SAMPLE ANALYSIS AND QUALITY ASSURANCE PLAN

for

URINARY ARSENIC AND BLOOD LEAD AMONG RESIDENTS OF VBI70 NEIGHBORHOODS

June 2002

Produced by:

US Environmental Protection Agency, Region 8
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APPROVAL PAGE

This Sampling Analysis and Quality Assurance Project Plan, written for the University of Colorado Health Science Center arsenic/lead exposure and biomonitoring study, has been prepared at the request of the U.S. Environmental Protection Agency, Region 8, by Syracuse Research Corporation. Study investigations and activities addressed in this Project Plan are approved without condition.

Technical Approval Bonnie Lavelle USEPA Project Manager	Date
William Brattin, PhD Syracuse Research Corporation	Date

1.0 INTRODUCTION

USEPA Region 8 will assist the University of Colorado Health Science Center (UCHSC) in their survey of soil arsenic and lead exposure among residents of VBI70 neighborhoods and will be responsible for the chemical analysis of all samples collected during the survey. This document details the sample handing, analysis, and quality assurance procedures that will be followed during this support activity.

2.0 ANALYSIS OF URINE

Samples of urine will be collected by UCHSC staff from study participants in accord with the protocols described in Appendices G, H, and I. Each collection container will be labeled in the field with a unique sample identification number using the following format:

UCHSC VBI70 Uxxxx

One copy of the label will be applied directly to the sample collection vessel. A second copy of the label will be applied to the survey form used to record the name and address of the sample donor. A third copy of the label will be used to label the sample tube shipped to the laboratory (see below).

2.1 Sample Holding and Preparation

a. Each urine sample will be held under refrigeration at approximately 4°C in its original collection container. Under these conditions, the holding time for un-preserved urine is 3 months. Samples should be maintained in a restricted access area at all times

b. Either daily or once per week (whichever is most convenient), all samples scheduled for analysis will be well mixed by swirling the urine container, and 3-4 mL will be removed by pipette and transferred to a 15-mL plastic screw-cap tube. This tube will be labeled with the same number as that given to the original parent sample (see above). After transfer of the aliquot, the cap of the tube will be screwed on tight to prevent leakage, and the tube will be placed in a test tube rack. This sub-sample will be submitted to the analytical laboratory for analysis. The remainder of the sample will be stored in a refrigerator until the sample has been successfully analyzed by the laboratory.

2.2 Sample Shipment to the Laboratory

Packaging

Samples will be packaged for shipment to the analytical laboratory in test tube racks. Each rack of tubes will be placed inside a large plastic bag to ensure that the tubes do not fall out of the rack and to help contain any spills or leaks. Sealed bags will be placed in a cooler and secured against excess movement by addition of plastic bubble wrap. Adsorbent material should be added around the bags in case of any spills or leaks. The cooler will be kept cool by inclusion of 3-4 frozen "blue ice" packages. Once filled, the cooler will be sealed with tape and a signed custody seal will be placed across the opening of the shipping container in order to ensure that no tampering occurs during the shipping process.

Shipping

National Medical Services (NMS) will perform all analyses for urinary arsenic. The shipping address and contact information is presented below:

National Medical Services
3701 Welsh Rd
P.O. Box 433A
Willow Grove, Pennsylvania 19090-0437
1-800-522-6671

Each cooler will be shipped to the laboratory by overnight transport. The laboratory shall be notified by phone to expect each shipment, and shipping should always occur so that someone is present at the laboratory to receive the shipment. Shipment on a Monday or Tuesday is generally preferred.

No special labeling is required for urine samples.

Chain of Custody

Each cooler shipped to the laboratory will be accompanied by a Chain of Custody (COC) form. These COC forms are to be prepared in triplicate on carbonless forms using the approach specified in SOP No. MK-VBI70-02. SRC will provide the field team with COC forms. Each

COC form will identify the samples included in the sample delivery group (SDG) (i.e., in the cooler) and the required analyses. Each complete COC form will be reviewed for accuracy and clarity by UCHSC staff before shipment, and then signed. The pink (bottom) copy of the form will be kept by UCHSC as documentation of the date and contents of the shipment. The white (top) and yellow (middle) copies of the COC form will be sealed inside the shipping container but inside a plastic zip-lock bag to avoid damage from moisture.

When the cooler arrives at the laboratory, the laboratory sample custodian will document the date and time of receipt. The cooler will be opened and the contents inspected. The chain-of custody form will be reviewed, and any tubes that are missing, broken, or otherwise questionable or compromised will be noted on the COC form. The laboratory will notify EPA's contractor (Syracuse Research Corporation, SRC) of any such problems immediately, and SRC will instruct the laboratory or arrange for replacement samples to be shipped, as needed. Once all samples have been reviewed and all issues have been resolved, the laboratory sample custodian will sign and date the COC form. Both remaining COC form copies (white and yellow) will be appropriately filed by the laboratory sample custodian.

