation. She will also check data acquisition procedures, and confer with Battelle staff. The Battelle QM will prepare an initial TSA report and will submit the report to the EPA QA Manager (with no corrective actions documented) and VTC within 10 business days after completion of the audit. A copy of each final TSA report (with corrective actions documented) will be provided to the EPA AMS Center Project Officer and QM within 20 business days of completion of the audit. At EPA's discretion, EPA QA staff may also conduct an independent on-site TSA during the verification tests. The TSA findings will be communicated to technical staff at the time of the audit and documented in the TSA reports.

C1.3 Data Quality Audits

The Battelle QM, or designee, will audit at least 10% of the sample results data acquired in the verification tests and 100% of the calibration and QC data versus the QAPP requirements. Two ADQs will be conducted for this project: Data collected on the first day of testing will be audited within 10 business days of receipt and assessed using a project-specific checklist. The remaining data will be audited at the conclusion of testing and will be completed within 10 business days of receipt of all test data. During these audits, the Battelle QM, or designee, will trace the data from initial acquisition (as received from the vendor's technology), through reduction and statistical comparisons, to final reporting. All calculations performed on the data undergoing the audit of data quality (ADQ) will be checked. Data must undergo a 100% validation and verification by technical staff (i.e., VTC, or designee) before it will be assessed as part of the data quality audit. All QC data and all calculations performed on the data undergoing the audit will be checked by the Battelle QM or designee. Results of each ADQ will be documented using the checklist and reported to the VTC and EPA within 10 business days after completion of the audit. A final ADQ that assesses overall data quality, including accuracy and completeness of the technical report, will be prepared as a narrative and distributed to the VTC and EPA within 10 business days of completion of the audit.

C1.4 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 all audits will be submitted to EPA within 10 business days as noted above. Assessment reports will include the following:

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

C2 REPORTS TO MANAGEMENT

During the laboratory evaluation, any QAPP deviations will be reported immediately to EPA. The Battelle QM and/or VTC, during the course of any assessment or audit, will identify to the technical staff performing experimental activities any immediate corrective action that should be taken. A summary of the required assessments and audits, including a listing of responsibilities and reporting timeframes, is included in Table 13. If serious quality problems exist, the Battelle QM will notify the AMS Center Manager, who is authorized to stop work. Once the assessment reports have been prepared, the VTC 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 QM will ensure that follow-up corrective action has been taken. The QAPP and final report are reviewed by the EPA AMS Center QM and the EPA AMS Center Project Officer. Upon final review and approval, both documents will then be posted on the ETV website (www.epa.gov/etv).

Table 13. Summary of Quality Assessment and Control Reports[1]

Assessment	Prepared By	Report Submission Timeframe	Submitted To
Lead100/AND1000 TSA (within the first week of testing)	Battelle	10 business days after TSA is complete TSA response is due to QM within 10 business days TSA responses will be verified by the QM and provided to EPA within 20 business days	EPA ETV AMS Center
ADQ (Day 1 data)	Battelle	ADQ will be completed within 10 business days after receipt of first data set	EPA ETV AMS Center
ADQ (Remaining data and verification report)	Battelle	ADQ will be completed within 10 business days after completion of the verification report review	EPA ETV AMS Center

[1] Any QA checklists prepared to guide audits will be provided with the audit report.

SECTION D

DATA VALIDATION AND USABILITY

D1 DATA REVIEW, VALIDATION, AND VERIFICATION REQUIREMENTS

The key data review requirements for the verification test are the collection of QC samples as outlined in the QAPP, a comparison of raw data sheets and comments against final data to flag any suspect data, and a review of final data to resolve any questions about apparent outliers. The QA audits, as described within this document, are designed to ensure the quality of these data. The key data verification requirements for this test are stated in Section C1 of this QAPP. The data generated during this test will be reviewed by a Battelle technical staff member within two weeks of receipt or generation of the data. The reviewer will be familiar with the technical aspects of the verification test but will not be the person who generated the data. This process will serve both as the data review and the data verification and will ensure that the data have been recorded, transmitted, and processed properly. Furthermore, this process will ensure that the monitoring systems data were collected under appropriate testing conditions.

D2 VALIDATION AND VERIFICATION METHODS

Data verification is conducted as part of the data review as described in Section C1 of this QAPP. A visual inspection of handwritten data will be conducted to ensure that all entries were properly recorded or transcribed and any erroneous entries were properly noted. All calculations used to transform the data will be reviewed to ensure the accuracy and the appropriateness of the calculations. Calculations performed manually will be reviewed and repeated using a handheld calculator or commercial software (e.g., Microsoft® Excel). Calculations performed using standard commercial office software (e.g., Microsoft® Excel) will be reviewed by inspection of the equations used for the calculations and verification of selected calculations by a handheld calculator. Calculations performed using specialized commercial software (i.e., for analytical instrumentation) will be reviewed by inspection and, when feasible, verified by a handheld calculator or standard commercial office software.

To ensure that the data generated from this test meet the goals of the test, a number of data validation procedures will be performed. Sections B and C of this QAPP provide a description of the validation safeguards employed for this verification test. Data validation efforts include the completion of QC activities and the performance of a TSA as described in Section C. The data from this test will be evaluated relative to the measurements to ensure that the DQOs are met. Data failing to meet these criteria will be flagged in the data set and not used for evaluation of the technologies, unless these deviations are accompanied by descriptions of their potential impacts on the data quality.

An ADQ will be conducted by the Battelle QAO to ensure that data review, verification, and validation procedures were completed and to ensure the overall quality of the data.

D3 RECONCILIATION WITH USER REQUIREMENTS

This QAPP and the resulting ETV verification report will be subjected to review by the vendor, EPA, and expert peer reviewers. These reviews will ensure that this QAPP and the resulting verification report meet the needs of potential users of the Lead100/AND1000. Performance data for the Lead100/AND1000, collected under conditions where the QC requirements for the replicate and PEA samples were met, will be presented in the final verification report without any further comment. Performance data and variable measurements that do not meet these criteria will be noted, and a discussion of the possible impact of the failed requirements on the performance evaluation will be presented in the final verification report. The final verification report will be submitted to EPA in Word (DOC) and Adobe portable document format (PDF) and subsequently posted on the ETV Web site (www.epa.gov/etv).

SECTION E

REFERENCES

E1 REFERENCES

1. *Quality Management Plan for the ETV Advanced Monitoring Systems Center, Version 8.* U.S. Environmental Technology Verification Program, Battelle, April 2011.
2. *Environmental Technology Verification Program Quality Management Plan.* January, 2008(EPA/600/R-08/009).
3. EPA. 2012. Lead in Drinking Water. Available at: http://water.epa.gov/drink/info/lead/index.cfm.
4. World Health Organization. 2012. Water Sanitation Health. Available at: http://www.who.int/water_sanitation_health/diseases/lead/en/.
5. Touring Ohio Magazine. 2012. Scioto River. Available at: http://www.touring-ohio.com/central/columbus/scioto-river.html.
6. *SOP ENV-ADM-009, Standard Operating Procedure for Sample Chain-of-Custody* Battelle, September 2007.
7. *Standard Methods for the Examination of Water and Wastewater, 22nd Edition*, American Public Heath Association/American Water Works Association, Rice, E.W., Baird, R.B., Eaton, A.D. and Clesceri, L.S. (ed.)

Appendix A

AND1000 Fluorimeter for Water Testing (AND-Prod-1000-2-2012)

ANDalyze

Document: AND-Prod-1000-2-2012

AND1000 Fluorimeter for Water Testing

User Manual

February 3, 2012   Edition 1

Table of Contents

2 Safety Information

Please read this entire manual before unpacking, setting up or operating this equipment. Pay attention to all danger, warning and caution statements. Failure to do so could result in serious injury to the operator or damage to the equipment. Make sure that the protection provided by this equipment is not impaired, do not use or install this equipment in any manner other than that specified in this manual. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the manufacturer may be impaired.

2.1 Use of Hazard Information

DANGER:

Indicates a potentially or imminently hazardous situation which, if not avoided, could result in death or serious injury.

CAUTION:

Indicates a potentially hazardous situation that may result in minor or moderate injury.

Important:

Indicates a situation which, if not avoided, may cause damage to the instrument. Information that requires special emphasis.

Note:

Information that supplements points in the main text.

2.2 Precautionary Labels

Read all labels and tags attached to the instrument. Personal injury or damage to the instrument could occur if not observed. A symbol, if noted on the instrument, will be included with a danger or caution statement in the manual. This symbol, if noted on the instrument, references the instruction manual for operation and/or safety information. Electrical equipment marked with this symbol may not be disposed of in European public disposal systems after 12 August of 2005. In conformity with European local and national regulations (EU Directive 2002/96/EC), European electrical equipment users must now return old or end-of life equipment to the manufacturer for disposal at no charge to the user.

Note: For return for recycling, please contact the equipment producer or supplier for instructions on how to return end-of-life equipment, producer-supplied electrical accessories and all auxiliary items for proper disposal.

2.3 Chemical and Biological Safety

DANGER: Potential Chemical/ Biological Exposure Hazards. Handling chemical samples, standards and reagents can be dangerous. Users of this product are advised to familiarize themselves with safety procedures and the correct use of chemicals, and to carefully read all relevant Material Safety Data Sheets.

- Normal operation of this instrument may involve the use of hazardous chemicals or biologically harmful samples.

- The user must observe all cautionary information printed on the original solution containers and safety data sheet prior to their use.

- All waste solutions must be disposed in accordance with local and national law.

- The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

3 General Information

3.1 Overview of Product

The AND1000™ Fluorimeter enables field testing to be done in two short steps, eliminating or significantly reducing test expenditures, complexity and wait times experienced in traditional heavy metals water testing. The Fluorimeter uses an extremely sensitive measurement technique to determine the levels of heavy metal contaminants. ANDalyze's proprietary Catalytic DNA sensors use a DNAzyme reaction that fluoresces in the presence of a target contaminant substance such as lead. The fluorescence of the reaction is measured to determine the concentration of the target heavy metal and is reported in parts per billion (ppb). Using the product to test for metals is a simple, quantitative test that allows for detection at or below US EPA standards in drinking water. The test is performed by taking a 1 milliliter water sample, injecting it through the sensor, and into the AND1000 fluorimeter. This sample is then automatically analyzed and reports results in less than two minutes.

3.2 General Features

- Fluorescence Based Sensing - Reaction produces quantitative fluorescence based results.
- Sensor Kit - Each disposable color-coded sensor is designed for a specific heavy metal test target. Kits provide sampling tubes, syringes and cuvettes.
- Rugged & Convenient Construction - Tough, light weight, impact-resistant shell protects electronics and sensors.
- Key Pad - Quick, simple menu navigation and one button push for sample analysis.
- Data Capture & Reporting - Time and date stamped with sample number. Data downloadable through a USB cable.
- Water Resistant - Enclosure rating - IP54
- Portable - Battery operated and rechargeable through USB cable or traditional adapter.

Note: See full technical specifications in section 9.

3.3 Analyte Sensors

To help differentiate the materials specific to each analyte, the packaging has been individually labeled and the sensor housings and buffer caps have been color coded.

Green – Lead (Pb) **Orange – Uranium (U)** **Blue – Copper (Cu)** **Gray – Mercury (Hg)**

Note: The same (blue) sensors are used for both the high and low ranges of copper testing

3.4 Operating Environment and Storage

Important: The following conditions are necessary to ensure correct instrument operation and accurate results:

- Place the instrument firmly on a flat and even surface during operation.
- Maintain an ambient temperature of 10 to 40 ºC (50 to 104ºF) for proper instrument operation. For the most accurate results and highest precision a range of 20 to 30ºC (68 to 86ºF) should be maintained.
- The relative humidity should be less than 80%; moisture should not condense on the instrument.
- Do not operate or store the instrument in extremely dusty, damp or wet locations.
- Keep the surface of the instrument, the cell compartment and all accessories clean and dry.
- Sensors are stable up to 1 year if stored at <23˚C (74˚F), <50 % R.H. away from direct sunlight. For best results, store in the refrigerator (4˚C/39˚F).
- Buffer solutions are stable up to 6 months if stored at <23 ˚C (74˚F) but if stored in the refrigerator (4˚C/39˚F), can be used for up to 1 year.
- Sensors and buffers must be brought to room temperature before use.

3.5 Water Testing Guidelines

Note: This is a drinking water test kit. If you are testing other matrices such as surface water, ground water or industrial water; contact ANDalyze customer service for additional application notes.

Water Sampling

- For best results use freshly collected sample (unpreserved) for analysis. We recommend that you use the sample within 1 hour (maximum of 2 hours) of collection to minimize any metal loss to the walls of the sample container. This is particularly important for testing trace lead levels.
- Once the sample is mixed with ANDalyze sample buffer, test within 15 minutes.

pH Range

- Tests have shown that environmental samples preserved in acid to a pH < 2 cannot usually be brought to an appropriate pH when mixed with the ANDalyze buffer. These samples must be first neutralized with NaOH to a suitable pH before mixing with ANDalyze buffer. Please contact ANDalyze customer service for instructions related to pre-treatment of highly acidic or highly basic samples.

4 Set-Up and Operating Procedures

4.1 Charging the Battery

Important: Before using the instrument assure that the fluorimeter device is charged.

To Confirm the Battery Level:

1. Turn Power ON by pressing and Holding the ON/OFF button. (See Yellow Arrow on Photo to Right)

2. Once device is initiated, confirm the battery indicator status is green (See Red Arrow on Photo to Right). The battery indicator should not be red (as seen in the photo to right)

-

4.2 Home Testing Screen

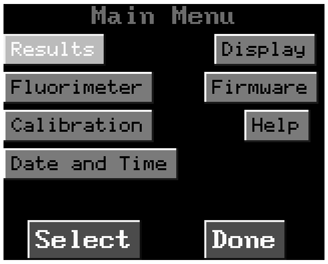
Options can be accessed by:

- Using the **up/down** buttons and pressing **SELECT** button when the arrow is to the left of the desired option.
- OR by pressing the button located just below the screen under the desired options at bottom.

