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Certification of Total Arsenic in Blood and Urine Standard Reference Materials by Radiochemical Neutron Activation Analysis and Inductively Coupled Plasma - Mass Spectrometry

Rick L. Paul[1,*], **W. Clay Davis**[1], **Lee Yu**[1], **Karen E. Murphy**[1], **William F. Guthrie**[2], **Dennis D. Leber**[2], **Colleen E. Bryan**[1], **Thomas W. Vetter**[1], **Gulchekhra Shakirova**[3], **Graylin Mitchell**[3], **David J. Kyle**[3], **Jeffery M. Jarrett**[3], **Kathleen L. Caldwell**[3], **Robert L. Jones**[3], **Steven Eckdahl**[4], **Michelle Wermers**[4], **Melissa Maras**[4], **C. D. Palmer**[5], **M.F. Verostek**[5], **C. M. Geraghty**[5], **Amy J. Steuerwald**[5], and **Patrick J. Parsons**[5,6]

[1]Analytical Chemistry Division, National Institute of Standards and Technology, Gaithersburg, MD 20899

[2]Statistical Engineering Division, National Institute of Standards and Technology, Gaithersburg, MD 20899

[3]Inorganic and Radiation Analytical Toxicology Branch, Centers for Disease Control and Prevention, Atlanta, GA 30341

[4]Mayo Clinic, Rochester, MN 55905

[5]Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center, New York State Department of Health (NYSDOH), Albany, NY 12201-0509

[6]Dept of Environmental Health Sciences, School of Public Health, University at Albany, 12201-0509

Abstract

A newly developed procedure for determination of arsenic by radiochemical neutron activation analysis (RNAA) was used to measure arsenic at four levels in SRM 955c Toxic Elements in Caprine Blood and at two levels in SRM 2668 Toxic Elements in Frozen Human Urine for the purpose of providing mass concentration values for certification. Samples were freeze-dried prior to analysis followed by neutron irradiation for 3 h at a fluence rate of $1\times10^{14}\mathrm{cm}^{-2}\mathrm{s}^{-1}$. After sample dissolution in perchloric and nitric acids, arsenic was separated from the matrix by extraction into zinc diethyldithiocarbamate in chloroform, and ^{76}As quantified by gamma-ray spectroscopy. Differences in chemical yield and counting geometry between samples and standards were monitored by measuring the count rate of a ^{77}As tracer added before sample dissolution. RNAA results were combined with inductively coupled plasma – mass spectrometry (ICP-MS) values from NIST and collaborating laboratories to provide certified values of (10.81 ± 0.54) μg/kg and (213.1 ± 0.73) μg/kg for SRM 2668 Levels I and II, and certified values of (21.66

*Corresponding author: rick.paul@nist.gov, telephone: (301)975-6287, fax: (301)208-9279.

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± 0.73) μg/kg, (52.7 ± 1.1) μg/kg, and (78.8 ± 4.9) μg/kg for SRM 955c Levels 2, 3, and 4 respectively. Because of discrepancies between values obtained by different methods for SRM 955c Level 1, an information value of < 5 μg/kg was assigned for this material.

Keywords

neutron activation analysis; arsenic; toxic elements; biologicals; radiochemistry; standard reference materials

INTRODUCTION

Exposure of humans to arsenic, even at relatively low levels, has been linked to a variety of health problems, including heart disease, skin damage, and lung, bladder, and kidney cancers.[1–3] As a consequence, arsenic standards for drinking water have been lowered from 50 μg/L to 10 μg/L in much of the world.[4] Monitoring of arsenic levels in drinking water, soils, food, and biological tissues is therefore vital to the public health, and this monitoring requires reliable methods for determination of arsenic at low levels as well as certified reference materials for validation of these methods.

In response to the need for reference materials, the National Institute of Standards and Technology has established a continually expanding portfolio of biological Standard Reference Materials (SRMs) certified for arsenic content.[5] Inductively coupled plasma-quadrupole mass spectrometry (ICP-qMS), is routinely used at NIST for determination of elements at low levels. Determination of arsenic by this method may suffer from interference by chlorine at lower μg/kg levels, though to compensate for this, collision cell technology (CCT) or high resolution sector field (HR) ICP-MS is used. Certified values (values for which NIST has the highest confidence, where all sources of uncertainty or bias have been evaluated[6]) are usually assigned only when two or more independent methods (methods with few or no sources of bias in common) have been used to provide measurements.

Neutron activation analysis (NAA), which has few sources of uncertainty or bias in common with non-nuclear methods of analysis, has played a key role in the certification of arsenic and other elements in biological Standard Reference Materials (SRMs). Arsenic is quantified by counting of the principal ^{76}As gamma-ray line (559 keV) emitted from ^{76}As, $t_{1/2}$ = 1.09379 d ± 0.00045 d, formed upon neutron capture of ^{75}As. Quantitative measurement is based on comparison with As standards prepared from high-purity materials irradiated in the same manner. In instrumental neutron activation analysis (INAA), the sample is irradiated with neutrons, and then counted after some optimal time period to allow decay of shorter-lived radionuclides, thus eliminating uncertainties arising from sample dissolution or extraction of arsenic from the matrix. Unfortunately arsenic determination by INAA below ≈ 100 μg/kg is often hampered by the presence of significant amounts of ^{24}Na ($t_{1/2}$ = 15 h), ^{82}Br ($t_{1/2}$ = 35.3 h), or ^{32}P ($t_{1/2}$ = 14.3 d) resulting in high count rates, high dead time, and elevated spectrum baseline due to Compton scattering and Bremsstrahlung radiation from ^{32}P. Better As detection limits for NAA may be achieved by using high throughput counting or Compton suppression[5], but the best detection limits are obtained

using radiochemical neutron activation analysis (RNAA), with separation of arsenic from the sample matrix prior to counting. Several RNAA procedures for determination of arsenic in biological materials were recently evaluated, including absorption of arsenic on hydrated manganese dioxide and solvent extraction into chloroform containing zinc diethyldithiocarbamate, ($ZnDDC_2$).[7] The solvent extraction procedure was found to yield the best results, with limits of detection down to ≈ 0.1 μg/kg.