2.3 Sample Analysis

2.3.1 Total Non-Dietary (Inorganic) Arsenic

All samples of urine will be analyzed for total non-dietary arsenic. This total includes both trivalent and pentavalent forms of inorganic arsenic (As+3, As+5), as well as the primary urinary metabolites of these forms (monomethyarsonate (MMA), and dimethylarsinate (DMA)). Complex organic arsenicals found in seafood (e.g., arsenobetaine) are not included in the total.

Details of the sample preparation and analysis are proprietary. In general, iodine is added to the sample and the target analytes are extracted into an organic phase under acidic conditions. A portion of this extract is analyzed for arsenic via ICP-MS. The detection limit is approximately 1.0 ug/L.

Each urine sample will also be analyzed for creatinine.

2.3.2 Total Urinary Arsenic

In some cases, samples of urine may require re-analysis to determine if an elevated level of arsenic might be due to dietary arsenic. SRC will identify samples requiring total urinary arsenic analysis and provide the list of samples to the laboratory.

NMS will analyze specified urine samples for total arsenic by ICP-MS. This method detects all forms of arsenic, including arsenobetaine and other related organic forms that occur in the diet. Details of the analytical method used by NMS for total urinary arsenic analysis are proprietary.

The detection limit is approximately 1 ug/L. Data will be reported to SRC as above.

2.4 Quality Control

Quality Control (QC) consists of the collection of data that allow a quantitative evaluation of the accuracy and precision of the data collected during the project. QC samples that will be collected for urinary arsenic during this project include the following types of samples:

Laboratory-Based QC Samples

The analytical laboratory will collect several types of data that help assess the accuracy and precision of the sample preparation and analysis procedure. The analytical laboratory will use BioRad laboratory control samples. These samples are prepared from human urine and contain inorganic and organic forms of arsenic. Specifically, following each calibration of the instrument, the analytical laboratory will analyze one blank, one BioRad Level 1 (target = 56 ug/L, range 42 - 70ug/L), and one BioRad Level 2 (target = 137 ug/L, range 103-172 ug/L) laboratory control sample. After every 10 analyses, the analytical laboratory will analyze one LCS, alternating between high and low levels. Furthermore, one non-dietary LCS (certified for MMA, DMA, As+5) and one dietary LCS (certified for MMA, DMA, As+5, and arsenobetaine) will be analyzed once a week to ensure the inorganic arsenic is correctly extracted and no dietary arsenic is included in the analyses. If any of the LCS values are outside the specified acceptance criteria (2 standard deviations from the mean based on 20 measurements), all samples analyzed since the last successful LCS analysis will be re-analyzed.

Field-Based QC Samples

Field-based QC samples are samples that are prepared by the study team and are submitted to the analytical laboratory in a blind fashion. That is, the laboratory is not aware the sample is a QC sample, and should treat the sample in the same way as a field sample. Two types of blind field QC sample will be submitted in this program:

Field Splits

A field spilt sample is prepared by withdrawing a second 3-4 mL aliquot of a parent urine sample and submitting that to the laboratory under a different (and unique) sample number from the first aliquot. The results of field split sample analysis help evaluate analytical precision (reproducibility). Split samples will be prepared at a rate of approximately 5%, and submitted in random order.

PE Samples

Performance Evaluation (PE) samples are samples of urine that contain a known and certified level of a contaminant. The results of PE sample analysis help evaluate analytical accuracy. In brief, the "blank" PE sample were prepared by collecting urine from adult volunteers who had not ingested seafood for at least three days. Note that the concentration of total inorganic arsenic in this sample is not zero, but is about 4 ug/L. Other PE samples were be prepared by spiking this "blank" urine with known incremental concentrations (5 ug arsenic/L and 15 ug arsenic/L) of sodium arsenate (As+5), sodium arsenite (As+3), monomethylarsonate (MMA), dimethylarsenate (DMA), or arsenobetaine. One concentration (20 ug/L) of arsenobetaine was prepared. Thus, there are a total of 10 different PE samples for this program. Nominal concentrations for each PE sample were established by the laboratory preparing the PE samples. These results are shown in Table 1.

As part of the first sample shipment, two samples of each of these 10 PE samples will be submitted in random order. For subsequent weekly shipments, one of each standard type will be submitted in random order.

QC Acceptance Criteria

The acceptance criterion for field split samples is a Relative Percent Difference (RPD) of no more than 30%. This acceptance criterion may be revised as data become available.