Selectable Options:

- **Metal**
  Selects which metal will be tested.
- **Site**
  Uses site specific calibrations for accurate tests.
- **Start**
  To begin a test of the indicated metal.
- **Menu**
  To change or view internal settings. See below

Metal: Lead
Site: CHM DI
Time: 05/24 14:30



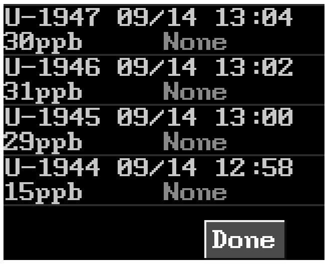
The Menu Screen can be accessed by pressing the button located below the screen under **Menu.**

Selectable Options:

1. **Results**
2. **Fluorimeter**
3. **Calibration**
4. **Date and Time**
5. **Display**
6. **Firmware**
7. **Help**

Hardware Version: 10
Software Version: 10
Contrast : 15
Dark Mode : OFF

-



```
U-1947 09/14 13:04
30ppb       None
U-1946 09/14 13:02
31ppb       None
U-1945 09/14 13:00
29ppb       None
U-1944 09/14 12:58
15ppb       None
```

Display

- Contrast can be changed to adjust to conditions.
- Dark Mode

Fluorimeter

- View and Set parameters for each specific metal.
- Edit values using up/down keys and SELECT.

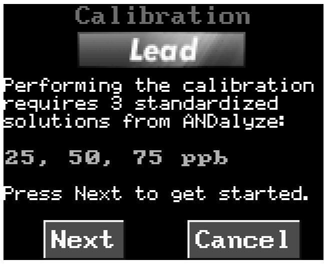
- **Window**: Determines the method of calculating the rate of fluorescence increase over time. We use a moving average method to calculate maximum rate. (If the value of window and samples is same, then the average slope is calculated)

- **Samples**: Determines time up to which data is collected. A value of 100 is approximately 20 seconds. Increase sample value, to increase data collection time. Generally an increase in samples can increase sensitivity especially in matrices such as waste water.

- **Scale**: Slope of the line that correlates the rate of fluorescence increase vs. metal concentration. This value is determined by the 3-point calibration

Calibration

- For laboratory calibration ONLY. Requires (3) standardized solutions from manufacturer.



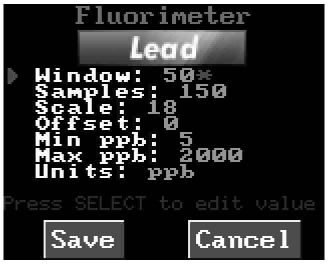
Performing the calibration requires 3 standardized solutions from ANDalyze:

25, 50, 75 ppb

Press Next to get started.

Help

- Displays How-To video showing a sample calibration.



Window: 50*
Samples: 150
Scale: 18
Offset: 0
Min ppb: 5
Max ppb: 2000
Units: ppb

Press SELECT to edit value

- **Offset**: Intercept of the line that correlates the rate of fluorescence increase vs. metal concentration. Currently it is set to be zero

- **Min ppb**: At concentration <min ppb, “Below Limit” is displayed

- **Max ppb**: At concentration >max ppb, “Above Max” is displayed

- **Units**: Determines units for displaying metal concentration. You can change this to ppb, ppm, µM

Firmware

- Displays the current version of software. Can be upgraded through USB online.

Version: 2.0.2

Firmware can be updated over the USB plug.

Date and Time

- Set the date and time to maintain accurate history. Edit using the arrow keys and **Save**

09/14/11
13:56:46

4.4 Firmware Update

ANDalyze is continually adding new features and options to the AND1000 through new firmware updates. To update your AND1000 firmware, please visit andalyze.com and log in to register your device. Once logged in, you will see the option to update your device. Follow the instructions.

4.5 InstraComm Lite

This software allows users to launch and record live sample results, download all sample results from the instrument and perform related configuration tasks. The InstraComm application was developed by Autonomous Innovations, Inc. and a complimentary "Lite" version has been made available with the purchase of ANDalyze's AND1000. To download your complimentary version of InstraComm Lite, please visit andalyze.com and log in to register your device. For additional information and operating instructions see InstraComm Lite Solutions Notes (Available separately).

5 Site Set-Up and Management

This section provides information concerning the set-up and management of Sites. As different sample locations contain different water characteristics (sample matrix) it is recommended to create a new site for each sample area. This allows the user to track and compare the results at each location while also normalizes the results through an on-site calibration.

5.1 New Site Set-Up

When testing water at a new location, the instrument has to be calibrated for accurate readings. Completing the On-Site Calibration requires two separate solutions and tests. At the end of the process, the fluorimeter is calibrated for testing water at that site and also provides the lead test results for this site. A calibration may only be accurate when originally performed. Do not rely on a calibration to be accurate over long time periods as environmental samples can vary greatly.

1. Scroll to the "**Site**" field and press "**Select**".
2. Using the up-down keys, select "**New Site**".
3. Enter the name of the location (8 characters max) and press "**Save**".


Ready to Analyze
Metal: Lead
Site: CHM DI
Time: 05/24 14:30
Press SELECT to Edit


Select Site
None
New Site
Factor: n/a
Press ◀▶ to change action.


New Site
Enter up to 8 characters.
CHM DI

5.2 On Site Calibration

The On-Site calibration process adjusts for changes in sample matrix (such as ionic strength and pH) by calculating the recovery of a known spike in that matrix. When a user performs an on-site calibration for Lead, for example, the fluorimeter software calculates the ppb of lead in the sample (a) and the ppb of lead in sample spiked with 25 ppb lead (b) using the calibration curve from the 3 point calibration (initially completed at factory or in laboratory). It then calculates an accuracy factor based on % recovery. Accuracy factor = 25/b-a. This factor is stored for a particular site that the user enters. The sample concentration displayed after an on-site calibration = a * accuracy factor.

Important: On-Site calibration must be performed if testing is being done:

- At a new location which has not been previously saved.
- To adjust for significant changes in sampling temperature.
- When beginning to use a new lot of sensors.

See the specific Testing and Calibration Manual for detailed instructions.

5.3 Re-Calibrating and Deleting Sites

On the testing screen, scroll to the **SITE** field and press the **SELECT** button on the center of the keypad.

Ready to Analyze

Metal: Lead

Site: None

Time: 05/20 17:02

Press SELECT to Edit

On the Select Site window, Using the up-down keys, scroll to the specific site. Use the right/left keys to change action at the bottom left corner to **CALIB.** Or **DELETE**

Select Site

None
New Site
CHM DI

Factor : 0.174369

Press ◀▶ to change action.

To Re-Calibrate a Saved Site: Select **CALIB.** and press the button below it. Follow instructions for on-site calibration stated above.

Select Site

None
New Site
CHM DI

Factor : 0.174369

Press ◀▶ to change action.

To Delete a Saved Site: Select **DELETE** and press the button below it.

Select Site

None
New Site
CHM DI

Factor : 0.174369

Press ◀▶ to change action.

6 Maintenance

- **CAUTION:** Always disconnect power from the instrument before attempting any cleaning operations.

- **Important:** Under no circumstances should the instrument, display or the accessories be cleaned with solvents such as white spirit, acetone, etc.

Fluorimeter

- Clean the enclosure, sample cell compartments and all accessories with a soft damp cloth. A mild soap solution can also be used. Do not get excess water in the sample cell compartments. Do not insert a brush or sharp object into sample cell compartment to avoid damaging the mechanical components. Dry the cleaned parts carefully with a soft cotton cloth.

Display

- Take care not to scratch the display. Do not touch the screen with ballpoint pens, pencils or similar pointed objects.
- Clean the display with a soft, lint-free and oil-free cotton cloth.
- Diluted window cleaner liquid can also be used.

Sample Chamber

- Splashes or spills on and in the instrument should be cleaned up immediately. Remove any liquid inside the sample chamber by using a non-lint swab. Do not tip the instrument to empty liquid from the sample chamber.

Sensors

- Never reuse sensors! Once the sensor bag is exposed to air, it should be used immediately (within 30 minutes) as exposure to humidity, air and/or heat can affect its effectiveness. Dispose used sensor and all other used consumables such as sample tubes, syringes, plastic pipettes after each analysis.

Pipettes

- The 100µL automatic pipette can be reused as it uses disposable pipette tips. Pipette tips should be disposed of immediately after use.

7 Customer Service Contact Information

Contact us by Email:
info@andalyze.com

By Telephone:
+1 888 388 0818 or +1 217 328 0045

Business Service Hours:
9:00am to 5:00pm Central Standard Time (USA)

Company Address:
ANDalyze Inc.
2109 S Oak Street, Suite 102
Champaign, IL 61820 USA

Website:
www.andalyze.com

8 Consumables and Replacement Items

- Fluorimeter (Product: AND1000 fluorimeter)
  - Capable of measuring multiple metals.
  - Includes: Fluorimeter
    USB to MINI-B Cable
    100µL Fixed Volume Pipette and Tips

- Sensor Kit (Products: Lead100, Uranium100, Copper100,Mercury100)
  - Equipment for (30) Tests and/or Calibrations
    Kit Includes: (30) Sensor Bags with Sensor & Cuvette
    (30) Sample Tubes (with buffer)
    (35) 1 mL Syringes
    (30) Disposable Transfer Pipettes
    8 mL Analyte Standard Solution
    Lead Test - 1.1ppm Pb^{2+}
    Uranium Test - 1.3ppm UO_2^{2+}
    Copper (Low Range) - 1.4ppm Cu^{2+}
    Copper (High Range) - 0.9ppm Cu^{2+}
    Instruction Manuals
    Material Safety Data Sheets (MSDS)

9 Technical Specification for AND1000 Fluorimeter

The *AND1000 Fluorimeter* is a platform centric hand-held device for heavy metals testing for drinking water and industrial water supplies. It uses an extremely sensitive measurement technique to determine the levels of heavy metal contamination.

AND1000 Fluorimeter

Instrument Specifications:

Light Source:	Light Emitting Diode
Detector:	Photomultiplier Tube
Filters:	Excitation 485nm Emission 535nm

Certifications:

CE Marked: Complies with the following European Union Directives:

- Low Voltage Electrical Equipment Directive 2006/95/EC
- Electromagnetic Compatibility (EMC) Directive 2004/108/EC

Power Specifications

Power:	Battery Operated
Power Recharging:	via USB cable
Power usage:	The battery lasts over 225 tests on a full charge (at 45 seconds/test).

Mechanical Specifications

Dimensions:	Width: 3.6 inches/9.14 cm Depth: 8.0 inches/ 20.3 cm Height: 2.25 inches/ 5.72 cm Weight: 1.25 lb/565 g
IP rating	IP54 (water resistant)

Data specifications

Interface:	USB 2.0
Data:	Downloaded in csv format

Note: *All specifications are subject to change without notice.*

10 Limited Warranty for Fluorimeter Device

ANDalyze provides the following limited warranty for the ANDalyze AND1000 Fluorimeter device (the "**Fluorimeter Device**"). ANDalyze warrants that the Fluorimeter Device (including the software used on the Fluorimeter Device), when used in accordance with the user documentation, will operate in all material respects in conformity with the specifications stated in the user documentation for a period of ninety (90) days from the date of your receipt (the "**Warranty Period**"). If it does not, your sole remedy and ANDalyze's total liability for such material nonconformity in the Fluorimeter Device will be, at ANDalyze's option and discretion, to repair or replace the Fluorimeter Device at ANDalyze's expense or to refund the purchase price (but not any taxes, export or shipping fees) and subject to the limitations in The foregoing remedy is subject to the Limitation of Liability in Section 15 of these terms. To qualify, you must notify ANDalyze during the Warranty Period of any problems that you experience with the Fluorimeter Device. ANDalyze will have no liability for any nonconformity of which you fail to notify ANDalyze prior to the expiration of the Warranty Period. This warranty does not apply to (i) Fluorimeter Device which has been used in a manner other than as authorized under these Terms and the documentation provided with the Fluorimeter Device (including the product brochure and fluorimeter specifications); (ii) any software on the Fluorimeter Device that has been modified by you or any party other than ANDalyze or which has been improperly installed to the extent such modification or improper installation caused the breach of warranty; (iii) failures caused by accident, neglect, failure to maintain a suitable operating environment, tampering, or any other event other than ordinary use.

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Appendix B

Lead Testing and On-Site Calibration for Water Testing (AND-Lead-100-02-2012)

Document: AND-Lead-100-02-2012

Lead Testing and On-Site Calibration for Water Testing

February 8th, 2012 Edition 1

Table of Contents

2 General Information

Important: Before continuing, please read the entire *AND1000 User Manual*. Pay attention to all danger, warning and caution statements. Failure to do so could result in serious injury to the operator or damage to the equipment. Make sure that the safety features of this equipment are not impaired; do not use or install this equipment in a manner other than that specified in this manual. If the equipment is used in a manner not specified by the manufacturer, the safety features of this equipment may be impaired and injury to the operator or damage to the equipment may result.

2.1 Water Testing Guidelines

Note: The below steps are for testing for dissolved, bio-available metal ions in drinking water samples. These steps can also be used for other matrices such as surface, ground, industrial and wastewater which have been pre-treated. Contact ANDalyze customer service for additional application notes on pre-treatment methods.

<u>Water Sampling</u>

- For best results use freshly collected sample (unpreserved in acid) for analysis. We recommend that you use the sample within 1 hour (maximum of 2 hours) of collection to minimize any metal loss to the walls of the sample container. This is particularly important for testing trace lead levels (Contact ANDalyze customer service for the citation to a peer reviewed publication about loss of lead to container walls). Large volumes (1L) may be stored up to 12 hours in HDPE containers in a refrigerator or cooler with ice packs if required.
- Once the sample is mixed with ANDalyze sample buffer, test within 15 minutes.