Reflecting the importance of monitoring As and other toxic elements in humans, NIST has developed the clinical reference materials, SRM 955c Toxic Elements in Caprine Blood and SRM 2668 Toxic Elements in Frozen Human Urine. The concentration of toxic elements in blood and urine is routinely measured to track population exposure trends and to monitor occupational exposure. ICP-MS had previously been used to provide As reference values for SRM 955c, but the values were not certified for lack of data from a second method. Reference values represent the best estimate of the true value, but because NIST has not fully investigated known or suspected sources of bias, the value does not qualify as a certified value. [6] The newly evaluated RNAA solvent extraction procedure was used for the first time together with ICP-MS to provide arsenic mass concentrations to certify these SRMs.

MATERIALS AND METHODS

SRMs preparation

SRM 955c Toxic Elements in Caprine Blood was prepared by the New York State Department of Health (NYSDOH) according to NIST design and specification. The SRM consists of vials of frozen goat blood at four concentration levels. Briefly, Level 1 is composed of base blood from undosed adult goats and contains endogenous (trace) levels of metals. Levels 2, 3, and 4 are composed of blood pools collected from lead-dosed goats and supplemented with As (as As^{+3}), Cd (as Cd^{+2}) and Hg (as Hg^{+2}, $MeHg^{+2}$, and $EtHg^{+}$). Levels 2, 3, and 4 were spiked to contain approximately 20 μg/L, 50 μg/L, and 80 μg/L of arsenic respectively. Homogeneity assessment of the arsenic in Levels 1 and 3 was previously performed by ICP-qMS at NIST.

SRM 2668 Toxic Elements in Frozen Human Urine was developed in collaboration with the Centers for Disease Control and Prevention (CDC). Each unit of the SRM consists of five vials of frozen urine of each of two levels: Level 1, with approximately 10 μg/L of arsenic, population geometric mean is similar to the U.S. (≈ 50^{th} -95^{th} percentile distribution) and Level 2, with approximately 200 μg/L of arsenic, represents an elevated urine concentration (>95th percentile) based on National Report on Human Exposure to Environmental Chemicals (2005–2006 National Health and Nutrition Examination Survey). [8]

Preparation and irradiation of samples, standards, and controls for RNAA

RNAA measurements were made on all four levels of SRM 955c and on both levels of SRM 2668. Multiple vials of each material were obtained for analysis. Each vial of blood or urine contained between 1.5 mL and 2 mL of liquid, corresponding to a mass of approximately 2 g of blood or 1.7 g of urine. For the urine SRM, six vials of each level were sampled, with

each vial representing one sample. Twelve samples of Level 1 blood and six samples of Level 3 blood were analyzed, each vial again representing one sample. Since no previous homogeneity testing was performed on the Level 2 and Level 4 blood, vial contents for these materials were divided approximately in half, with each vial used to provide two samples. Four vials of each of these two blood levels were sampled, for a total of eight samples of each.

All samples were prepared according to the following procedure. A series of linear polyethylene (LPE) bags were prepared by heat-sealing polyethylene film. The bags were cleaned by soaking in 1 mol/L nitric acid for approximately 24 h, and were then soaked in ultra-pure (triply-distilled) water for another 24 h, and finally air-dried in a clean hood for at least 48 h. Each bag, with an opening at the top large enough to facilitate easy transfer of the liquid, was positioned vertically within a Teflon bottle of approximately the same diameter as the bag. A cap was placed on the bottle, and the mass of the bottle and bag recorded. All vials of blood and urine were kept frozen until preparation for analysis. Prior to sample preparation, vials were removed from the freezer, and the blood or urine was allowed to equilibrate to room temperature for several hours. The caps were removed from the Teflon bottles and contents from each vial were added to a polyethylene bag, care being taken to make sure that no liquid leaked from the bag into the containment bottle. The bottle was then capped, and again weighed using an analytical balance. The liquid mass was determined by difference, i.e., the mass of sample was equal to the mass of the Teflon bottle + liquid-filled bag minus the mass of the Teflon bottle + empty bag. The capped bottles containing the sample-filled bags were transferred to a freezer at −80 °C and kept there overnight.

In order to minimize problems encountered in encapsulation and irradiation of liquids (such as poorly defined irradiation geometries, sample volatilization, and buildup of pressure within the irradiation vessel) the blood and urine samples were freeze-dried prior to analysis. This freeze-drying was performed solely to minimize problems associated with irradiation of liquids; no quantitative results were obtained from the process. The bottles containing the liquid-filled bags were removed from the freezer, the caps removed, and the bottles transferred to a freeze dryer at a nominal pressure of 13.3 Pa (0.1 Torr) and a nominal temperature of − 40 °C. Samples were freeze-dried for a period of about two weeks, during which time the temperature was raised by 5 °C approximately every two days. When a nominal temperature of 0 °C was reached, the bottles were removed from the freeze dryer, allowed to warm to room temperature, the polyethylene bags removed from the bottles, and each bag sealed at the top, with care taken to ensure that none of the contents were lost. Each bag and Teflon bottle was inspected for signs of leakage during the freeze-drying process; no leaks were detected. Each sample-containing bag was then sealed inside a second acid-washed LPE bag, and each doubly encapsulated sample was then sealed into a third polyethylene bag made of acid-washed tubular polyethylene to ensure containment during irradiation.