For all PE samples except arsenobetaine analyzed for total inorganic arsenic, the acceptance criteria will be ± 20% of the nominal value shown in Table 1. For arsenobetaine analyzed for total inorganic arsenic, the acceptance criteria are equal to the criteria for blank urine, with an increment of no more than 10% of the spiked arsenobetaine level. For the arsenobetaine PE sample analyzed for total arsenic, the acceptance criteria is ± 20% of the nominal value shown in Table 1. These acceptance criteria may be revised as data become available.

QC Assessment and Response Actions

Results for QC samples will be reviewed by SRC promptly upon receipt from the laboratory. Any deviation of a QC sample from the acceptance criteria above will be evaluated and a corrective action selected. If deviations are minor (only slightly outside the acceptance bounds) and are not consistent over time or sample type, no action will be required. If deviations are consistent (occurring in two or more consecutive weeks) or if deviations are not trivial, SRC will immediately contact the laboratory to discuss possible causes and appropriate laboratory corrective actions.

2.5 Reporting

NMS will submit a weekly report to SRC which includes a Microsoft Excel summary of the results for each sample, including the total inorganic arsenic concentration (ug/L), urinary creatinine concentration (g/L), and total inorganic arsenic concentration normalized for creatinine (ug/g). The report will also include a summary of the laboratory QC samples for each SDG.

3.0 ANALYSIS OF BLOOD

3.1 Sample Collection

In brief, the tip of the participant's finger is carefully washed and dried, and the finger is pierced using a sterile lancet. Once a drop of blood has collected on the finger tip, the blood is drawn into a plastic capillary tube that is held in a microtainer. When the capillary is filled, the capillary is tipped upright, allowing the blood to drain into the microtainer. The capillary and the lancet are discarded as biomedical waste, and the microtainer is sealed with the attached cap.

Each microtainer will be assigned a unique sample identifier using the following format :

UCHSC VBI70 Bxxxx

One copy of the label is applied to the microtainer by wrapping it around the bottom portion of the microtainer. A second copy of the label is applied to the field survey form that records the name and address of the sample donor. A database will be used to record and correlate the sample identification number and the name and address of the sample donor.

All microtainers will be stored in bubble wrap envelopes (20-25 microtainers per envelope) and stored refrigerated at 4°C.

3.2 Sample Preparation

No sample preparation is necessary prior to sample shipment.

3.3 Sample Shipment to the Laboratory

Packaging

Samples will be shipped to the analytical laboratory in plastic bubble wrap envelopes. Each envelope will be sealed to ensure that the tubes do not fall out during shipment. Envelopes will be placed in a cooler and secured against excess movement by addition of extra plastic bubble wrap. The cooler will be kept cool by inclusion of 3-4 frozen "blue ice" packages. Once filled, the cooler will be sealed with tape and a signed custody seal will be placed across the opening of the shipping container in order to ensure that no tampering occurs during the shipping process.

Shipping

All analyses of blood will be performed by Tamarac Medical, Inc. The shipping address and contact information for the laboratory are provided below:

Tamarac Medical, Inc.
7000 South Broadway #2C
Littleton, CO 80122
(800) 842-7069

Delivery of the samples from UCHSC to the laboratory will occur about once per week. Transport will normally by courier sent from the laboratory. UCHSC staff will contact the laboratory when a shipment is ready, and arrange for the details of the shipment. In the event that this method is not available, a UCHSC staff member will contact the laboratory and transport the samples to the analytical facility at an agreed upon time.

Chain of Custody

Each cooler shipped to the laboratory will be accompanied by a Chain of Custody (COC) form. These COC forms are to be prepared in triplicate on carbonless forms using the approach specified in SOP No. MK-VBI70-02. Each COC form will identify the samples included in the sample delivery group (SDG) (i.e., in the cooler) and the required analyses. Each complete COC form will be reviewed for accuracy and clarity by UCHSC staff before shipment, and then signed.

One copy of the form will be kept by UCHSC as documentation of the date and contents of the shipment. The other copies of the COC form will be sealed inside the shipping container but inside a plastic zip-lock bag to avoid damage from moisture.

When the cooler arrives at the laboratory, the laboratory sample custodian will document the date and time of receipt. The cooler will be opened and the contents inspected. The chain-of custody form will be reviewed, and any tubes that are missing, open, or otherwise questionable or compromised will be noted. The laboratory will notify SRC of any such problems immediately.

3.4 Sample Analysis

Each blood sample will be analyzed for lead using the method of Miller (1987). See SOP # 01 for method details. In brief, the method uses graphite furnace atomic absorption spectrometry (GFAAS) to analyze blood samples for lead. In order to stabilize the blood, all blood samples will be combined with known volumes of Metexchange reagent. This method requires a minimum volume of 50 uL of blood, with 100 uL being desirable. The detection limit is 2.0 ug/dL.