<u>pH Range</u>

The ANDalyze sample buffer that is provided in the sample tubes brings the pH of the test solution to pH 7.0. The water sample must be in the range of pH 5 – pH 8. If you have a sample which is acidic or basic, mix with the ANDalyze buffer and check the pH of this buffered test solution. It should be ~pH 7.0 for best results.

Note: Our tests have shown that environmental samples preserved in acid to a pH < 2 cannot be brought to a pH of 7.0 when mixed with the ANDalyze buffer. These samples have to be first neutralized with NaOH to a pH ~5 before mixing with ANDalyze buffer. Contact ANDalyze customer service for additional application notes on pre-treatment methods.

2.2 Sensor Bag: Cuvette and Sensor

Sensor Bag: Each sensor bag contains a cuvette, a sensor and a desiccant. These are **<u>single use</u>** and must be discarded. The desiccant should be blue in color. If it has turned completely pink in color, the sensor may not perform well.

2.3 Inserting Cuvette and Sensor

Cuvette: The cuvette has an arrow which should face you when inserted. Insert the cuvette completely so that the fluorimeter lid can close. The meter and cuvette design helps to prevent improper orientation.

Sensor: The square portion of the sensor can be placed on the cuvette in any orientation with the round sections facing upward. Sensors can only be used once and should be disposed of immediately after use.

2.4 Sample Injection and Measurement

The AND 1000 Fluorimeter should be **laid flat** on a stable surface during a measurement

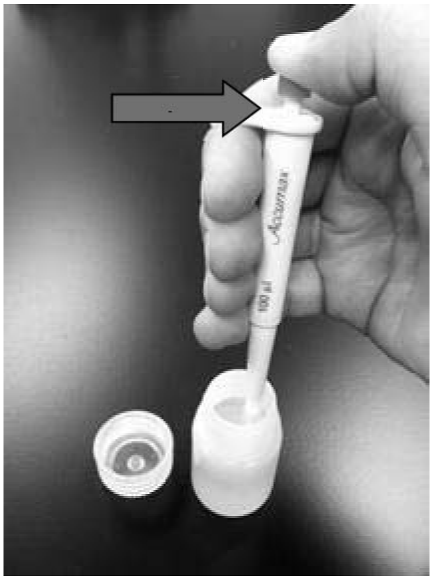
A buffered solution is prepared in a sample tube as described in the On-Site Calibration section (3) and test section (4) and this is used for measurements

A new 1 mL syringe should be used to withdraw 1 mL buffered sample water from a sample tube. This syringe can be attached to the top of the housing as shown in the picture.

The sample should be injected through the housing into the cuvette at a constant speed of **3 – 5 seconds.** The syringe and housing should be immediately removed and the sample door closed. The **START** button located just below the screen should be pressed to start any measurement

Important: After each analysis, discard all components used during the analysis including cuvette, sensor housing, sample tube, and syringes to avoid cross contamination.

2.5 Pipette Use Guidelines



2. Depress the plunger button on the top to the first stop (see photo at left). **DO NOT depress all the way to the pipette body.**

3. Immerse the clean tip into the solution to be withdrawn.

4. Release the pressure slowly to withdraw the solution into the tip.

Note: Make sure that the pipette tip continues to be immersed in the solution during release so as to not expose the tip point to air.

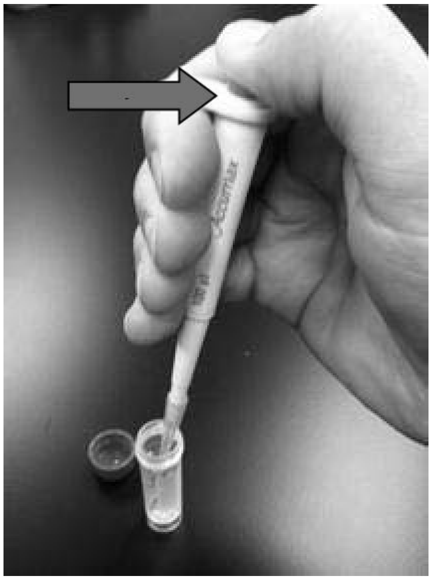
5. Remove the pipette from the solution.

6. Immerse the tip into the liquid present in the sample tube where the withdrawn solution is to be dispensed.

7. Slowly depress the operating button **ALL THE WAY** to dispense the liquid contained in the pipette tip. (See photo at right)

8. Remove the pipette and discard the used tip.

Note: Dispose of tips immediately after use to prevent possible contamination of the pipette.



3 On-Site Calibration

3.1 Required Materials

- AND1000 Fluorimeter
- (2) Sensor Bags with Sensor & Cuvette
- (2) 1mL Syringe
- (2) Sample tubes (with buffer)
- Lead Standard Solution (1.1 ppm)
- 100 µL Fixed Volume Pipette and Tips
- (1) Clean sample collection cup (Not Provided)

3.2 Preparing for On-Site Calibration

When testing water at a new location, the instrument has to be calibrated for accurate readings. Completing the On-Site Calibration requires two separate solutions and tests. At the end of the process, the fluorimeter is calibrated for testing water at that site and also provides the test results for the site. A calibration may only be accurate when originally performed, as changes in the sample matrix, temperature, and sensor lot will affect the results. Do not rely on a calibration to be accurate over days as environmental samples can vary greatly. If in doubt, perform On-site Calibration

Important: On-Site Calibration must be performed if testing is being done:

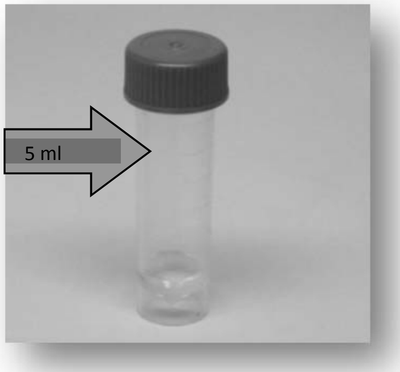
- At a new location which has not been previously saved.
- To adjust for significant changes in sampling temperature.
- When beginning to use a new lot of sensors.

1. **Site:** Select the site to be tested or enter a new site as described in Section 5 of the *AND1000 User Manual*

2. **Collect Sample Water** – In a clean sample cup, collect a small volume of water to be tested.

3. **Prepare Calibration Solutions** – Slowly pour the test water into **two sample tubes** containing the liquid buffer up to the 5 mL mark. If required, use one of the provided disposable plastic pipettes to transfer water accurately, rinsing the pipette in the solution prior to use. **Do not exceed the 5 mL mark for accurate results**.

   Important: Open tube carefully so that liquid buffer does not spill out.



One tube will be used for analyzing a sample spiked with Lead and the other for analyzing an unspiked sample.

3.3 On-Site Calibration: Sample with Lead Spike (Step 1 of 2)

1. Attach a **new** tip to the 100µL fixed volume pipette.

2. Withdraw 100µL of the Lead Standard Solution from the bottle using the pipette.

3. **Mix** - Transfer the 100µL spike into one of the sample tubes filled with buffered sample water.

4. **Shake -** Close cap and shake well. For most accurate results, wait 5 minutes before testing. Incubation is **required** for all environmental water samples.

   Important: Open tube carefully so that liquid does not spill out.

Withdrawing Standard Solution Using Pipette

Transferring Spike Solution to Sample Tube

5. **Pouch –** Open the sensor bag which contains the sensor (colored plastic housing) and plastic cuvette.

6. **Cuvette –** Place the plastic cuvette in the AND1000 Fluorimeter.

7. **Sensor** - Place colored plastic sensor on the cuvette. (Any orientation). Make sure sensor is seated securely on the cuvette.

8. Enter the Site Calibration screen (as seen in photo to the right) which will appear once a new site has been entered (See Section 5 of *AND1000 User Manual*).

Important: Do **not** press **START** until the sample is ready. Follow steps below.

Note: If the unit is left on for more than 2 minutes without any activity, a screen-saver (black screen) will be activated; press any button to resume operation (Do not press and hold ON/OFF). The instrument automatically turns off if not used for more than 10 minutes.

Site Calibration

Lead

Site: SITE1

Step 1 of 2:

Please insert sample water spiked with Lead.

9. **Test** - With one of the provided 1 mL syringes, draw 1 mlL buffered sample water from the spiked sample tube into the syringe. Attach the syringe to the housing over the cuvette in the instrument. Maintaining a constant speed (over 3-5 seconds), carefully squeeze the sample water through the housing into the cuvette. Quickly remove sensor housing and syringe and close sample chamber door. Press the **START** button located just below the screen. Remove the cuvette when complete.

3.4 Site Calibration: Unspiked Sample Test (Step 2 of 2)

Site Calibration

Lead

Site: CHM DI

Step 2 of 2:

Please insert plain sample-water.

The Site Calibration screen (as seen in photo to the right) will appear once Step 1 of 2 has been completed for a new site.

DO NOT press START until the second sample is ready for injection!

1. With one of the provided 1 mL syringes, draw 1 mL buffered sample water from the **<u>second</u> unspiked sample tube** into a new syringe.
2. Attach the syringe to the housing over the cuvette in the instrument.
3. Maintaining a constant speed (over 3-5 seconds), carefully squeeze the sample water through the housing into the cuvette.
4. Quickly remove sensor housing and syringe and close sample chamber door.
5. Press the **START** button located just below the screen. Remove the cuvette when complete.

3.5 Applying Results

Calibration Results

Lead

Site: CHM DI

Factor: 0.174369

Sample-water conc:

BELOW LIMIT

The results display the site name, a calibration factor associated with that site, and the Lead test results for the water sample used during this process. (The results displayed are for the sample water and not for the sample water containing the spike). To save the factor associated with this site, press **APPLY**.

On-Site Calibration from this site has been saved. You are now ready to test more samples from this site.

4 Testing a Sample

This section will walk you through the process of testing a water sample for the desired analyte. Please read this entire section before beginning the test as parts of the test will be time sensitive. Be aware and pay attention to all notes.

4.1 Required Materials

- AND1000 Fluorimeter
- (1) Sensor Bag with Sensor & Cuvette
- (1) 1 mL Syringe
- (1) Sample tube (with buffer)
- (1) Clean sample collection cup (Not Provided)

Note: Before using the AND1000 assure that the fluorimeter is charged. (See Section 4.1 in the *AND1000 User Manual* for Battery Charging Information)

4.2 Testing a Sample

1. **Start-Up** - Initialize the instrument by pressing the ON/OFF button. Instrument will initialize in about 1-2 seconds.

2. **Metal Type** - Ensure that the fluorimeter is on the correct metal screen.

3. **Site** – Confirm that the site being tested is correct.

Ready to Analyze

Metal: Lead

Site: CHM DI

Time: 05/24 14:30

Press SELECT to Edit

Note: Unit may go into screen-saver mode after 2 minutes. Press any button to resume (Do not press and hold ON/OFF).

Do Not press START until the sample is ready!

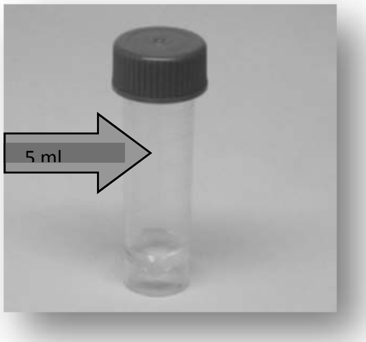
4. **Pouch -** Open the sensor bag which contains the sensor (colored plastic housing) and plastic cuvette.

Important: The bag contains a transparent desiccant pouch. This should be blue in color. If desiccant has turned completely pink in color, the sensor may not perform well.

5. **Cuvette** - Place the plastic cuvette in the instrument.

6. **Sensor** - Place colored plastic sensor on the cuvette. (Any orientation). Make sure the sensor is seated securely on the cuvette.

–



9. **Syringe** - With one of the provided syringes, draw 1 mL of buffered sample water from the sample tube into syringe.

10. Attach the syringe to the housing over the cuvette in the instrument. (Syringe tip will fit into top of sensor)

Note: If the unit is left on for more than 2 minutes without any activity, a screen-saver (black screen) will be activated, press any button to resume operation (Do not press and hold ON/OFF). The instrument automatically turns off if not used for more than 10 minutes.

11. Maintaining a constant speed (over 3-5 seconds), carefully squeeze the buffered sample water through the housing into the cuvette.

12. Quickly remove sensor housing and syringe and close sample chamber door.

13. Press the **START** button located just below the screen.

14. Remove cuvette when complete.

4.3 Results

The sample will be tested, and results displayed in parts per billion (ppb) or parts per million (ppm) in under a minute.

To save the results, press the **SAVE** button.

Note: A reading of “Below 2ppb Limit” indicates the amount of Lead in the sample is below the minimum detectable levels of the instrument.


Analysis Complete
Please Remove Cuvette
Metal: Lead
26ppb
ID: PB-11

5 Technical Specifications for ANDalyze Sensors

5.1 Detection in Drinking Water

ANDalyze's proprietary catalytic DNA sensor uses a DNAzyme reaction that fluoresces in the presence of the target contaminant (lead, uranium, copper, etc). The fluorescence of the reaction is measured using the AND 1000 fluorimeter to determine the concentration of the free analyte ion (Pb^{2+}, UO_2^{2+}, Cu^{2+}, etc.) in solution and is reported in parts per billion (ppb) or parts per million (ppm).

Materials Used

- AND1000 Fluorimeter
- Lead100 Sensor Kit

Sensor

Performance

Lead dilutions containing 0, 5, 10, 15, 25, 50, 75, 100, 150, 200 ppb Pb^{2+} were prepared in DI water. Five replicates were used for each test at each dilution.