For preparation of standards, a solution containing (54.37 ± 0.31) μg/g of arsenic, was prepared by gravimetric dilution of SRM 3103a Arsenic Standard Solution. Standards were prepared by using a disposable pipet to deposit 2 to 3 drops of the solution onto Whatman 41

filter paper. The pipet was weighed before and after dispensing in order to determine the mass of solution deposited. Filter papers were allowed to dry for several days in a clean hood. Each filter paper containing the dried solution was formed into a disk using a stainless steel die and hydraulic press. Filter paper standards were triply encapsulated in polyethylene film as described above.

Control materials were prepared by pressing approximately 0.3 g portions of Standard Reference Materials, SRM 1575a Pine Needles and SRM 1577c Bovine Liver, into 12.7 mm diameter pellets using a stainless steel die and hydraulic press. All control materials were triply sealed into polyethylene bags.

Samples, control materials, standards, and iron foil neutron flux monitors (each ≈ 6 mg) were packed into polyethylene rabbits (irradiation vessels). Each rabbit typically contained two or three blood or urine samples, two standards, one or two controls and two flux monitors. Each rabbit was irradiated in the NIST reactor pneumatic tube irradiation facility, RT-1, for a total time of 3 h at a reactor power of 20 MW, which provided a thermal neutron fluence rate of approximately 1.0×10^{14} $cm^{-2}s^{-1}$. In order to compensate for a nearly linear drop-off of flux as a function of distance along the length of container, i.e., distance from the reactor core, each rabbit was flipped, i.e. rotated 180° at the midpoint of the irradiation and reinserted. Rabbits were allowed to sit for at least three days prior to sample processing in order to allow decay of ^{24}Na in order to minimize radiation exposure to the analyst.

Preparation of ^{77}As tracer solution

Prior to processing of irradiated samples, a solution containing ^{77}As ($t_{1/2}$ = 1.6179 d)[9] was prepared for the purpose of estimating corrections arising from differences in arsenic yield (fraction of As recovered from the sample) and counting geometry from sample to sample and between samples and standards. The preparation of this solution has been described previously.[7, 10] Approximately 50 mg of high purity (> 99.9 % based on measurement of impurities) GeO_2 was added to each of two polypropylene snap-top vials. The vials were placed in a polyethylene irradiation vessel (rabbit) and irradiated in the NIST reactor pneumatic tube irradiation facility, RT-2, for a total time of 6 h at a reactor power of 20 MW, which provided a thermal neutron fluence rate of approximately 3×10^{13} $cm^{-2}s^{-1}$. The rabbit was flipped at the midpoint of irradiation as described above. Approximately 3 d after irradiation, the contents of each polypropylene vial were transferred to a Teflon beaker. Each 50 mg GeO_2 portion was dissolved by heating in 5 mL of 1 mol/L potassium hydroxide solution.* Each dissolved portion was then processed in the following manner to separate As from the matrix: 1 mL of 2.5 mol/L H_2SO_4 was added to neutralize the solution, followed by addition of 5 mL H_2O, 4.5 mL concentrated H_2SO_4, and 5 mL 2 mol/L potassium iodide solution. The 20 mL total solution was transferred to a 125 mL separatory funnel, and As was extracted into 10 mL of toluene. The aqueous phase was discarded and the toluene phase washed with two 20 mL portions of 4 mol/L H_2SO_4/0.5 mol/L KI solution. Arsenic was then back-extracted into 10 mL of 10 % (volume fraction) H_2SO_4.

*Purity of reagents is not crucial in post-irradiation chemistry: since only radioactive nuclides are being measured, there is little possibility of contamination by reagents. Therefore all chemicals used in post-irradiation processing of tracers, samples, standards, and controls were reagent grade.

The 10 mL portions from the GeO_2 dissolutions were combined and the 20 mL portion heated to boiling on a hot plate while 30 % (volume fraction) H_2O_2 was added to reduce iodide to I_2. After no more I_2 vapors were observed, the solution was evaporated to approximately 2 mL, then allowed to cool, transferred to a polypropylene bottle, and the acid residue diluted to approximately 17 mL with singly deionized H_2O (referred to as "H_2O throughout the remainder of the post irradiation chemistry procedure). This solution was then set aside as a tracer to be used in the arsenic RNAA procedure. Because the nuclide is relatively short-lived, a new solution was prepared for each set of blood or urine irradiations.

Digestion of samples, standards, and controls

Approximately 3 days after irradiation, samples, standards, and controls were removed from the rabbits. In order to avoid sample loss and assure complete transfer of each blood or urine sample to the dissolution beaker, the following procedure was used. Each sample bag was transferred to a Teflon beaker containing 0.1 mL of a solution with approximately 1 mg/g As (to serve as carrier) and 1 mL of the ^{77}As tracer solution. Approximately 10 mL of water were added to the beaker (sufficient to completely cover the encapsulated sample). A razor blade knife was then used to slice open the bags, any residue remaining on the knife was rinsed into the beaker with additional water, and 10 mL of concentrated HNO_3 were added to each beaker. Beakers were covered with Teflon lids and placed on a hot plate with a surface temperature of approximately 150 °C to 200 °C for approximately 4 h to 5 h, during which time most of the freeze-dried sample was leached off the bags. Lids were removed, and each bag was transferred to a second Teflon beaker containing 10 mL of concentrated HNO_3 and 10 mL H_2O, and the beakers covered and heated for an additional 2 hours. Lids were again removed, the bag removed from the beaker and rinsed with H_2O (rinse added to the beaker), and the bag transferred to a counting vial for determination of any ^{76}As remaining. The two acid fractions from each sample were combined into one beaker, the beaker placed on a hot plate at approximately 200 °C to 250 °C, and the solution evaporated to near dryness. Next 10 mL of HNO_3 and 10 mL of $HClO_4$ were added to each beaker, and the beaker was heated (covered) at 150 °C to 250 °C for approximately 12 h. The lids were removed, the surface temperature increased to 250 °C to 280 °C, and the volume of each solution reduced to about 1 mL to 2 mL. After beakers were allowed to cool to room temperature, an additional 10 mL of HNO_3 and 10 mL of $HClO_4$ were added to each beaker and the beaker again heated on a hot plate at 150 °C to 250 °C for approximately 12 h. Lids were again removed, the hot plate surface temperature increased to 250 °C to 280 °C, and the volume of each solution reduced to about 1 mL to 2 mL. Each digest was then set aside for further processing.