3.5 Quality Assurance

Laboratory-Based QC Samples

Following each calibration, Tamarac will analyze one low (8.0 ug/dL), one medium (20.0 ug/dL), and one high (40.0 ug/dL) LCS. Upon a successful run, Tamarac will analyze a one set of 10 field samples followed by two sets of 11 field samples. Following each series, Tamarac will analyze one medium and one high LCS. Once each series of samples have been analyzed with all LCS samples passing established acceptance criteria, Tamarac will re-calibrate the machine and repeat the steps above. The acceptance criterion is defined as an LCS that is within 2.0 ug/dL of the nominal concentration.

In the event that a QC sample result is outside the acceptance criteria, Tamarac will re-calibrate the machine and re-analyze the LCS. If the LCS fails twice, Tamarac will stop the machine and take appropriate measures to correct the problem.

Field-Based QC Samples

Field Duplicate

Field duplicate samples are collected at the same time as the primary sample. In this case, the field duplicate sample is a second sample of blood drawn from the same individual, by filling a second capillary tube immediately after filling the first tube. These samples will be collected opportunistically from those individuals with sufficient blood flow after a single finger prick. Each field duplicate sample is assigned a unique sample identifier that is not related to the sample identifier for the primary field sample. Field duplicate samples will be collected and submitted in random order at a frequency of about 5%, if possible.

PE Samples

PE samples for blood lead analysis will be provided by the Centers for Disease Control and Prevention. Consensus (nominal) concentrations of lead in these samples were determined by CDC using GFAAS or ICP-MS.

A summary of these samples is listed below:

CDC Sample ID	Nominal Value (ug/dL)
194	0.4
1494	4.5
994	8.9
396	14.8

PE samples will be prepared for submittal to the laboratory by withdrawing 50-75 uL samples from the CDC stock samples using the sample capillary sampling device as is used to collect blood samples from program participants. Microtainers containing PE samples will be assigned random sample numbers and submitted blind to the laboratory along with the field samples. Each week, one sample of each PE sample above (i.e., a total of 4) will be submitted. In the first shipment of samples, two of each PE sample will be submitted.

QC Acceptance Criteria

Field Duplicates

The acceptance criterion for field split samples is a maximum difference of 2.0 ug/dL between the primary sample and the field duplicate sample. This acceptance criteria value may be revised as data become available.

PE Samples

In accord with recommendations from CDC, the acceptance criterion for all blood PE samples with nominal concentrations below 40 ug/dL is ± 4 ug/dL. This acceptance criteria value may be revised as data become available.

QC Assessment and Response Actions

Results for QC samples will be reviewed by SRC promptly upon receipt from the laboratory. Any deviation of a QC sample from the acceptance criteria above will be evaluated and a corrective action selected. If deviations are minor (only slightly outside the acceptance bounds) and are not consistent over time or sample type, no action will be required. If deviations are consistent (occurring in two or more consecutive weeks) or if deviations are not trivial, SRC will

immediately contact the laboratory to discuss possible causes and appropriate laboratory corrective actions.

3.6 Reporting

Tamarac will provide EPA's contractor (SRC) a weekly electronic (Microsoft Excel) report of sample results and a hard copy machine printout of all QC results. Results will be delivered to SRC via email. However, any sample results above 10 ug/dL will either be phoned or faxed to SRC immediately following sample analysis.

When a sample of blood from a program participant is reported to exceed a value of 10 ug/dL, SRC will promptly report the sample number, name, and address to UCHSC staff, who will seek to arrange for collection of a second (confirmation) sample from the individual. Whenever possible, this sample will be a venous sample collected in a vacutainer by a trained pediatric phlebotomist. This sample will be submitted to Tamarac with a unique identifier number for analysis for lead, as above. If the second sample confirms a blood lead level above 10 ug/dL, SRC will provide Tamarac with the name and address of the donor, and in accord with Colorado State law (6 CCR-1009-7), Tamarac will report the value to the Colorado Department of Public Health and Environment (CDPHE) within 30 days.

5.0 FIELD AUDIT ACTIVITIES

As part of the overall Quality Assurance Plan for this project, EPA will provide random audits of field data and sample collection procedures by UCHSC staff. This will include ensuring that all surveys are administered properly and that responses are recorded correctly and with consistency between different teams, that biological samples are collected properly, and that biological sample identifier information is correct and complete. Audits will include observations of each different field team's activities, including initial visits and return visits for repeat sample collection. Audits will be concentrated in the beginning of the program, but will continue at random times throughout the program.

Any issues or problems observed by the field auditors will be reported to EPA and the UCHSC team leader, both verbally and in written (memo) form, along with any suggestions for addressing those problems.