Limit of Detection (LOD)

1 ppb Pb^{2+}
Based on 3 sigma method

Limit of Quantification (LOQ)

2 ppb Pb^{+}
Based on 10 sigma method

Linear Detection Range

2 - 100 ppb Pb^{2+}

Precision

Standard: 15 ppb Pb^{2+}
95% Confidence Limits: 13 – 17 ppb Pb^{2+}

Coefficient of Variation (CV):

0–200 ppb Pb^{2+} ±15%

Note: All specifications are subject to change without notice.

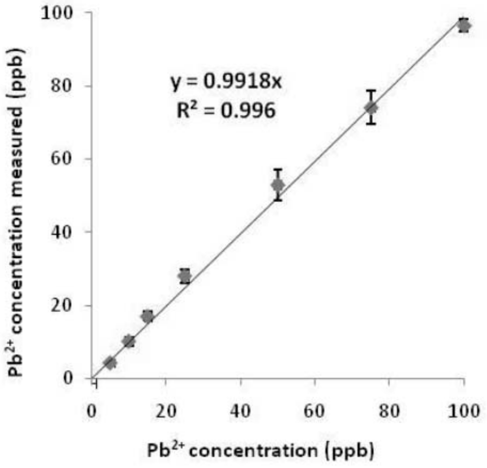
5.2 Interference

Interference tests were done with a 30ppb Pb^{2+} solution plus the potential interfering ion. The interference tolerance levels represent the concentration above which the lead concentration is changed to ±10%. Data represents an average of at least three replicates. For each interference test, an on-site calibration with the particular water matrix (containing the interfering ion) was performed.

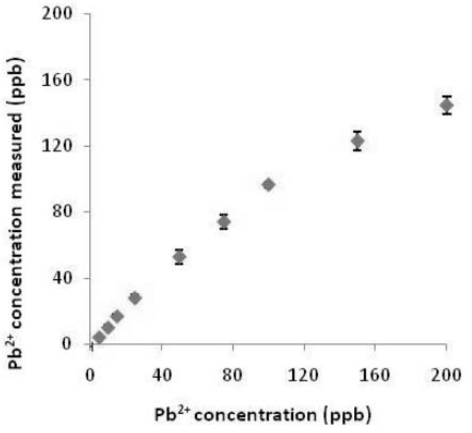
Interfering ion	Interference level
Calcium, Ca^{2+}	500 ppm
Magnesium, Mg^{2+}	500 ppm
Zinc, Zn^{2+}	100 ppm
Aluminum, Al^{3+}	0.1 ppm
Copper, Cu^{2+}	0.5 ppm
Iron, Fe^{3+}	0.04 ppm
Cadmium, Cd^{2+}	15 ppm
Mercury, Hg^{2+}	0.03 ppm
Manganese, Mn^{2+}	150 ppm
Ammonium, NH_4^{+}	2000 ppm
Carbonate, CO_3^{2-}	100 ppm
Phosphate, PO_4^{3-}	50 ppm
Chloride, Cl^{-}	2000 ppm
Sulfate, SO_4^{2-}	100 ppm
Nitrate, NO_3^{-}	5000 ppm

5.3 Product Accuracy Ranges

The graphs below depict the average measured Pb^{2+} concentration as displayed on the AND1000 fluorimeter (y-axis) vs. the known concentration of the lead standards (x-axis). Error bars depict the standard deviation from five measurements. Figure 1 shows the linear range of 0 - 100 ppb Pb^{2+}. For higher concentrations of Pb^{2+} (tested up to 200 ppb Pb^{2+}), the accuracy decreases (Graph 2). Samples containing higher than 100 ppb Pb^{2+} can be diluted 1:1 and re-analyzed.



Graph 1: Average Pb^{2+} Conc. vs. Known Conc.



Graph 2: Accuracy vs. Linear Detection Range

6 Consumables and Replacement Items

- Fluorimeter (Product: AND1000 fluorimeter)
  - Capable of measuring multiple metals.
  - Includes: Fluorimeter
    USB to MINI-B Cable
    100µL Fixed Volume Pipette and Tips

- Sensor Kit (Products: Lead100)
  - Equipment for (30) Tests and/or Calibrations
    Kit Includes: (30) Sensor Bags with Sensor & Cuvette
    (30) Sample Tubes (with buffer)
    (35) 1 mL Syringes
    (15) Disposable Transfer Pipettes
    8 mL Lead Standard Solution (1.1 ppm Pb^{2+})
    Instruction Manuals
    Material Safety Data Sheets (MSDS)

7 Customer Service Contact Information

Contact us by Email:
info@andalyze.com

By Telephone:
+1 888 388 0818 or +1 217 328 0045

Business Service Hours:
9:00am to 5:00pm Central Standard Time (USA)

Company Address:
ANDalyze Inc.
2109 S Oak Street, Suite 102
Champaign, IL 61820 USA

Website:
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3-Point Laboratory Calibration

Laboratory calibration or renewal of AND1000 fluorimeter factory calibration

Problem Statement

The AND1000 fluorimeter is a field test device designed to obtain a quick result on-site, and as such, a single point standard is used for on-site calibration. However, as with any analytical technique, a calibration performed with 3 concentrations will yield more accurate results over the entire detection range. A 3-point calibration is recommended for laboratory evaluations and is available as an option on the AND1000 fluorimeter. 3-Point calibrations are typically completed at the factory as part of the production and quality control process and are not described in the user manual. This solution note is made available to the end user as under certain circumstances it may be required for the end user to perform a 3-point calibration. Proper calibration is critical to the accuracy of the AND1000 fluorimeter so please follow these instructions closely and if any issues arise, please contact ANDalyze. The protocol for laboratories to perform a 3-point calibration is as follows:

Important: On-site calibration factors associated with various sites are dependent on the 3-point calibration performed using distilled water at the factory. If you change the 3 point calibration, previously saved on-site calibrations factor will no longer remain valid and will have to be re-done.

Materials: Required Equipment & Solutions (For EACH metal to be calibrated)

- AND1000 Fluorimeter
- (3) Sensor Bags with Sensor & Cuvette
- (3) 1 mL Syringes
- (3) Samples Tubes (with buffer
- (3) 3-Point Calibration Metal Standard Solutions (specific to metal) prepared freshly from a high concentration metal stock*
    - Lead – 25 ppb, 50 ppb, 75 ppb
    - Uranium – 10 ppb, 30 ppb, 50 ppb
    - Copper Low – 50 ppb, 100 ppb, 150 ppb
    - Copper High - 0.75 ppm, 1.5 ppm, 2.25 ppm
- Pipette with pipette tips to make 3-Point Calibration Metal Standard Solutions. The 100 µL fixed-volume pipette supplied with the AND1000 fluorimeter is insufficient for preparation of these solutions.

* **ANDalyze test kits do not contain a high concentration stock** for preparation of solutions for 3-point calibration. Users can prepare the calibration solution from an acid-preserved metal stock solution purchased from a standard chemical company or contact ANDalyze customer service to obtain metal stock solution for 3-point calibration.

Preparation of Standard Metal Solutions

Use a high concentration metal standard (*e.g*, 1000 ppm) for making calibration solutions so that the acid used to preserve these standards (1-5% usually) is sufficiently diluted when you make the ppb level solutions and therefore does not require any neutralization (samples must be pH 5-8 for successful analysis). Also, volume changes to the sample upon addition of standard will be negligible.

Note: Calibration solutions prepared in water should be used within 1 hour of preparation to avoid changes in concentration from metal adsorbing to the walls of the container. Once mixed with ANDalyze buffer, it must be used within 5 – 15 minutes.

For example: To make 50 mL of the following 3-Point Calibration Lead Standard Solutions:

25 ppb Pb^{2+}: 50 mL water + 125 µL, 10 ppm Pb^{2+}
50 ppb Pb^{2+}: 50 mL water + 250 µL, 10 ppm Pb^{2+}
75 ppb Pb^{2+}: 50 mL water + 375 µL, 10 ppm Pb^{2+}

High concentration metal stocks can be purchased from any scientific supply company, such as Sigma-Aldrich:

- Lead Standard for ICP 1000 µg/mL (ppm): Sigma Aldrich product number: 41318
- Uranium atomic absorption standard solution 1000 µg/mL (ppm): GFS Chemicals product number: 1829
- Copper Standard for ICP 1000 µg/mL (ppm): Sigma Aldrich product number: 68921

Perform a New 3-Point Calibration for the Lead Sensor

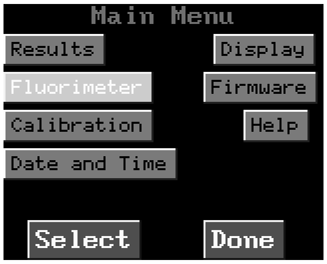
Prepare 25, 50, and 75 ppb Lead solutions in water starting with a high concentration Lead standard (preferably a 1000 ppm commercial stock solution, first diluted to 10 ppm, to dilute out acid present in the standards) as described above.

Method:

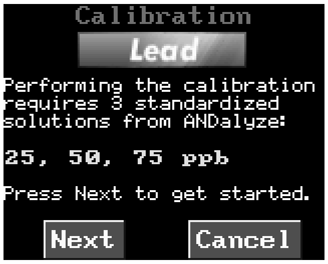
1. In the Lead screen, press **MENU**

Ready to Analyze
Metal: Lead
Site: CHM DI
Time: 05/24 14:30
Press SELECT to Edit

2. Select **CALIBRATION** in the main menu



3. At the calibration screen the user is prompted to insert 25, 50, 75 ppb Lead in three separate steps. Press **NEXT**.



Calibration
Lead
Performing the calibration requires 3 standardized solutions from ANDalyze:
25, 50, 75 ppb
Press Next to get started.

4. **A Pre-Calibration Screen will appear**. Press **OK.** The AND1000 fluorimeter will perform the required pre-calibration and proceed to the next step.

5. **The first screen prompts the user to insert a 25 ppb sample**. This should be the 25 ppb lead sample that you have prepared in distilled water, mixed in with the ***sample buffer present in the ANDalyze sample tubes***. Inject 1 mL of the buffered sample through the sensor housing as per instructions in the *Testing and On-site Calibration* manual user manual.

6. **Press next after the test is completed** and follow the same instructions for the 50 and 75 ppb lead solutions.

7. **To accept the calibration**, select **APPLY**

Note: After a 3-Point Calibration is performed with a given matrix On-site Calibration is not required before testing the matrix. In this case only the "site" field on the fluorimeter should be set to "none"

To Perform a New 3-Point Calibration for the Copper or Uranium Sensor:

Perform steps 1 – 7 as described above for Lead, where step 1 should correspond to the metal that you are interested in calibrating.

For the Copper sensor (0.6 – 3 ppm) range: Prepare 0.75, 1.5, and 2.25 ppm copper solutions and proceed as per instructions on the screen and in the test manual for testing a sample solution.

For the Copper sensor (40- 200 ppb) range: Prepare 50, 100, and 150 ppb copper solutions and proceed as per instructions on the screen and in the test manual for testing a sample solution.

For the Uranium sensor: Prepare 10, 30, and 50 ppb uranium solutions and proceed as per instructions on the screen and in the test manual for testing a sample solution.

How Does an On-site Calibration Work?

When a user performs an on-site calibration, the fluorimeter software calculates (a) the lead concentration (ppb) in the sample and (b) the lead concentration (ppb) in the sample spiked with 25 ppb lead, using the calibration curve from the 3-Point Calibration. The fluorimeter software then calculates an accuracy factor based on % recovery. Accuracy factor = 25/(b-a). This factor is stored for a unique site that is entered by the user. The sample concentration displayed after an on-site calibration = a * accuracy factor

Environmental Water Testing: Surface Water, Groundwater, Hard Water, Wastewater, & Seawater

Matrix-specific sample preparation and testing methods for environmental waters

Problem Statement

1. ANDalyze metal test kits are designed for use out of the box with drinking water; however they can be used for environmental water analysis with some minor protocol modifications.
2. Below are instructions for testing samples obtained from sources such as:
   - Surface Water (rivers, lakes, ponds)
   - Ground Water (wells, aquifers)
   - Hard or Very Hard Water (multiple sources)
   - Treated Wastewater – Finished or treated and diluted tenfold
   - Seawater (from the surface, not the sediment/water column interface)

Materials

Each matrix type may require one or more of the following pre-treatment kits. Read guidelines for each matrix. Kits may be purchased from ANDalyze wherever indicated or individual components for pre-treatment may be purchased through a scientific supply company.

ANDalyze Dilution Kit
- 50 mL Self-standing sample tube
- 5 mL Fixed Volume Pipette
- Reagent grade water

ANDalyze pH Adjustment Kit
- Sodium Hydroxide Neutralization Solution, 1% (w/w) sodium hydroxide in a dropper bottle
- Nitric Acid Neutralization Solution, 1.5% (v/v) nitric acid in a dropper bottle
- pH paper

ANDalyze Iron Interference Kit
- Sodium Hydroxide Neutralization Solution, 1% (w/w) sodium hydroxide in a dropper bottle
- Hydrogen Peroxide Solution, 30% (w/w) hydrogen peroxide in a dropper bottle

ANDalyze Filtration Kit **(Available now from ANDalyze)**
- 0.2 µm Nylon filter, 25 mm diameter (Nalgene)
- 20 mL Syringe
- 50 mL Self-standing sample tube

Solution Statement

ANDalyze kits may be used to test many different environmental waters. Each matrix may require different pretreatment steps. Please see the general protocols below for: (1) Dilution, (2) pH Adjustment, (3) Filtration, and (4) Environmental Water On-site Calibration. Matrix-specific instructions, including necessary protocols, are presented following the protocols.