Standard and control disks were removed from the polyethylene bags and each added to a Teflon beaker containing 0.1 mL of the 1 mg/g As solution and 1 mL of the ^{77}As tracer solution. Approximately 0.2 g of unirradiated Bovine Liver were added to each beaker containing a standard disk in order to minimize errors due to differences in matrix between samples and standards. To all beakers containing standards and control materials were added 10 mL concentrated HNO_3 and 10 mL H_2O, and each beaker heated (covered) at 150 °C to 200 °C for 4 to 5 h. Lids were removed, and each beaker was heated at approximately 200 °C to 250 °C until the solution was evaporated to near dryness. Two additional digestions

with 10 mL $HClO_4$ and 10 mL HNO_3 were then performed as described in the sample procedure above.

Separation of arsenic

Arsenic in the digests was separated by solvent extraction.[7, 11] Five milliliters of concentrated H_2SO_4 were added to the digest in each beaker. The beakers were heated uncovered at a temperature of 250 °C to 275 °C for about 30 min in order to expel traces of $HClO_4$. Each beaker was removed from the hot plate and cooled to room temperature, followed by dropwise addition of 15 mL H_2O and 1.5 mL of 0.2 mol/L KI solution to reduce As^{5+} to As^{3+}. Beakers were again covered and heated to boiling for 10 to 15 min. Lids were removed, 1.5 mL of 0.8 mol/L ascorbic acid added to complete the conversion of As to As^{3+}, and the beakers removed from the hot plate and allowed to cool for 30 min.

Each solution was transferred to a 60 mL separatory funnel and extracted with 10 mL of 0.025 mol/L $Zn(DDC)_2$ in chloroform for about 2 min. The chloroform layer (bottom layer) was drained off into a beaker, and the aqueous phase again extracted for 2 min with 5 mL of $Zn(DDC)_2$ /chloroform solution. The chloroform phase was drained off, the aqueous phase discarded, and the combined 15 mL chloroform phase transferred back to the separatory funnel. The chloroform phase was next washed with 5 mL of 2 mol/L H_2SO_4 containing 100 mg of $ZnSO_4$ for 2 min, and the chloroform layer drained into a 20 mL plastic liquid scintillation counting vial (aqueous phase discarded). Each vial was then set aside for determination of ^{76}As and ^{77}As by gamma-ray spectroscopy.

Counting and Data Reduction

Gamma radiations from all processed samples, standards, and controls were collected using a high-purity germanium detector with associated electronics, with vials counted at the face of the detector. Rinsed polyethylene bags containing blood or urine residue were counted in the same geometry. Blood and urine samples and rinsed bags were counted for approximately 5 h each; counting times varied for standards and controls. Quantitative determination of arsenic was achieved using the 559 keV line from decay of ^{76}As ($t_{1/2}$=1.09379 d ± 0.00045 d).[12] Peaks were integrated and fitted using commercial software. Sample and control yield/counting geometry corrections were determined relative to standards by comparing the ^{77}As count rate for each sample or control with the average ^{77}As count rate of the processed standards. Because samples masses and activities were determined relative to standards, it was not necessary to know the activity of the tracer or to calculate absolute yields. Correction factors determined in this manner generally ranged from about 0.9 to 1.1.

Arsenic mass fractions were calculated from ^{76}As count rates measured in samples and standards, sample masses, and As mass in standards, corrected by yield/counting geometry factors as determined from ^{77}As count rates. Count rates were corrected for pulse pileup (where applicable) and radioactive decay. No detectable As was measured in the bag residues for the blood; an upper limit for the As content was calculated for each bag (less than 2 ng for all bags) and this number was then used to calculate the As uncertainty due to

incomplete digestion in the bag. A small amount of arsenic was detected in the bag residues for urine, resulting in a correction of approximately 0.5 μg/L applied to the measured values.

Arsenic mass fractions (in μg/kg) for blood and urine were calculated based on the mass of the blood and urine measured at room temperature prior to freeze-drying. All values were then converted to mass concentrations (in μg/L) using the density of the thawed liquid sample at room temperature (liquid temperature at 22 °C). A Mettler/Paar model DMA 35 density meter was used to measure a density of 1.012 g/mL ± 0.002 g/mL for the SRM 2668 urine. The calibration of the density meter was verified by measuring the density of deionized water at room temperature. The density of the SRM 955c blood was determined by a semi-gravimetric method using a Lang-Vey pipet standardized with water. The density for each level was determined to 6 decimal points, the average value being approximately 1.052 g/mL. Individual values for the four levels are listed on the Certificate of Analysis.

Mass fractions for pine needle and bovine liver control materials were calculated on a dry mass basis. The dry mass of each sample was calculated by multiplying the measured mass (as-received mass) of the sample, by a drying factor determined by drying replicate portions of each SRM over magnesium perchlorate to constant weight.