6.0 REFERENCES

Colorado Department of Public Health and Environment (CDPHE). 1999. Rules and regulations pertaining to the detection, monitoring, and investigation of environmental and chronic diseases. http://www.cdphe.state.co.us/op/regs/100907.pdf

Miller et al. 1987. Determination of lead in blood using electrothermal atomic absorption spectrometry, l'vov platform, and matrix modifier. Analyst 112:1701-1704

TABLE 1

NOMINAL VALUES AND ACCEPTANCE CRITERIA FOR URINE PE SAMPLES

Spiking Material	Spiked Level (ug As/L)	Measured Conc (ug As/L)		Acceptance Criteria (ug/L)		
		mean	stdev	Method	Low	High
None	--	4.4	0.4	a, b	3.5	5.3
As(+3)	5	9.6	0.2	a, b	7.7	11.6
	15	20.7	0.6	a, b	16.5	24.8
As(+5)	5	10.3	0.6	a, b	8.3	12.4
	15	20.0	0.0	a, b	16.0	24.0
MMA	5	7.4	0.3	a, b	5.9	8.9
	15	14.7	0.6	a, b	11.7	17.6
DMA	5	8.6	0.3	a, b	6.9	10.3
	15	17.0	0.0	a, b	13.6	20.4
Arsenobetaine	20	24.3	0.6	a	3.5	7.3
				b	19.5	29.2

a = Total inorganic (non-dietary) arsenic

b = Total arsenic

VBI70 SURVEY OF ARSENIC AND LEAD EXPOSURE AMONG RESIDENTS

Date: May 2002 (Rev. # 0) **SOP No. 01**

Title: Analysis of Blood Lead Samples by Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

Total Pages 5

APPROVALS:

Syracuse Research Corp, Inc.
Author

Study Director Date

SYNOPSIS: This SOP describes the method to be used for collection of blood samples for lead analysis.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8		
Study QA Officer		
Syracuse Research Corp.		

BLOOD LEAD ANALYSIS USING GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

(Reference: Miller et al., Analyst, Vol. 112, 1701, 1987)

Graphite furnace atomic absorption spectrometry (GFAAS) is a method whereby specific wavelengths of light are passed through vaporized media (solid and aqueous) to ascertain the concentration of an element. GFAAS is dependant on two factors: the accurate measurement of the light intensity and the assumption that the light absorbed by the element is proportional to its concentration.

1.0 EQUIPMENT AND MATERIALS

- Perkin-Elmer model 5100 atomic absorption spectrometer and graphite furnace
- Model PRS-10 printer/sequencer
- Metexchange reagant
- 100 uL pipet and pipet tips
- 2-ml conical autosampler cups
- Vortex Mixer
- Whole blood lead standards

2.0 PREPARATION OF SAMPLES FOR ANALYSIS

Proper handling of blood samples and other biological fluids is essential not only for valid analytical assessments to be made, but also to ensure the health of technicians. As human blood is capable of transmitting disease, every sample will be handled as if potentially infectious. Technicians will be cautioned and instructed to maintain clean working areas, not to eat or drink in those areas, and to clean up any spills. They will also be required to wear lab coats and disposable gloves when handling these human blood samples.

Specimen quality, homogeneity, and proper handling are important factors in determining the accuracy of any of the tests. The presence of clots in the samples interfere in the methods to be performed (as they would in any hematological method) and produce inaccurate volume measurements when pipetting.
Therefore, the quality of the blood samples will be evaluated visually before any sample preparation is performed. Capillary tubes containing clots will not be analyzed. The bad samples will be recorded and subsequently stored. Any other peculiarities of a sample, such as lipemia,

hemolysis or any discoloration from normal, will be noted and recorded on the data form and analyzed at the discretion of the Lab Director (Donna Clark).

All sample preparations and analyses will be carried out under a temporary type hood to avoid air-borne contamination. Since thorough mixing and homogeneity of the sample is essential, whole blood samples will be always placed on an orbital mixer for approximately 30 minutes before any aliquot is removed. To maintain homogeneity, samples will be recapped and inverted before another portion of blood is to be removed.

The blood obtained by fingerstick, and contained in the lead-free microtainers will be dispensed into a known volumes of Metexchange reagent the same day the sample is received by the laboratory. The blood and reagent mixture is stable at room temperature for at least one year, which is more than sufficient to permit sample analysis.

3.0 PROCEDURE AND CALIBRATION

Anlysis of blood samples will occur using a Perkin-Elmer model 5100 GFAAS. Prior to analysis, the instrument parameters must be adjusted to the following:

Parameter	**Parameter Specifications**
Resonance radiation source	Hollow-cathode lamp
Lamp current	10 mA
Wavelength	283.3 nm, slit 0.7 (low)
Sample Volume	20 uL

Instrument calibration can be accomplished by analyzing a standard blood lead solution and performing a comparison to known values. To do this, Add 50 uL of whole blood to 200 uL of Metexchange reagent. After the Metexchange reagent has been combined with the blood, perform a series of 7 dilutions in 2 ml autosampler cups. Add 50, 100, 250, 500, 750, or 1000 ug/L of lead standards. Mix well and analyze. Create a calibration graph by plotting concentration vs. absorbance.