Note: Our tests have shown that the percent recovery for lead in environmental samples is ~60 % for less than 25 ppb lead and 75-125 % for 25-100 ppb lead. The percent recovery for uranium in environmental samples is > 60% for less than 30 ppb uranium and 75 – 125 % for 30 – 60 ppb uranium. Copper is less well characterized in environmental matrices, though the copper sensor is tolerant of high salt conditions.

General Protocols

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(1) General Protocol - Dilution

Dilution is needed for accurate readings if the target metal ion is present at a concentration higher than the linear detection range stated in the *Testing and Calibration* manual. Linear detection ranges are noted below:

Lead100 sensor – 2-100 ppb Lead
Uranium100 sensor – 2-60 ppb Uranium
Copper High Range – 0.6-3 ppm Copper
Copper Low Range – 40-200 ppb Copper
Mercury100 Range – 2-50 ppb Mercury

The ANDalyze Copper sensor is available in two ranges and therefore dilution is usually not required.

1. **Dilution is best performed using standard laboratory glassware** and reagent grade water – one volume sample to nine volumes reagent grade water.
2. **Dilution may also be performed in the field**, with a decrease in accuracy, by withdrawing 5 mL sample with a 5 mL fixed volume pipette, adding the aliquot to a 50 mL self-standing tube, and filling to the 50 mL mark with reagent grade water. Shake well.
3. **If the sample is diluted**, on-site calibration must be performed with the diluted sample.

(2) General Protocol - pH Adjustment

ANDalyze Lead, Mercury and Copper sensors perform best when the sample pH is between 5 and 8 (pH 4-7 for Uranium). Samples with a pH greater than 8 or below 5 will not test reliably for Lead, Mercury, or Copper (greater than 7 or below 4 for Uranium). It is required to adjust the pH into this range before sample preparation steps and testing can continue. Samples above pH 10 should not be tested even with pH adjustment.

1. **Check the sample pH** using pH paper.
2. **Prepare the following solutions** if pH adjustment is required
    1. Sodium Hydroxide Neutralization Solution, 1% (w/w) sodium hydroxide
    2. Nitric Acid Neutralization Solution, 1.5% (v/v) nitric acid
3. **Adjust the sample pH**
    1. **If the sample is below pH 5** (or pH 4 for U) addition of a dilute sodium hydroxide solution is necessary. To a 50 mL volume of sample add the Sodium Hydroxide Neutralization Solution dropwise with stirring or with shaking between addition of each drop. Do not titrate beyond pH 5 for Lead, Mercury, and Copper and pH 4 for Uranium.

       Note: pH change from 4-5 is rapid, requiring a half drop or less. **Check the pH multiple times during titration.** The number of drops required depends heavily on matrix constituents. As few as four drops may be sufficient to increase pH from 3 to 4, or many more may be required.

    2. **If the sample is above pH 8** for Lead, Mercury, and Copper (above pH 7 for Uranium) addition of a dilute nitric acid (1.5 %) solution is necessary. Samples above pH may be unsuitable for testing even with pH adjustment as metal ion may have already precipitated out.

       Note: pH change from 9-8 is rapid, requiring a half drop or less depending on matrix. **Check the pH multiple times during titration.** The number of drops required depends heavily on matrix constituents. As few as four drops may be sufficient to decrease pH from 10 to 7, or many more may be required.

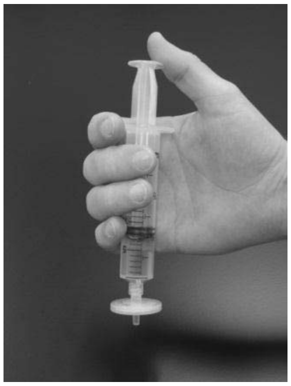
Note: For highly basic water samples, acidification may be insufficient to solubilize precipitated metals.

(3) – General Protocol - Filtration

1. **Obtain an ANDalyze Filtration Kit.** Before testing or spiking any environmental water sample, it must be filtered to remove suspended solids.

2. **Filter the water sample.** Draw ~20 mL water sample into a 20 mL syringe, securely attach the filter, and dispense into the self-standing vial.

   Note: If the sample is collected off-site and transported to a laboratory for testing, ensure that the sample is stirred (*e.g.*, stir-bar in the bottom of a 1 L HDPE Nalgene bottle filled with sample on a stir plate) while filling the syringe to ensure homogeneity.



3. The sample should be clear and the filter may no longer be white.

   Note: If a sample contains a great deal of suspended solids the syringe filter may clog after elution of 10-20 mL sample. In this case, discard the clogged filter and use a fresh filter to continue filtering the sample.

(4) General Protocol - Environmental Water On-site Calibration

On-site calibration is performed for all new samples and any time a matrix may have changed, *e.g*. new sampling day, change in matrix composition, new sensor batch, temperature change, etc. If in doubt, perform On-site Calibration.

Important: For environmental samples, it is required to incubate the calibration spike with the sample for at least 5 minutes as the spiked metal takes some time to reach equilibrium between dissolved and bound states. Failure to allow spike incubation in the sample will lead to lower recovery.

1. **Perform on-site calibration as described in the Product Manual.** After adding the 100 µL standard metal solution spike as per the instructions in the product manual, shake, and **let it sit for ~ 5 minutes** before the analysis is performed.

2. **Use all spiked solutions** within 15 minutes.

Note: The ANDalyze test kit is designed to test for bioavailable metals and not total metals without acid digestion, which is beyond the scope of this procedure.

Matrix-Specific Protocols

Surface Water Testing Protocol

ANDalyze has performed extensive testing of our kits in surface waters such as rivers, lakes, and streams. Some surface waters, such as runoff from industrial sites, heavily contaminated bodies of water, mine runoff, or areas affected by acid rain may exceed interference levels and the acceptable pH range. Special care may be needed in handling as well as testing these samples. Please contact ANDalyze with any questions.

Important: Testing of surface waters from rivers, lakes, and streams usually does not require dilution, pH adjustment, or iron interference removal. If required, perform those steps as stated in the protocols. Filtration, however, is always required.

1. **Check the pH using pH paper** and adjust if required.
2. **Filtration** is required as per the Filtration Protocol.
3. **Perform Environmental Water On-site Calibration** following the Environmental Water On-site Calibration Protocol (with incubation of calibration spike) and instructions in the *Testing and On-site Calibration* manual.

Groundwater Testing Protocol

Important: Testing of raw or treated groundwater usually does not require dilution or pH adjustment. If required, perform those steps as stated in the protocols. Iron interference may be an issue and the solution color should be noted – yellow/orange color may be indicative of iron. Filtration is always required.

1. **Check the pH using pH paper** and adjust if required.
2. **Verify that iron interference is not an issue.** If interference is suspected, follow the Iron Interference Solution Note.
3. **Filtration** is required as per the Filtration Protocol.
4. **Perform Environmental Water On-site Calibration** following the Environmental Water On-site Calibration Protocol (with incubation of calibration spike) and instructions in the *Testing and On-site Calibration* manual.

Note: ANDalyze has performed extensive testing of our kits in ground waters from across the U.S.A as well as in artificial matrices based on those in Standard Methods for the Examination of Water & Wastewater, Centennial Edition. As a general rule, performance in soft waters exceeds that of very hard waters, which are much more likely to exceed the interference level or pH range.

Hard Water Testing Protocol

ANDalyze, Inc. has performed extensive testing of our kits in simulated hard waters, including hard and moderately hard waters according to Standard Methods for the Examination of Water & Wastewater, Centennial Edition.

Important: Testing of hard waters usually does not require dilution, pH adjustment, or iron interference removal. If required, perform those steps as stated in the protocols. Filtration, however, may be required.

1. **Check the pH using pH paper** and adjust if required.
2. **Filtration** is required as per the Filtration Protocol **IF** the water is cloudy.
3. **Perform Environmental Water On-site Calibration** following the Environmental Water On-site Calibration Protocol (with incubation of metal spike) and instructions in the *Testing and On-site Calibration* manual.

Wastewater Testing Protocol

Important: ANDalyze has tested multiple **finished or treated** wastewater matrices and analysis can be challenging depending on matrix constituents. **Raw wastewater** is **NOT** suitable for testing. Dilution of the sample (1:10) is absolutely required, which increases the **effective detection range tenfold**, *e.g.* the detection range for Lead after dilution is 20-1000 ppb. Analyte concentrations measured at the low end of the sensor ranges are **qualitative** rather than quantitative and the relative standard deviation of results is larger than experienced in drinking water.

3. **Precautions!** Wastewaters are complicated matrices and may contain interferences beyond other metal ions.
   - **Chelators** such as EDTA will cause false negatives. Chelators are present in many cleaning products and industrial processes, so check wastewater components carefully.
   - **Fluorescent compounds** will give a high background signal and results may be unreliable
   - **Very high concentration of other metal ions** – Example: Known metals from a metal finisher plant
   - **Wear personal protective equipment**. Wastewaters may have extreme pH values and contain hazardous components. Wear appropriate laboratory attire and use a fume hood as appropriate.

2. **Follow the Dilution Protocol to dilute the sample tenfold.** Remember that your effective detection range has increased tenfold.
3. **Check the pH using pH paper** and adjust if required.
4. **Verify that iron interference is not an issue.** If interference is suspected, follow the Iron Interference Solution Note.
5. **Filtration** is required as per the Filtration Protocol. If filters clog rapidly then pre-filtration through Whatman 3MM paper or acid digestion may be necessary.
6. **Perform Environmental Water On-site Calibration** following the Environmental Water On-site Calibration Protocol and instructions in the *Testing and On-site Calibration* manual.
   1. **If on-site calibration fails, repeat.**
   2. **If on-site calibration fails again**, further dilution may be necessary. Dilute the sample another tenfold (total 100-fold dilution). Be aware that the analyte concentration may be out of the linear detection range upon 100-fold (total) dilution.

Note: Some waste water matrices have many interferences and cannot be effectively analyzed without acid digestion or other treatment procedures, which are beyond the scope of this solution note.

Seawater Testing Protocol

Important: Sample preparation steps for seawater are normally not necessary beyond filtration unless the sample is taken from a polluted area. This protocol is intended for analysis of seawater from the surface or water column with minimal sediment. Analyte concentrations measured are **qualitative/ semi quantitative** rather than quantitative and the relative standard deviations of results are larger than experienced in drinking water.

1. **Filtration** is required as per the Filtration Protocol.
2. **Perform Environmental Water On-site Calibration** following the Environmental Water On-site Calibration Protocol and instructions in the *Testing and On-site Calibration* manual.
3. Dilution (tenfold) may be performed if spike recovery is very low.

Note: Bittern Water is a concentrated solution left over after crystallization of NaCl from seawater. It contains very high concentrations of interfering ions, notably magnesium. ANDalyze test kits may work in bittern water upon dilution of the sample by at least ten-fold if not 100-fold. Contact ANDalyze for further details.

Iron Interference with the Lead100 Sensor

Minimization of interference from soluble iron in environmental water

Problem Statement

The presence of soluble iron has a negative effect on sensor performance. The interference level for the ANDalyze Lead sensor is defined as the level of an ion at which the signal of a 30 ppb Pb^{2+} solution is changed by ±10%. The interference level for soluble Fe(III) is 40 ppb. Insoluble iron in the form of precipitates may be removed, at least in part, through natural settling or filtration. Steps to minimize interference are provided below.

Materials

ANDalyze pH Adjustment Kit
- Sodium Hydroxide Neutralization Solution, 1% (w/w) sodium hydroxide in a dropper bottle
- Nitric Acid Neutralization Solution, 1.5% (v/v) nitric acid in a dropper bottle
- pH paper

ANDalyze Iron Interference Kit
- Sodium Hydroxide Neutralization Solution, 1% (w/w) sodium hydroxide in a dropper bottle
- Hydrogen Peroxide Solution, 30% (w/w) hydrogen peroxide in a dropper bottle

ANDalyze Filtration Kit **(Available now from ANDalyze)**
- 0.2 µm Nylon filter, 25 mm diameter (Nalgene)
- 20 mL Syringe
- 50 mL Self-standing sample tube

Solution Statement

Important: Ground water samples visibly orange in color are likely to contain high levels of iron (low ppm), though lower levels may not be easily detected with the naked eye. If it is suspected that the water sample to be tested contains interfering levels of iron, it may be prudent to test the iron concentration using a commercial iron test kit.

Important Safety Note: Refer to the product manual for general guidelines on safety, proper use, and general sample testing procedures before using the testing protocol listed in this solution note. Be very careful when handling the sodium hydroxide solution and the hydrogen peroxide solution. Wear gloves and eye protection. Exposure to concentrated hydrogen peroxide will result in burns. See the manufacturer Material Safety Data Sheet for further information.

Note: Although no individual ion may exceed the interference levels, a combination of many interfering ions close to the maximum levels may have an effect meeting or exceeding that of a single ion at the interference level.

pH Adjustment Protocol

ANDalyze Lead sensors perform best when the sample pH is between 5 and 8. Samples with a pH greater than 8 or below 5 will not test reliably for Lead. It is required to adjust the pH into this range before sample preparation steps and testing can continue. **Samples above pH 10 should not be tested for Lead**, even with pH adjustment.

1. **Check the sample pH** using pH paper.
2. **Prepare the following solutions** if pH adjustment is required
    1. Sodium Hydroxide Neutralization Solution, 1% (w/w) sodium hydroxide
    2. Nitric Acid Neutralization Solution, 1.5% (v/v) nitric acid
3. **Adjust the sample pH**
    1. **If the sample is below pH 5** addition of a dilute sodium hydroxide solution is necessary. To a 50 mL volume of sample add the Sodium Hydroxide Neutralization Solution dropwise with stirring or with shaking between addition of each drop. Do not titrate beyond pH 5 for Lead.

       Note: pH change from 4-5 is rapid, requiring a half drop or less. **Check the pH multiple times during titration.** The number of drops required depends heavily on matrix constituents. As few as four drops may be sufficient to increase pH from 3 to 4, or many more may be required.