RESULTS AND DISCUSSION

SRM 2668 Toxic Elements in Frozen Human Urine

Table 1 gives mass concentration values for As measured by RNAA in the Level I and Level II urine. Standard deviations for both materials do not exceed the combined uncertainties from counting statistics, yield determination, and correction for undissolved sample, giving no reason to suspect material heterogeneity. Expanded uncertainties (U) were calculated by combining uncertainties from individual sources in quadrature to obtain a combined standard uncertainty (u), which was then multiplied by a coverage factor of 2 to obtain the expanded uncertainty.[13] The following sources of uncertainty were evaluated, with associated relative uncertainties for the urine and blood measurements in parentheses. (1) Uncertainties associated with measurement replication for samples, calculated as $1s/\sqrt{n}$ (see Tables 1, 3, and 4). (2) Uncertainties associated with measurement replication for standards, calculated as $1s/\sqrt{n}$ (0.5 % to 1 %). (3) Uncertainties in the masses of elements in the standard, estimated based on uncertainties in metal purity, the mass of each element, the mass of solution, and the mass of solution deposited on the filter papers (0.6 %). (4) Uncertainties associated with peak integration, estimated based on the evaluation of two different peak fitting methods and the assumption of a rectangular distribution of results (5 % for Level I blood with low count rates and poor counting statistics, 0.1 % to 0.7 % for others). (5) Pileup correction uncertainties, estimated from the uncertainty in the pileup calibration constant (≤ 0.2 %). (6) Yield/counting geometry correction uncertainty for As, estimated from the average counting statistics given by the 239 keV ^{77}As peak and the uncertainty in the delivery volume of the 1 mL pipet used to transfer the ^{77}As solution to the digestion beakers (≈ 0.5 %). (7) Uncertainties due to incomplete digestion of As in the bag, higher uncertainties for lower arsenic levels (0.2 % to 2 %). The uncertainty in the measured As residue in the bag was used to determine this uncertainty for urine; the measured upper limit for As in the bag was used in the uncertainty determination for the blood. The detection

of ^{76}As in urine residue but not blood residue could be due to a number of factors including longer count times for the urine residue vs. blood residue resulting in lower As detection limits in the urine residue, lower total activity (including lower ^{82}Br) in the urine residue resulting in lower As detection limits, or more rigorous acid soaking of the blood filled bags resulting in greater removal of the arsenic. 8) All other sources of were deemed insignificant (< 0.1 % contribution from each).

Arsenic data used in the certification of the urine were provided by the RNAA and CCT-ICP-qMS measurements at NIST as well as ICP-MS data from four collaborating laboratories.[14, 15] Table 2 compares RNAA with other measured values. Values measured by RNAA for Level I agree with As mass fractions measured by ICP-MS at NIST and at collaborating laboratories. RNAA values for Level II are low compared with NIST CCT-ICP-qMS values (error bars do not quite overlap – see Table 2), but are in agreement with values from collaborating laboratories. No method-based explanation could be found to account for the apparent discrepancy between the NIST RNAA and ICP-qMS values. It is unlikely that the RNAA measurements for Level II are biased low due to loss during freeze-drying, irradiation, or dissolution (see discussion of SRM 955c below). It is also unlikely that the ICP-MS values are biased high due to polyatomic interferences to ^{75}As such as ^{40}Ar^{35}Cl and ^{40}Ca^{35}Cl. At NIST, ICP-qMS measurements of arsenic were performed using helium as a collision gas, with kinetic energy discrimination set automatically, which should alleviate the effects of polyatomic interferences. The ICP-qMS methods by the outside laboratories include similar approaches to eliminate these interferences. Furthermore, since the Level I and Level II urine contained approximately same amount of chlorine (2.73 mg/L ± 0.12 mg/L and 2.62 mg/L ± 0.10 mg/L), and presumably equal amounts of calcium as well since no calcium was added to the Level II urine†, if ^{40}Ar^{35}Cl and ^{40}Ca^{35}Cl are contributing to the bias, one would expect the same amount of bias at level I. Yet ICP-MS and RNAA agree for Level 1 As. Also, no bias was observed in analysis of control materials measured alongside the SRM 2668 samples. The apparent RNAA/ICP-MS discrepancy for Level II may be a result of material inhomogeneity. The material inhomogeneity, calculated with the statistical Monte Carlo methods in the certification data analysis, was incorporated into the expanded uncertainty of the certified values of arsenic; yet, this source of uncertainty was unaccounted for in the reported RNAA and the ICP-qMS values. If a prediction interval is added to NIST RNAA and CCT-ICP-qMS values to take into account the material inhomogeneity, then NAA and ICP-qMS values overlap for the Level II arsenic.

Certified mass concentrations are the weighted means of results from NIST and collaborating laboratories, found by leveraging a linear, Gaussian random effects statistical model and the DerSimonian-Laird procedure. The estimation procedures were supplemented by the parametric bootstrap for uncertainty propagation. The certified values for both levels are given in Table 2 along with expanded uncertainties.

†No calcium values were measured for SRM 2668, but analysis of calcium in SRM 2670a Toxic Elements in Urine (Freeze-Dried) yielded the same amount of calcium in both Low Level and High Level urine.

SRM 955c Toxic Elements in Caprine Blood

Tables 3 and 4 give mass concentration values for As measured by RNAA at the four concentration levels. Table 3 gives values measured for 12 vials of Level 1 blood and 6 vials of Level 3 blood. Table 4 gives arsenic values measured for 8 paired samples of each of Levels 2 and 4, with each vial of blood providing 2 samples, labeled “a” and “b”. Results from individual levels are discussed separately below.