To begin the analyzing a blood sample, inject 20 uL of prepared sample material into the GFAAS. Once the sample is injected, the GFAAS will begin the analysis. The four principle analytical steps and their corresponding instrument control settings are listed below:

Step	**Temperature/° C**	**Ramp time/s**	**Hold time/s**	**Argon flow-rate/mL min^{-1}**
Dry	180	10	25	300
Char	750	5	25	300
Atomise	2400	1	4	20
Cool	20	1	4	200

Record the absorbance readings.

The instrument platform should be cleaned daily and not to exceed 300 analyses between cleanings.

4.0 CALCULATIONS

Blood lead concentrations are calculated by comparing the absorbance to the calibration graph. All results should be reported in ug/L or ug/g lead in whole blood.

5.0 REFERENCES

Miller et al. 1987. Determination of lead in blood using electrothermal atomic absorption spectrometry, l'vov platform, and matrix modifier. Analyst 112:1701-1704

VBI70 SURVEY OF ARSENIC AND LEAD EXPOSURE AMONG RESIDENTS

Date: September 1999 (Rev. # 0) **SOP No. 02**

Title: Preparation of Urine for Arsenic Performance Evaluation Samples

Total Pages 7

APPROVALS:

Ed Hinderberger L.E.T., Inc.

Author

Date

Study Director

SYNOPSIS: This SOP details the procedure for collecting and preparing urine samples for use in estimating arsenic bioavailability in test materials.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8		
Study QA Officer		
Syracuse Research Corporation		

1.0 PURPOSE

Bioavailability of arsenic will be evaluated for several test materials. Arsenic doses will be administered to juvenille swine on a predetermined schedule. Urine samples will be collected, digested, and analyzed for arsenic.

2.0 SCOPE

The procedures described in this SOP are for the preparation and analysis of arsenic by hydride generation. These methods were developed by the L.E.T laboratory in Columbia Missouri and are intended primarily for use in this study. They may not apply to other types of analyses.

3.0 SAMPLE PREPARATION

Transfer 25 ml. of urine to an acid cleaned 100 ml. beaker. Add 3.0 ml. of Methanol, 5 drops of anti-foam agent, 10.0 ml of 40% (W/V) Magnesium Nitrate Hexahydrate, and 10.0 ml of concentrated trace metal grade Nitric acid (HNO_3). Cover with a watch glass and place on a hot plate to reflux for 8-12 hours at 70 - 80 C. or overnight. Increase heat to 200 C and slide the watch glass back to allow faster evaporation. Heat to complete dryness (8 - 12 hours). Cover with watch glass and allow to cool. Transfer samples to cool muffle furnace and run following program. Ramp to 500 C at 1 degree/minute then hold at 500 C for 3 hours, turn off and allow to cool. Remove samples and add 5 ml. D.I. Water and 5 ml concentrated trace metal grade Hydrochloric acid (HCl). Allow to gently boil until the white residue is dissolved. After dissolving the residue cool and dilute with D.I. water to 50.0 ml. Transfer to clean labeled 2 oz. bottles.

When samples are ready for analysis they are diluted for Hydride Generation AA with a solution of 10% HCl, 10% KI, and 5% Ascorbic Acid. The samples are initially diluted l/10 or 1/5 in 1O.O ml. depending on the detection limit desired and capped. A 1/10 dilution should give a detection limit of 2 mg/L and a 1/5 should give a detection limit of 1 mg/L. Samples should set at least 30 minutes before analysis, but overnight is preferred.

4.0 APPARATUS AND MATERIALS

4.1 Equipment

The basic analysis equipment required are the Perkin-Elmer 3100 atomic absorption spectrometer (AAS), the Perkin-Elmer FIAS 200 flow injection system, the Perkin-Elmer

autosampler (AS-90), computer, monitor, and printer.

4.2 Standards, Reagents, and Miscellaneous

Other materials required are acid cleaned beakers for sample digestion, hotplate, fumehoods, Class A volumetric glassware and pipets for sample dilution and standards preparation, and the following reagents:

Trace Metal Grade Hydrochloric Acid – Fisher
Trace Metal Grade Nitric Acid – Fisher
Anti-Foam Reagent
Methanol ACS grade - Fisher
Electrolytic Sodium Hydroxide - Fisher
Potassium Iodide A.C.S. grade - Fisher
1000 PPM Arsenic standard - Fisher
Sodium Tetrahyridoborate 98% - Alfa
Ascorbic Acid - Recrystalized
Arsenic Calibration Check Standard - ERA

Purity of reagents is essential in this analysis. Contamination in samples and standards is often the limiting factor in low level analysis. High purity reagents must be used to avoid introducing contaminants during sample digestion. Calibration and check standards should not be used beyond the certified expiration date. Reagents must be either analytical reagent (AR) or ultrapure grade and should be chosen based on instrument response. Ideally, peak area absorbance should be near zero or small compared to the reporting limit. Calibration standards are prepared from the 1000 ppm arsenic standard.