    2. **If the sample is above pH 8** for Lead, addition of a dilute nitric acid (1.5 %) solution is necessary. Samples above pH 10 should not be tested even with pH adjustment.

       Note: pH change from 9-8 is rapid, requiring a half drop or less depending on matrix. **Check the pH multiple times during titration.** The number of drops required depends heavily on matrix constituents. As few as four drops may be sufficient to decrease pH from 10 to 7, or many more may be required.

Note: For highly basic water samples, acidification may be insufficient to solubilize precipitated metals.

Peroxide Treatment Protocol

The addition of hydrogen peroxide converts soluble iron to insoluble iron which can then be removed by filtration.

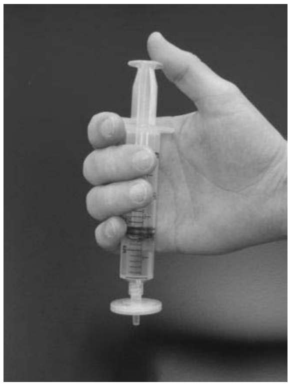
1. **Hydrogen peroxide addition.** Add 4 drops of the hydrogen peroxide solution to each ~50 mL water sample using a dropper, replace cap on the sample tube, mix well by inversion, then let sit on the bench for 20 min.
2. **After the 20 min incubation** continue with the Filtration Protocol described below.

Filtration Protocol

1. **Obtain an ANDalyze Filtration Kit.** Before testing or spiking any environmental water sample, it must be filtered to remove suspended solids.

2. **Filter the water sample.** Draw ~20 mL water sample into a 20 mL syringe, securely attach the filter, and dispense into the self-standing vial.

   Note: If the sample is collected off-site and transported to a laboratory for testing, ensure that the sample is stirred (e.g., stir bar in the bottom of a 1 L HDPE Nalgene bottle filled with sample on a stir plate) while filling the syringe to ensure homogeneity.



3. The sample should be clear and the filter may no longer be white.

   Note: If a sample contains a great deal of suspended solids the syringe filter may clog after elution of 10-20 mL sample. In this case, discard the clogged filter and use a fresh filter to continue filtering the sample.

After completion of the Filtration Protocol continue with On-site Calibration as described in the *Testing and On-site Calibration* manual.

Summary/Notes/References

It is expected that, upon completion of this procedure, the interference from Iron will be greatly reduced and that a more accurate reading of Lead concentration will be obtained. It should be noted that some co-precipitation of Iron and Lead may be possible.

Appendix C

Environmental Water Testing: Surface Water, Groundwater, Hard Water, Wastewater and Seawater Solution Notes (AND-Sol-Env-02-2012)

Environmental Water Testing: Surface Water, Groundwater, Hard Water, Wastewater, & Seawater

Matrix-specific sample preparation and testing methods for environmental waters

Problem Statement

1. ANDalyze metal test kits are designed for use out of the box with drinking water; however they can be used for environmental water analysis with some minor protocol modifications.
2. Below are instructions for testing samples obtained from sources such as:
   - Surface Water (rivers, lakes, ponds)
   - Ground Water (wells, aquifers)
   - Hard or Very Hard Water (multiple sources)
   - Treated Wastewater – Finished or treated and diluted tenfold
   - Seawater (from the surface, not the sediment/water column interface)

Materials

Each matrix type may require one or more of the following pre-treatment kits. Read guidelines for each matrix. Kits may be purchased from ANDalyze wherever indicated or individual components for pre-treatment may be purchased through a scientific supply company.

ANDalyze Dilution Kit

- 50 mL Self-standing sample tube
- 5 mL Fixed Volume Pipette
- Reagent grade water

ANDalyze pH Adjustment Kit

- Sodium Hydroxide Neutralization Solution, 1% (w/w) sodium hydroxide in a dropper bottle
- Nitric Acid Neutralization Solution, 1.5% (v/v) nitric acid in a dropper bottle
- pH paper

ANDalyze Iron Interference Kit

- Sodium Hydroxide Neutralization Solution, 1% (w/w) sodium hydroxide in a dropper bottle
- Hydrogen Peroxide Solution, 30% (w/w) hydrogen peroxide in a dropper bottle

ANDalyze Filtration Kit **(Available now from ANDalyze)**

- 0.2 µm Nylon filter, 25 mm diameter (Nalgene)
- 20 mL Syringe
- 50 mL Self-standing sample tube

Solution Statement

ANDalyze kits may be used to test many different environmental waters. Each matrix may require different pretreatment steps. Please see the general protocols below for: (1) Dilution, (2) pH Adjustment, (3) Filtration, and (4) Environmental Water On-site Calibration. Matrix-specific instructions, including necessary protocols, are presented following the protocols.

Note: Our tests have shown that the percent recovery for lead in environmental samples is ~60 % for less than 25 ppb lead and 75-125 % for 25-100 ppb lead. The percent recovery for uranium in environmental samples is > 60% for less than 30 ppb uranium and 75 – 125 % for 30 – 60 ppb uranium. Copper is less well characterized in environmental matrices, though the copper sensor is tolerant of high salt conditions.

General Protocols

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(1) General Protocol - Dilution

Dilution is needed for accurate readings if the target metal ion is present at a concentration higher than the linear detection range stated in the *Testing and Calibration* manual. Linear detection ranges are noted below:

Lead100 sensor – 2-100 ppb Lead
Uranium100 sensor – 2-60 ppb Uranium
Copper High Range – 0.6-3 ppm Copper
Copper Low Range – 40-200 ppb Copper
Mercury100 Range – 2-50 ppb Mercury

The ANDalyze Copper sensor is available in two ranges and therefore dilution is usually not required.

1. **Dilution is best performed using standard laboratory glassware** and reagent grade water – one volume sample to nine volumes reagent grade water.
2. **Dilution may also be performed in the field**, with a decrease in accuracy, by withdrawing 5 mL sample with a 5 mL fixed volume pipette, adding the aliquot to a 50 mL self-standing tube, and filling to the 50 mL mark with reagent grade water. Shake well.
3. **If the sample is diluted**, on-site calibration must be performed with the diluted sample.

(2) General Protocol - pH Adjustment

ANDalyze Lead, Mercury and Copper sensors perform best when the sample pH is between 5 and 8 (pH 4-7 for Uranium). Samples with a pH greater than 8 or below 5 will not test reliably for Lead, Mercury, or Copper (greater than 7 or below 4 for Uranium). It is required to adjust the pH into this range before sample preparation steps and testing can continue. Samples above pH 10 should not be tested even with pH adjustment.

1. **Check the sample pH** using pH paper.
2. **Prepare the following solutions** if pH adjustment is required
    1. Sodium Hydroxide Neutralization Solution, 1% (w/w) sodium hydroxide
    2. Nitric Acid Neutralization Solution, 1.5% (v/v) nitric acid
3. **Adjust the sample pH**
    1. **If the sample is below pH 5** (or pH 4 for U) addition of a dilute sodium hydroxide solution is necessary. To a 50 mL volume of sample add the Sodium Hydroxide Neutralization Solution dropwise with stirring or with shaking between addition of each drop. Do not titrate beyond pH 5 for Lead, Mercury, and Copper and pH 4 for Uranium.

       Note: pH change from 4-5 is rapid, requiring a half drop or less. **Check the pH multiple times during titration.** The number of drops required depends heavily on matrix constituents. As few as four drops may be sufficient to increase pH from 3 to 4, or many more may be required.

    2. **If the sample is above pH 8** for Lead, Mercury, and Copper (above pH 7 for Uranium) addition of a dilute nitric acid (1.5 %) solution is necessary. Samples above pH may be unsuitable for testing even with pH adjustment as metal ion may have already precipitated out.

       Note: pH change from 9-8 is rapid, requiring a half drop or less depending on matrix. **Check the pH multiple times during titration.** The number of drops required depends heavily on matrix constituents. As few as four drops may be sufficient to decrease pH from 10 to 7, or many more may be required.

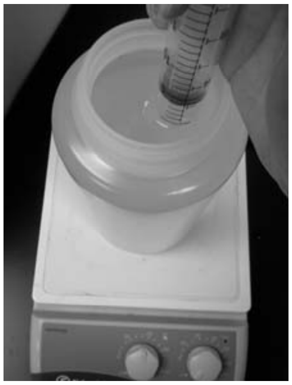
Note: For highly basic water samples, acidification may be insufficient to solubilize precipitated metals.

(3) – General Protocol - Filtration

1. **Obtain an ANDalyze Filtration Kit.** Before testing or spiking any environmental water sample, it must be filtered to remove suspended solids.

2. **Filter the water sample.** Draw ~20 mL water sample into a 20 mL syringe, securely attach the filter, and dispense into the self-standing vial.

   Note: If the sample is collected off-site and transported to a laboratory for testing, ensure that the sample is stirred (*e.g.*, stir-bar in the bottom of a 1 L HDPE Nalgene bottle filled with sample on a stir plate) while filling the syringe to ensure homogeneity.



3. The sample should be clear and the filter may no longer be white.

   Note: If a sample contains a great deal of suspended solids the syringe filter may clog after elution of 10-20 mL sample. In this case, discard the clogged filter and use a fresh filter to continue filtering the sample.

(4) General Protocol - Environmental Water On-site Calibration

On-site calibration is performed for all new samples and any time a matrix may have changed, *e.g*. new sampling day, change in matrix composition, new sensor batch, temperature change, etc. If in doubt, perform On-site Calibration.

Important: For environmental samples, it is required to incubate the calibration spike with the sample for at least 5 minutes as the spiked metal takes some time to reach equilibrium between dissolved and bound states. Failure to allow spike incubation in the sample will lead to lower recovery.

1. **Perform on-site calibration as described in the Product Manual.** After adding the 100 µL standard metal solution spike as per the instructions in the product manual, shake, and **let it sit for ~ 5 minutes** before the analysis is performed.

2. **Use all spiked solutions** within 15 minutes.

Note: The ANDalyze test kit is designed to test for bioavailable metals and not total metals without acid digestion, which is beyond the scope of this procedure.

Matrix-Specific Protocols

Surface Water Testing Protocol

ANDalyze has performed extensive testing of our kits in surface waters such as rivers, lakes, and streams. Some surface waters, such as runoff from industrial sites, heavily contaminated bodies of water, mine runoff, or areas affected by acid rain may exceed interference levels and the acceptable pH range. Special care may be needed in handling as well as testing these samples. Please contact ANDalyze with any questions.

Important: Testing of surface waters from rivers, lakes, and streams usually does not require dilution, pH adjustment, or iron interference removal. If required, perform those steps as stated in the protocols. Filtration, however, is always required.

1. **Check the pH using pH paper** and adjust if required.
2. **Filtration** is required as per the Filtration Protocol.
3. **Perform Environmental Water On-site Calibration** following the Environmental Water On-site Calibration Protocol (with incubation of calibration spike) and instructions in the *Testing and On-site Calibration* manual.

Groundwater Testing Protocol

Important: Testing of raw or treated groundwater usually does not require dilution or pH adjustment. If required, perform those steps as stated in the protocols. Iron interference may be an issue and the solution color should be noted – yellow/orange color may be indicative of iron. Filtration is always required.

1. **Check the pH using pH paper** and adjust if required.
2. **Verify that iron interference is not an issue.** If interference is suspected, follow the Iron Interference Solution Note.
3. **Filtration** is required as per the Filtration Protocol.
4. **Perform Environmental Water On-site Calibration** following the Environmental Water On-site Calibration Protocol (with incubation of calibration spike) and instructions in the *Testing and On-site Calibration* manual.

Note: ANDalyze has performed extensive testing of our kits in ground waters from across the U.S.A as well as in artificial matrices based on those in Standard Methods for the Examination of Water & Wastewater, Centennial Edition. As a general rule, performance in soft waters exceeds that of very hard waters, which are much more likely to exceed the interference level or pH range.

Hard Water Testing Protocol

ANDalyze, Inc. has performed extensive testing of our kits in simulated hard waters, including hard and moderately hard waters according to Standard Methods for the Examination of Water & Wastewater, Centennial Edition.

Important: Testing of hard waters usually does not require dilution, pH adjustment, or iron interference removal. If required, perform those steps as stated in the protocols. Filtration, however, may be required.

1. **Check the pH using pH paper** and adjust if required.
2. **Filtration** is required as per the Filtration Protocol **IF** the water is cloudy.
3. **Perform Environmental Water On-site Calibration** following the Environmental Water On-site Calibration Protocol (with incubation of metal spike) and instructions in the *Testing and On-site Calibration* manual.

Wastewater Testing Protocol

Important: ANDalyze has tested multiple **finished or treated** wastewater matrices and analysis can be challenging depending on matrix constituents. **Raw wastewater** is **NOT** suitable for testing. Dilution of the sample (1:10) is absolutely required, which increases the **effective detection range tenfold**, *e.g.* the detection range for Lead after dilution is 20-1000 ppb. Analyte concentrations measured at the low end of the sensor ranges are **qualitative** rather than quantitative and the relative standard deviation of results is larger than experienced in drinking water.

3. **Precautions!** Wastewaters are complicated matrices and may contain interferences beyond other metal ions.
    - **Chelators** such as EDTA will cause false negatives. Chelators are present in many cleaning products and industrial processes, so check wastewater components carefully.
    - **Fluorescent compounds** will give a high background signal and results may be unreliable
    - **Very high concentration of other metal ions** – Example: Known metals from a metal finisher plant
    - **Wear personal protective equipment**. Wastewaters may have extreme pH values and contain hazardous components. Wear appropriate laboratory attire and use a fume hood as appropriate.