ICP-MS measurements used in the certification campaign were provided by NIST and by a number of collaborating laboratories. NIST CCT-ICP-qMS measurements were performed on duplicate 1 g portions from eight vials of each of Level 1 and Level 3 blood. In order to minimize the interference to ^{75}As from the polyatomic ions $^{40}Ar^{35}Cl$ and $^{40}Ca^{35}Cl$, a collision cell was employed using 8 % mole fraction H2 and 92 % He as collision gas. The non-NIST measurements were provided as part of a study conducted by the NYSDOH. Single, blinded vials of SRM 955c Levels 1 through 4 were distributed for analysis in addition to regularly scheduled proficiency testing (PT) samples as part of a special laboratories with 8 to 9 laboratories employing ICP-MS and 1 laboratory employing high resolution (HR)-ICP-MS.[14] Prior to any RNAA measurements being performed, the NIST and non-NIST ICP-MS measurements had been used to provide reference values of (2.07 ± 0.63) μg/L, (21.9 ± 1.7) μg/L, (54.9 ± 3.4) μg/L, and (77.5 ± 4.5) μg/L for arsenic mass concentrations of Levels 1, 2, 3, and 4, respectively. Reference values for Levels 2 and 4 were based solely on data from the non-NIST reference laboratories, while reference values for the Level 1 and Level 3 blood were based on NIST CCT-ICP-qMS measurements, with corroborating data from the reference laboratories. Values were listed as reference values rather than certified values because the methods of analysis used (in all cases ICP-MS) were not sufficiently independent to eliminate matrix-induced interference as a source of bias. Figure 1 illustrates that mean values obtained by CCT-ICP-qMS from NIST and the non-NIST reference laboratories for the Level 1 and Level 3 blood agree within the plotted error bars (expanded uncertainties), although the error bars for the Level I blood measurements for NYSDOH labs represent a relative expanded uncertainty of 76 % due to the As content being close to the LOD for most ICP-MS laboratories.

The RNAA value measured here for Level 3 is in agreement with both NIST and non-NIST reference laboratory ICP-MS values, and RNAA values for Levels 2 and 4 are in agreement with the mean value obtained from the reference laboratories within the expanded uncertainties (Figure 1). No heterogeneity was observed in the Level 2 material by RNAA, but analyses of the Level 4 blood indicated vial-to-vial heterogeneity. Because of this, additional uncertainty was factored into the total uncertainty of the certified value (see below).

Arsenic mass concentrations measured by RNAA in Level I blood are low compared with ICP-MS values determined at NIST and with the average value obtained from the NYSDOH laboratories (Figure 1). With the exception of one outlying value, individual RNAA values ranged from about 0.15 μg/L to 0.4 μg/L. Neglecting the outlier, a weighted mean and standard deviation of (0.280 ± 0.083) μg/L were calculated for the 11 non-outlying values, which is nearly 10 times lower than the mean values reported by ICP-MS. The relative standard deviation of 29.4 %, is significantly greater than the combined uncertainties from

counting statistics, peak fitting, yield/geometry correction, and losses due to incomplete dissolution. This could reflect heterogeneity in the SRM, but there are other possibilities. Samples could have been contaminated by transference of ^{76}As contamination from the bags to beakers. This would have a significant effect on the results given the very low level of As in the blood samples. Even though the bags were thoroughly cleaned, it is possible that minute amounts of spot contamination of arsenic were still present in some of the bags, which could have been transferred to the beakers along with the blood samples. This uncertainty is difficult to assess since each bag may have contained a different amount of contamination. Lastly, there is the case of the outlying value of 5.68 μg/L obtained for one sample in the second data set. This value is about 20 times the mean of the other eleven samples. This could be further evidence of heterogeneity of the SRM, or could reflect a contaminated vial or a badly contaminated bag used in sample packaging. Although, nothing unusual was noted in the processing of this sample that could account for the high value, it is possible that contamination from the bag or through contact with standards may have occurred.

Contamination of samples during processing by RNAA cannot explain why the mean value obtained by RNAA is nearly 10 times lower than the mean values by ICP-MS. One possible explanation for the discrepancy is that RNAA values are biased low due to loss of arsenic either during freeze-drying, irradiation, or dissolution prior to equilibration with ^{77}As tracer. No apparent arsenic losses were observed for the Levels 2, 3, and 4 bloods, as indicated by agreement between RNAA and ICP-MS values. However, those materials were spiked with As(III), while arsenic may be dominant in more volatile forms in the natural matrix material. Arsenic measured at natural levels in the Level I urine, processed in precisely the same manner as the Level I blood, showed no low bias for RNAA, indicating insignificant losses compared to the ICP-MS methods. Moreover, the same dissolution method was used in the RNAA measurement of arsenic in bovine liver and pine needles controls, and again no low bias was observed compared with the certified value (Figure 2). Hence it does not appear that a 10-fold low bias in the RNAA measurements is likely to be caused by arsenic loss during processing.

Another possible explanation for the discrepancy between RNAA and ICP-MS data is that the ICP-MS arsenic values are biased high, possibly due to inadequate correction for an isobaric interference. As mentioned earlier, for ICP-MS measurements performed at NIST, collision cell technology was used to minimize interferences from ^{40}Ar^{35}Cl and ^{40}Ca^{35}Cl. The CCT-ICP-qMS cell conditions are optimized to kinetically discriminate against the polyatomic interference. In an attempt to resolve the discrepancy between the RNAA and ICP-MS measurements, additional measurements of the Level 1 blood were performed at NIST using high resolution (HR) ICP-MS. This method is capable of higher mass resolution with capabilities exceeding those of quadrupole ICP-MS. Arsenic concentrations measured at NIST in six samples using this method ranged from 0.64 μg/L to 0.91 μg/L with a mean value and expanded uncertainty of 0.79 μg/L ± 0.24 μg/L, significantly lower than the mean value obtained by CCT-ICP-qMS, but not as low as the RNAA value (Figure 1). The limits of detection for measurement of As by RNAA and ICP-MS are relatively comparably in the absence of interferences: a detection limit of 0.1 μg/L was calculated for ICP-MS as 3 times

the uncertainty in the blank measurements, nearly identical to that determined using the RNAA procedure.[7]