6.0 ANALYTICAL PROCEDURES

6.1 Instrument Setup

Turn on the Perkin-Elmer 3100, FIAS 200, AS-9O, Computer, Monitor and Printer. Make sure the quartz cell is the one used for Arsenic, if not then change to the correct cell. Use the mouse whenever possible on the computer. When Dosshell comes on the screen, double click on Perkin-Elmer 3100. On the next screen double click on the aa inst.exe icon. On the next screen, when the icons for the 3100, FIAS 2OO and AS-90 are lit, click on the MHS-FIAS box, and then double click on the AUTO icon. Click on Element file, then double click on ASTST.MEL. When the four screens are completed, click on the FIAS control box and click on the Cell On/Off box to turn on the cell. The method will probably have less drift if the cell is allowed to heat overnight at 900 deg. C;

if there is a rush it can be used as soon as the temperature reaches 900.
After overnight warm up or while the cell is heating to 900 deg. C, prepare the standards in 10% HCl, 10% KI, 5% Ascorbic Acid. Prepare the following standards: 0.0, 0.2, 1.0, 5.0, 10.0, and 15.0 PPB. Prepare the ERA standard at about 5.0 mg/L for use as a calibration check standard. Turn on the EDL power supply and light the EDL Arsenic lamp and adjust the wattage to 8-10 watts. Place the Arsenic lamp in the instrument and adjust the wavelength to 193.7 with a 0.7 High Slit. After 10-15 minutes, move mouse arrow to windows and click on Align Lamps. Optimize the lamp energy using the wavelength knob and the two lamp position knobs. Click on AGE/AIC if the energy goes over 3/4 of the scale. Click on the upper left corner to close Align Lamps and move the mouse arrow to windows and click on Continuous Graphics. The absorbance reading will probably not be zero, so it will be necessary to autozero the reading. Using the three position knobs on the furnace head adjust the position to give the lowest reading. It may be necessary to autozero during the adjustments. When the best position has been found move the mouse pointer to the upper left corner and click to close the Continuous Graphics box.
Make sure that the reaction cell and reagent tubes are the correct ones for Arsenic. Change all the peristaltic tubing on the FIAS pumps. Prepare fresh 10% HC1 carrier and O.2% NaBH4 in 0.5% NaOH. Approximately 2 liters of HCL will be needed for each liter of NaBH4.

Move arrow to Windows and click on ID/Weight Parameter. Enter all data on the samples to be run(sample number autosampler position and dilution) Up to 98 samples can be run on one autosampler load. When all the sample information has been entered and checked, move the mouse arrow to File and click on Save As.... enter the name of the file(usually the number of the first sample to be analyzed minus the 9 exp. L95080001 would be entered as L5080001) this is necessary since the file name can only have 8 characters. Click on OK to save the file with that name and then move the mouse arrow to the upper left and click to exit ID/Wt Parameter.

6.2 Calibration

Click on AS-90 Control box and enter the name and the Data File: (usually the same as the first sample to be run that day the first number). Enter ASSTD as the ID/Wt File name (this file has the assignments for the calibration, standards check, known QC check and detection limit determination). Fill all the tubes and place in the positions required by the ASSTD ID/Wt File. Place the appropriate tubes in the l0% HCl and NaBH4 solutions. Click on the FIAS Control box and start pump 2. Move the clamps into position to start pumping the reagents. Turn on the Argon, after you are sure no liquid is going to go through the tube that goes to the Hydride cell, connect it to the cell. Click on Pump 1 and position its clamp to start pumping. Make sure that the rinse(zero standard) cup has the 10% HCl, 10% KI, and 5% Ascorbic Acid zero standard. Click on the AS-90 Control box and enter 8 in the Samples to Run: line and click on the box. Click on Run

Samples. The 5.00 PPB standard should be run 3 times and the absorbance values will be given in the lower left screen. If the last two values are within 5% of one another then the standardization can start. Click on Save Data On/Off and Printer On/Off(the boxes should be black if they are on) Click on Samples to Run ID/Wt box, and then on Run All. This should start the standardization. The standards run are S1 - 0.00, S2 - 5.00, and S3 - 15.00, this will calibrate from zero to 15.00 PPB. After the standardization the 5.00 standard in cup 8 will be run, if it is within 5% of 5.00 (4.75-5.25) the analysis will proceed. The next samples are a 10.00 standard and the known sample (ERA ~5.0 mg/L). If these are within the acceptable limits then the next ten samples will be the 0.2 PPB standard to determine the detection limit. Do a mean and standard deviation of the ten values (three times the standard deviation will be used as the detection limit). If the detection limit is not at least 0.1 PPB, then try to determine the problem and start the calibration over after solving the problem. The 5.00 PPb standard is run every ten samples and if it is not within the range 4.75 - 5.25 then the instrument will be restandardized and the 5.00 PPB standard rechecked, if it is still outside the range the analysis will stop.