2. **Follow the Dilution Protocol to dilute the sample tenfold.** Remember that your effective detection range has increased tenfold.
3. **Check the pH using pH paper** and adjust if required.
4. **Verify that iron interference is not an issue.** If interference is suspected, follow the Iron Interference Solution Note.
5. **Filtration** is required as per the Filtration Protocol. If filters clog rapidly then pre-filtration through Whatman 3MM paper or acid digestion may be necessary.
6. **Perform Environmental Water On-site Calibration** following the Environmental Water On-site Calibration Protocol and instructions in the *Testing and On-site Calibration* manual.
    1. **If on-site calibration fails, repeat.**
    2. **If on-site calibration fails again**, further dilution may be necessary. Dilute the sample another tenfold (total 100-fold dilution). Be aware that the analyte concentration may be out of the linear detection range upon 100-fold (total) dilution.

Note: Some waste water matrices have many interferences and cannot be effectively analyzed without acid digestion or other treatment procedures, which are beyond the scope of this solution note.

Seawater Testing Protocol

Important: Sample preparation steps for seawater are normally not necessary beyond filtration unless the sample is taken from a polluted area. This protocol is intended for analysis of seawater from the surface or water column with minimal sediment. Analyte concentrations measured are **qualitative/ semi quantitative** rather than quantitative and the relative standard deviations of results are larger than experienced in drinking water.

1. **Filtration** is required as per the Filtration Protocol.
2. **Perform Environmental Water On-site Calibration** following the Environmental Water On-site Calibration Protocol and instructions in the *Testing and On-site Calibration* manual.
3. Dilution (tenfold) may be performed if spike recovery is very low.

Note: Bittern Water is a concentrated solution left over after crystallization of NaCl from seawater. It contains very high concentrations of interfering ions, notably magnesium. ANDalyze test kits may work in bittern water upon dilution of the sample by at least ten-fold if not 100-fold. Contact ANDalyze for further details.

Appendix D

Iron Interference with Lead100 Sensor Solution Notes (AND-Sol-Lead-02-2012)

ANDalyze

Document: AND-Sol-Lead-02-2012

Iron Interference with the Lead100 Sensor

Minimization of interference from soluble iron in environmental water

Problem Statement

The presence of soluble iron has a negative effect on sensor performance. The interference level for the ANDalyze Lead sensor is defined as the level of an ion at which the signal of a 30 ppb Pb^{2+} solution is changed by ±10%. The interference level for soluble Fe(III) is 40 ppb. Insoluble iron in the form of precipitates may be removed, at least in part, through natural settling or filtration. Steps to minimize interference are provided below.

Materials

ANDalyze pH Adjustment Kit
- Sodium Hydroxide Neutralization Solution, 1% (w/w) sodium hydroxide in a dropper bottle
- Nitric Acid Neutralization Solution, 1.5% (v/v) nitric acid in a dropper bottle
- pH paper

ANDalyze Iron Interference Kit
- Sodium Hydroxide Neutralization Solution, 1% (w/w) sodium hydroxide in a dropper bottle
- Hydrogen Peroxide Solution, 30% (w/w) hydrogen peroxide in a dropper bottle

ANDalyze Filtration Kit **(Available now from ANDalyze)**
- 0.2 µm Nylon filter, 25 mm diameter (Nalgene)
- 20 mL Syringe
- 50 mL Self-standing sample tube

Solution Statement

Important: Ground water samples visibly orange in color are likely to contain high levels of iron (low ppm), though lower levels may not be easily detected with the naked eye. If it is suspected that the water sample to be tested contains interfering levels of iron, it may be prudent to test the iron concentration using a commercial iron test kit.

Important Safety Note: Refer to the product manual for general guidelines on safety, proper use, and general sample testing procedures before using the testing protocol listed in this solution note. Be very careful when handling the sodium hydroxide solution and the hydrogen peroxide solution. Wear gloves and eye protection. Exposure to concentrated hydrogen peroxide will result in burns. See the manufacturer Material Safety Data Sheet for further information.

Note: Although no individual ion may exceed the interference levels, a combination of many interfering ions close to the maximum levels may have an effect meeting or exceeding that of a single ion at the interference level.

pH Adjustment Protocol

ANDalyze Lead sensors perform best when the sample pH is between 5 and 8. Samples with a pH greater than 8 or below 5 will not test reliably for Lead. It is required to adjust the pH into this range before sample preparation steps and testing can continue. **Samples above pH 10 should not be tested for Lead**, even with pH adjustment.

1. **Check the sample pH** using pH paper.
2. **Prepare the following solutions** if pH adjustment is required
    1. Sodium Hydroxide Neutralization Solution, 1% (w/w) sodium hydroxide
    2. Nitric Acid Neutralization Solution, 1.5% (v/v) nitric acid
3. **Adjust the sample pH**
    1. **If the sample is below pH 5** addition of a dilute sodium hydroxide solution is necessary. To a 50 mL volume of sample add the Sodium Hydroxide Neutralization Solution dropwise with stirring or with shaking between addition of each drop. Do not titrate beyond pH 5 for Lead.

       Note: pH change from 4-5 is rapid, requiring a half drop or less. **Check the pH multiple times during titration.** The number of drops required depends heavily on matrix constituents. As few as four drops may be sufficient to increase pH from 3 to 4, or many more may be required.

    2. **If the sample is above pH 8** for Lead, addition of a dilute nitric acid (1.5 %) solution is necessary. Samples above pH 10 should not be tested even with pH adjustment.

       Note: pH change from 9-8 is rapid, requiring a half drop or less depending on matrix. **Check the pH multiple times during titration.** The number of drops required depends heavily on matrix constituents. As few as four drops may be sufficient to decrease pH from 10 to 7, or many more may be required.

Note: For highly basic water samples, acidification may be insufficient to solubilize precipitated metals.

Peroxide Treatment Protocol

The addition of hydrogen peroxide converts soluble iron to insoluble iron which can then be removed by filtration.

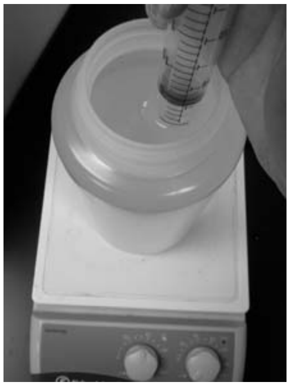
1. **Hydrogen peroxide addition.** Add 4 drops of the hydrogen peroxide solution to each ~50 mL water sample using a dropper, replace cap on the sample tube, mix well by inversion, then let sit on the bench for 20 min.
2. **After the 20 min incubation** continue with the Filtration Protocol described below.

Filtration Protocol

1. **Obtain an ANDalyze Filtration Kit.** Before testing or spiking any environmental water sample, it must be filtered to remove suspended solids.

2. **Filter the water sample.** Draw ~20 mL water sample into a 20 mL syringe, securely attach the filter, and dispense into the self-standing vial.

   Note: If the sample is collected off-site and transported to a laboratory for testing, ensure that the sample is stirred (e.g., stir bar in the bottom of a 1 L HDPE Nalgene bottle filled with sample on a stir plate) while filling the syringe to ensure homogeneity.



3. The sample should be clear and the filter may no longer be white.

   Note: If a sample contains a great deal of suspended solids the syringe filter may clog after elution of 10-20 mL sample. In this case, discard the clogged filter and use a fresh filter to continue filtering the sample.

After completion of the Filtration Protocol continue with On-site Calibration as described in the *Testing and On-site Calibration* manual.

Summary/Notes/References

It is expected that, upon completion of this procedure, the interference from Iron will be greatly reduced and that a more accurate reading of Lead concentration will be obtained. It should be noted that some co-precipitation of Iron and Lead may be possible.

Appendix E

3-Point Laboratory Calibration (AND-Sol-Cal-02-2012)

3-Point Laboratory Calibration

Laboratory calibration or renewal of AND1000 fluorimeter factory calibration

Problem Statement

The AND1000 fluorimeter is a field test device designed to obtain a quick result on-site, and as such, a single point standard is used for on-site calibration. However, as with any analytical technique, a calibration performed with 3 concentrations will yield more accurate results over the entire detection range. A 3-point calibration is recommended for laboratory evaluations and is available as an option on the AND1000 fluorimeter. 3-Point calibrations are typically completed at the factory as part of the production and quality control process and are not described in the user manual. This solution note is made available to the end user as under certain circumstances it may be required for the end user to perform a 3-point calibration. Proper calibration is critical to the accuracy of the AND1000 fluorimeter so please follow these instructions closely and if any issues arise, please contact ANDalyze. The protocol for laboratories to perform a 3-point calibration is as follows:

Important: On-site calibration factors associated with various sites are dependent on the 3-point calibration performed using distilled water at the factory. If you change the 3 point calibration, previously saved on-site calibrations factor will no longer remain valid and will have to be re-done.

Materials: Required Equipment & Solutions (For EACH metal to be calibrated)

- AND1000 Fluorimeter
- (3) Sensor Bags with Sensor & Cuvette
- (3) 1 mL Syringes
- (3) Samples Tubes (with buffer
- (3) 3-Point Calibration Metal Standard Solutions (specific to metal) prepared freshly from a high concentration metal stock*
    - Lead – 25 ppb, 50 ppb, 75 ppb
    - Uranium – 10 ppb, 30 ppb, 50 ppb
    - Copper Low – 50 ppb, 100 ppb, 150 ppb
    - Copper High - 0.75 ppm, 1.5 ppm, 2.25 ppm
- Pipette with pipette tips to make 3-Point Calibration Metal Standard Solutions. The 100 µL fixed-volume pipette supplied with the AND1000 fluorimeter is insufficient for preparation of these solutions.

* **ANDalyze test kits do not contain a high concentration stock** for preparation of solutions for 3-point calibration. Users can prepare the calibration solution from an acid-preserved metal stock solution purchased from a standard chemical company or contact ANDalyze customer service to obtain metal stock solution for 3-point calibration.

Preparation of Standard Metal Solutions

Use a high concentration metal standard (*e.g*, 1000 ppm) for making calibration solutions so that the acid used to preserve these standards (1-5% usually) is sufficiently diluted when you make the ppb level solutions and therefore does not require any neutralization (samples must be pH 5-8 for successful analysis). Also, volume changes to the sample upon addition of standard will be negligible.

Note: Calibration solutions prepared in water should be used within 1 hour of preparation to avoid changes in concentration from metal adsorbing to the walls of the container. Once mixed with ANDalyze buffer, it must be used within 5 – 15 minutes.

For example: To make 50 mL of the following 3-Point Calibration Lead Standard Solutions:

25 ppb Pb^{2+}: 50 mL water + 125 µL, 10 ppm Pb^{2+}
50 ppb Pb^{2+}: 50 mL water + 250 µL, 10 ppm Pb^{2+}
75 ppb Pb^{2+}: 50 mL water + 375 µL, 10 ppm Pb^{2+}

High concentration metal stocks can be purchased from any scientific supply company, such as Sigma-Aldrich:

- Lead Standard for ICP 1000 µg/mL (ppm): Sigma Aldrich product number: 41318
- Uranium atomic absorption standard solution 1000 µg/mL (ppm): GFS Chemicals product number: 1829
- Copper Standard for ICP 1000 µg/mL (ppm): Sigma Aldrich product number: 68921

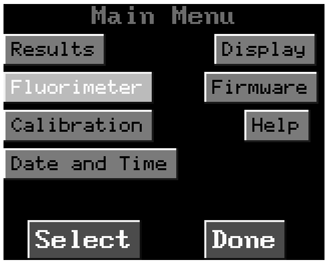
To Perform a New 3-Point Calibration for the Lead Sensor

Prepare 25, 50, and 75 ppb Lead solutions in water starting with a high concentration Lead standard (preferably a 1000 ppm commercial stock solution, first diluted to 10 ppm, to dilute out acid present in the standards) as described above.

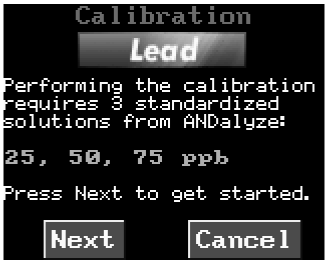
Method:

1. In the Lead screen, press **MENU**

2. Select **CALIBRATION** in the main menu



3. At the calibration screen the user is prompted to insert 25, 50, 75 ppb Lead in three separate steps. Press **NEXT**.



Performing the calibration requires 3 standardized solutions from ANDalyze:

25, 50, 75 ppb

Press Next to get started.

4. **A Pre-Calibration Screen will appear**. Press **OK.** The AND1000 fluorimeter will perform the required pre-calibration and proceed to the next step.

5. **The first screen prompts the user to insert a 25 ppb sample**. This should be the 25 ppb lead sample that you have prepared in distilled water, mixed in with the ***sample buffer present in the ANDalyze sample tubes***. Inject 1 mL of the buffered sample through the sensor housing as per instructions in the *Testing and On-site Calibration* manual user manual.

6. **Press next after the test is completed** and follow the same instructions for the 50 and 75 ppb lead solutions.

7. **To accept the calibration**, select **APPLY**

Note: After a 3-Point Calibration is performed with a given matrix On-site Calibration is not required before testing the matrix. In this case only the "site" field on the fluorimeter should be set to "none"

To Perform a New 3-Point Calibration for the Copper or Uranium Sensor:

Perform steps 1 – 7 as described above for Lead, where step 1 should correspond to the metal that you are interested in calibrating.

For the Copper sensor (0.6 – 3 ppm) range: Prepare 0.75, 1.5, and 2.25 ppm copper solutions and proceed as per instructions on the screen and in the test manual for testing a sample solution.

For the Copper sensor (40- 200 ppb) range: Prepare 50, 100, and 150 ppb copper solutions and proceed as per instructions on the screen and in the test manual for testing a sample solution.