Table 5 summarizes all measurements made on SRM 955c along with previously assigned reference values for the four levels and the final arsenic values for the new Certificate of Analysis. Table 6 summarizes the statistical evaluation of the certified values. Certified values have been assigned for levels 2, 3, and 4. No evidence of material heterogeneity was observed for the Levels 2 and 3 bloods. An additional standard uncertainty of 2.342 μg/L was added to the Level 4 data to account for vial-to-vial heterogeneity observed in the RNAA data. Because of method-to-method discrepancies between results obtained for the Level 1 blood, no certified value was assigned for this material. Instead, data from all methods were combined to provide an information value of < 5μg/L.

CONCLUSIONS

RNAA has proven an extremely valuable tool for providing measurement used in certification of arsenic at low levels in biological reference materials. By providing a method independent to ICP-MS, a newly developed RNAA extraction procedure enabled assignment of certified values for arsenic mass fractions at three of four levels in SRM 955c Toxic Elements in Caprine Blood and at two levels in SRM 2668 Toxic Elements in Frozen Human Urine. Because spectrometric interferences are minimized due to separation of interfering nuclides prior to counting, the method yields arsenic detection limits down to 0.1 μg/kg. The method is currently being used to value assign arsenic mass fractions in food SRMs at lower μg/kg levels and should continue to prove valuable for arsenic certification of future reference materials.

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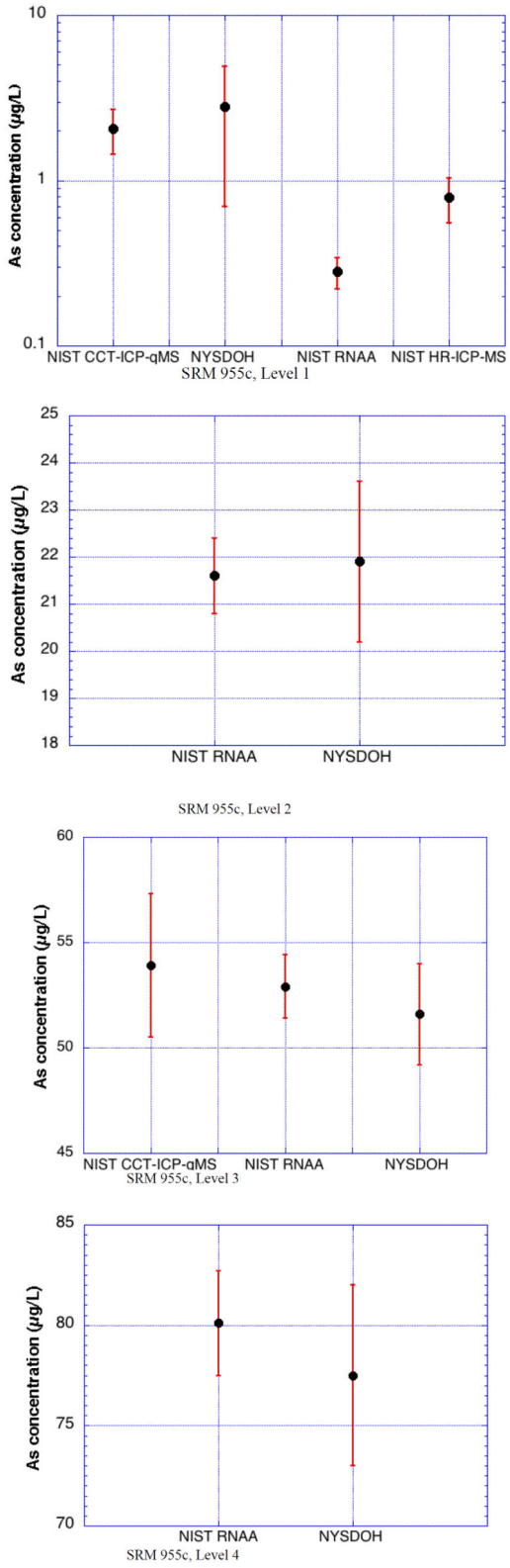

Figure 1.

Arsenic measured in SRM 955c Toxic Elements in Caprine Blood, by ICP-MS and RNAA at NIST and by ICP-MS by reference laboratories in collaboration with NYSDOH. Error bars represent expanded uncertainties.

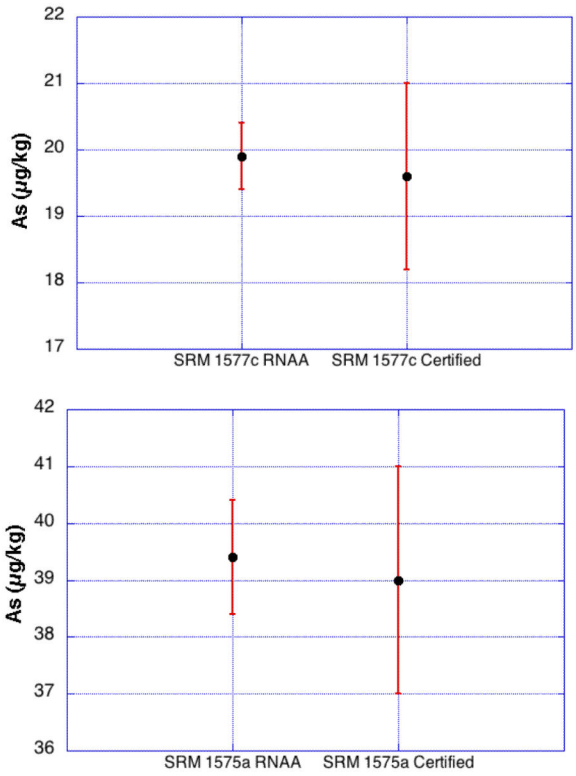

Figure 2.