6.2 Sample Analysis

After standardization, acceptance of the standard check samples, and detection limit, samples can then be analyzed. Move the mouse pointer to ID/Wt File: and enter the name of the file you want to run. Move the mouse arrow to reset sampler and click. Make sure that the Printer On/Off and Save Data On/Off are on. If it is necessary to turn data on again, make sure you append the data and do not write over the file. Place all the samples in the auto sampler in the positions matching the ID/Wt File:. Click on the box by ID/wt. to run the ID/wt file and then click

on Run Samples. An independent check standard (ERA ~5.0 mg/L) will be analyzed every 10 samples to verify instrument calibration (within 10% of accepted value). The instrument will check the 5.00 standard and if acceptable will then start the samples. The 5.0 standard is checked every 10 samples, and if it is not within the range 4.75 to 5.25 the instrument will be restandardized and if the 5.00 is still not in the range it will stop until the problem is resolved and restarted. Check the printer frequently to find any samples that are above 10.00 PPB, make the appropriate dilutions to be run after the other samples have been run.

7.0 DATA HANDLING

After all the samples and dilutions are run then the data can be reformatted. Move the mouse pointer to File and click on Exit to Benchtop. When in Benchtop, move the mouse pointer to Windows and click on Reformat Data, type the name of the data file and hit enter. Change the extension to AS, click on the box to it's left, then click on Header

Included. To go to the next page click, on Sample, then click on Sample Position, Dilution, Mean Conc (std units) and finally on Execute Reformat. The reformatted file is now saved. Exit by moving the mouse pointer to File and click on Exit to DOS. Click on OK when told that changes will be lost to reformat and OK when asked if OK to exit software. When back in Dosshell, double click on Command Prompt. When C:\DOS> is on the screen then type cd.. and enter. Then type aa_inst\aa_files\data and enter. Type print xxxxxxxx.as(your file name) and enter. Hit enter when asked for name of list device [PRN]. This should print out your reformatted data. Next place a 3.5" disc in the computer and type xcopy xxxxxxxx.as B:. This will transfer the file to disc for use in the calculations. The data are corrected for drift using quattro, then transferred to the main quattro file for final calculation. The formula is (corrected soln. conc. X dilution X final volume)/sample volume(ml.). the units are Ng/ml. or mg/L.

8.0 HEALTH AND SAFETY

Laboratory personnel should always follow established laboratory safety procedures. All digestions using perchloric acid should be performed in the perchloric acid fumehood.

9.0 QUALITY CONTROL

Quality control samples will be prepared at the following frequency:

Preparation Blank - 1/20 samples
Duplicate - 1/10 samples
Matrix Spike - 1/10 samples
NIST (#2670) Toxic Metals Freeze Dried Urine (Laboratory Control Standard) - 1/20 samples.

Contamination should be at a minimum in the blank samples and in no instance should it exceed the instrument detection limit. If contamination exceeds the instrument detection limit, the source of the contamination should be eliminated, the affected samples redigested and reanalyzed.

The difference between duplicate samples should not be greater than 1 mg/L if the values are <10 mg/L during the analysis run. At values $\geq$10 mg/L, the duplicate values should have a relative percent difference (RPD) of 20% or less.

The RPD is calculated using the following formula:

$$RPD = \frac{S - D}{\frac{S+D}{2}} \times 100$$

Where:

S = Original sample value.
D = Duplicate sample value.

Matrix spike recovery should be in the range 85-115% and the LCS recovery should be within the established control windows.

If duplicate precision, matrix spike, or LCS control limits are not met for a sample batch, potential problems should be investigated and solved. If sample preparation is found to be the problem, the affected samples should be redigested and reanalyzed. If the analytical instrumentation is the problem, the affected samples and QC should be reanalyzed once the problem is solved.

The calibration check standard (ERA ~5.0 mg/L) will be analyzed every ten samples. If this standard is not within $\pm$ 10% of the true value, the analysis will be stopped, any problems solved. The instrument will be recalibrated and all samples since the last good calibration check reanalyzed.

The data, calculations, quality control and reports are checked by the Quality Assurance Officer before a final report is released.