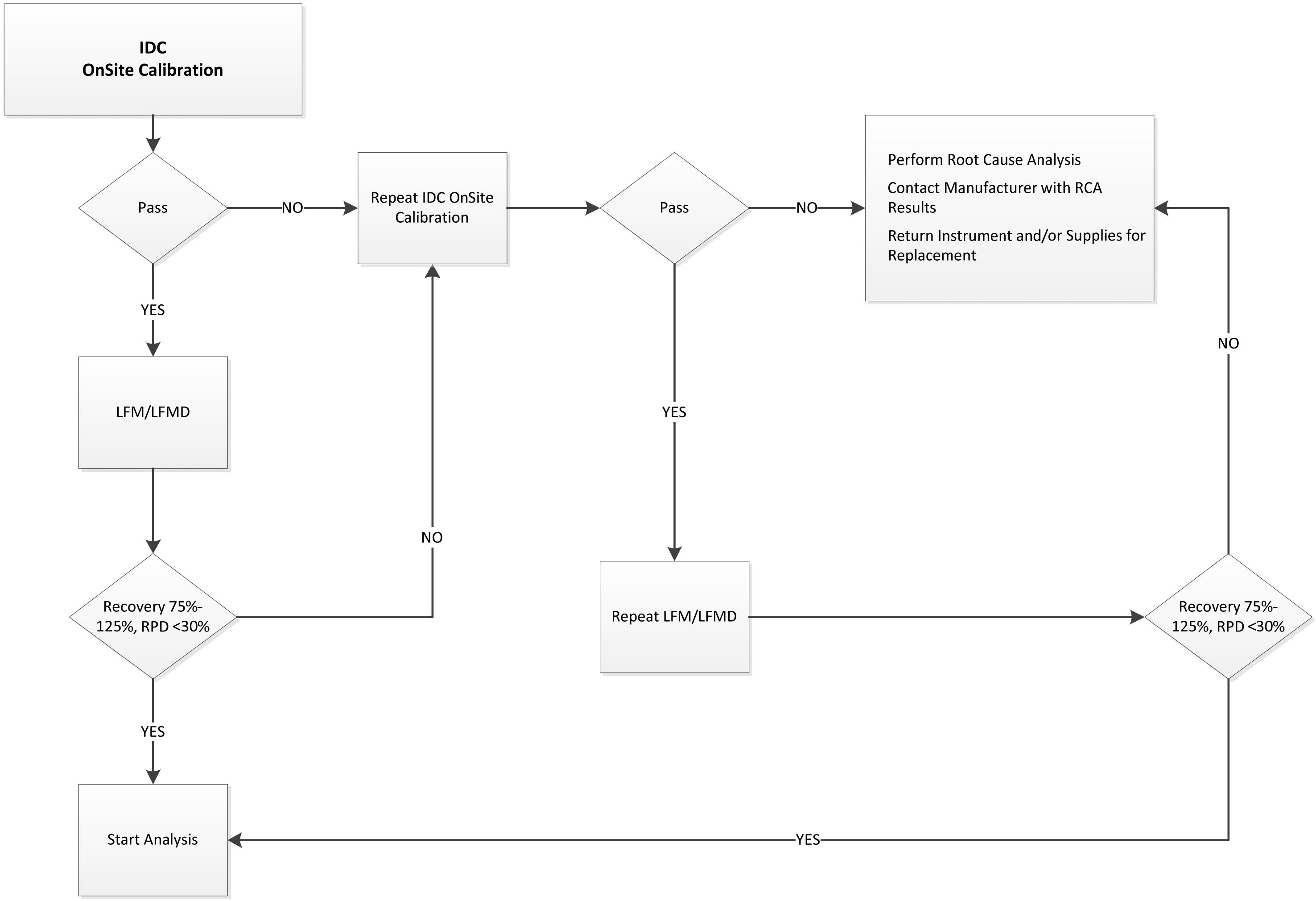
For the Uranium sensor: Prepare 10, 30, and 50 ppb uranium solutions and proceed as per instructions on the screen and in the test manual for testing a sample solution.

How Does an On-site Calibration Work?

When a user performs an on-site calibration, the fluorimeter software calculates (a) the lead concentration (ppb) in the sample and (b) the lead concentration (ppb) in the sample spiked with 25 ppb lead, using the calibration curve from the 3-Point Calibration. The fluorimeter software then calculates an accuracy factor based on % recovery. Accuracy factor = 25/(b-a). This factor is stored for a unique site that is entered by the user. The sample concentration displayed after an on-site calibration = a * accuracy factor

Appendix F

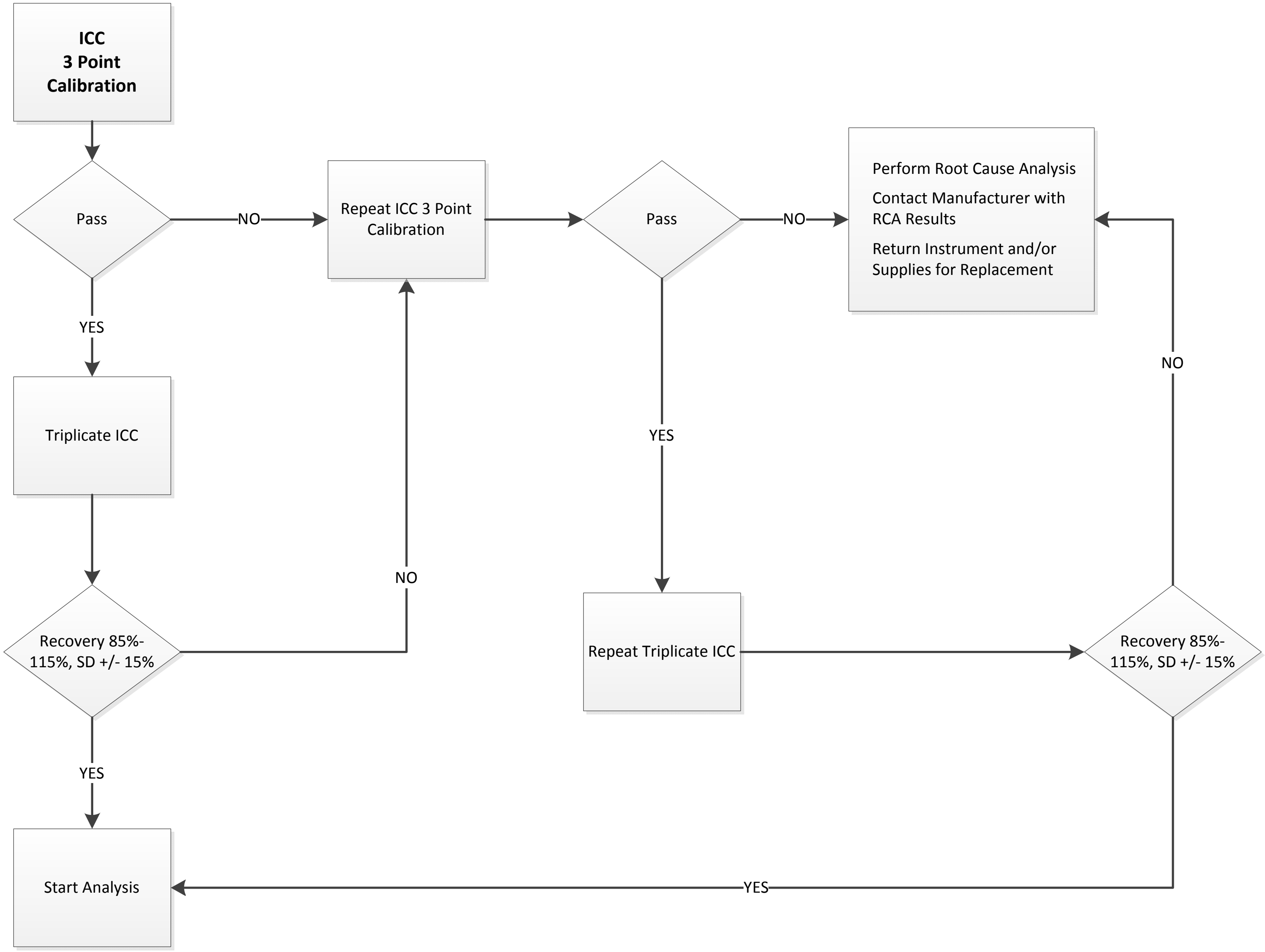
Root Cause Analysis Flowchart: On-site Calibration and IDC



IDC
OnSite Calibration
Pass
NO
YES
Repeat IDC OnSite Calibration
Pass
NO
Perform Root Cause Analysis
Contact Manufacturer with RCA Results
Return Instrument and/or Supplies for Replacement
LFM/LFMD
NO
YES
NO
Recovery 75%-125%, RPD <30%
Repeat LFM/LFMD
Recovery 75%-125%, RPD <30%
YES
Start Analysis
YES

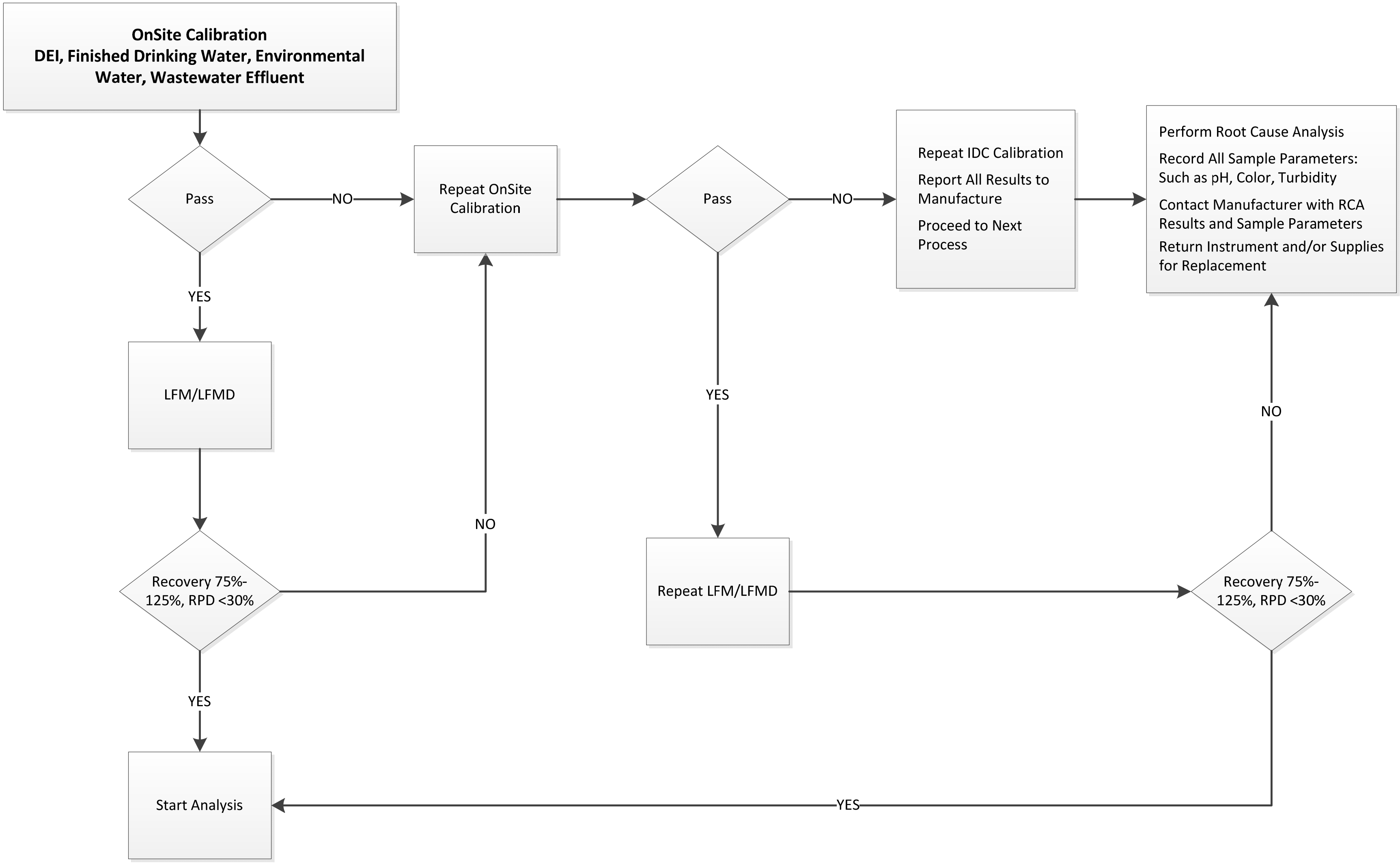
Appendix G

Root Cause Analysis Flowchart: On-Site Calibration, ICC and 3-Point Calibration


ICC
3 Point
Calibration
Pass
NO
Repeat ICC 3 Point Calibration
Pass
NO
Perform Root Cause Analysis
Contact Manufacturer with RCA Results
Return Instrument and/or Supplies for Replacement
YES
Triplicate ICC
NO
YES
NO
Recovery 85%-115%, SD +/- 15%
Repeat Triplicate ICC
Recovery 85%-115%, SD +/- 15%
YES
Start Analysis
YES

Appendix H

Root Cause Analysis Flowchart: On-Site Calibration, DEI, Finished Drinking Water, Environmental Water, Wastewater Effluent


OnSite Calibration
DEI, Finished Drinking Water, Environmental Water, Wastewater Effluent
Pass
NO
Repeat OnSite Calibration
Pass
NO
Repeat IDC Calibration
Report All Results to Manufacture
Proceed to Next Process
Perform Root Cause Analysis
Record All Sample Parameters: Such as pH, Color, Turbidity
Contact Manufacturer with RCA Results and Sample Parameters
Return Instrument and/or Supplies for Replacement
YES
LFM/LFMD
YES
NO
NO
Recovery 75%-125%, RPD <30%
Repeat LFM/LFMD
Recovery 75%-125%, RPD <30%
YES
Start Analysis
YES