Arsenic measured in controls SRM 1577c Bovine Liver (top) and SRM 1575a Pine Needles (bottom) by RNAA, compared with certified values. RNAA values represent the mean of 10 values, error bars represent expanded uncertainties (U).

Table 1

RNAA results for determination of As in SRM 2668 Toxic Elements in Frozen Human Urine, Levels I and II with evaluation of uncertainties. Uncertainties from counting statistics are given in jarentheses for each measurement.

Replicate Sample Number	As (μg/L) Level I	As (μg/L) Level II
1	10.80 (2.5)	210 (0.3)
2	10.41(2.5)	212 (0.2)
3	10.18 (2.2)	207 (0.3)
4	10.40(1.2)	211 (0.3)
5	10.13 (2.4)	208 (0.3)
6	10.75 (2.0)	*
Mean	**10.44**	**209**
Standard Deviation	**0.28**	**2**
Relative Standard Deviation (%)	**2.66**	**0.94**
Relative measurement replication uncertainty (%)	**1.1**	**0.42**
Final value (U)	**10.44 (0.47)**	**209 (4.8)**

*A sixth vial of the Level II urine was prepared for analysis, but sample was lost during chemical processing.

Table 2

Arsenic mass concentration values measured by RNAA and CCT-ICP-qMS at NIST and values contributed by outside laboratories along with expanded uncertainties (U). Uncertainties for collaborating labs are based on sample replication only, whereas NIST uncertainties represent the true expanded uncertainties. Certified values are weighted means of the results.

Level	As RNAA (μg/L)	As CCT-ICP-qMS NIST (μg/L)	As measured by outside laboratories by ICP-MS	As certified value (μg/L)
I	10.44 ± 0.47 (n=6)	10.53 ± 0.33	10.61 ± 0.18 11.6 ± 2.5 11.49 ± 0.61 14.6 ± 5.7	10.81 ± 0.54
II	209.6 ± 4.8 (n=5)	220.8 ± 5.3	213.405 ± 3.06 207.877 ± 5.75 218.132 ± 13.51 210.662 ± 16.59	213.1 ± 4.4

Table 3

RNAA results for determination of As in SRM 955c Toxic Elements in Caprine Blood, Levels 1 and 3, with uncertainties. Uncertainties from counting statistics for each sample are given in parenthesis for Level 1.

Sample designation	As (μg/L) Level 1	As (μg/L) Level 3
1	0.393 (10.2%)	52.5
2	0.284 (15.4%)	52.4
3	0.165 (22.5%)	52.0
4	0.380 (12.2%)	53.2
5	0.219 (23.1 %)	52.7
6	0.221 (22.7 %)	54.8
7	0.289 (15.0 %)	---
8	0.277 (16.0 %)	---
9	0.356 (10.6 %)	---
10	0.274 (19.2%)	---
11	5.68 (1.8%)*	---
12	0.146 (40.6%)	---
Mean	**0.280**	**52.9**
Standard Deviation	**0.083**	**1.0**
Relative Standard Deviation (%)	**29.4**	**1.9**
Average relative uncertainty from counting statistics (%)	**18.9**	**0.7**
Relative measurement replication uncertainty (%)	**8.9**	**0.79**
Final value (U)	**0.28 ± 0.06**	**52.9 (1.5)**

*Anomalous value not included in mean and standard deviation.

Table 4

RNAA results for determination of As in SRM 955c Toxic Elements in Caprine Blood, Levels 2 and 4, with uncertainties, showing paired samples (a and b) from the same vial.

Sample designation	As (μgL) Level 2	As (μg/L) Level 4
1a	21.9	77.9
1b	20.9	77.7
2a	21.3	82.0
2b	21.6	82.9
3a	21.6	80.5
3b	21.5	81.5
4a	(21.1)*	77.8
4b	22.4	(74.9)*
Mean	**21.6**	**80.1**
Standard Deviation	**0.5**	**2.2**
Relative Standard Deviation (%)	**2.15**	**2.78**
Average relative uncertainty from counting statistics (%)	**1.3**	**0.6**
Relative measurement replication uncertainty (%)	**0.81 %**	**1.05 %**
Final value (U)	**21.6 (0.8)**	**80.1 (2.6)**

*Bag leaked during irradiation; value not included in mean or final value.

Table 5

Arsenic mass concentrations (in μg/L) measured in SRM 955c. The previous reference value (determined using only NIST CCT-ICP-qMS and Reference Lab ICP-MS data) and the new certified value are given. All uncertainties are expanded uncertainties (U).

Level	CCT-ICP-qMS NIST	ICP-MS Ref. Labs	RNAA NIST	HR-ICP-MS NIST	Previously reported Reference Value from ICP-MS data	Certified Value from all data
1	2.07 ± 0.63	2.8 ± 2.1	0.28 ± 0.06 (n=11)	0.79 ± 0.24 (n=6)	2.07 ± 0.63[†]	< 5[i]
2	no data	21.9 ± 1.7	21.6 ± 0.8 (n=7)	no data	21.9 ± 1.7[‡]	21.66 ± 0.73
3	53.9 ± 3.4	51.6 ± 2.4	52.9 ± 1.5 (n=5)	no data	53.9 ± 3.4[†]	52.7 ± 1.1
4	no data	77.5 ± 4.5	80.1 ± 2.6 (n=7)	no data	77.5 ± 4.5[‡]	78.8 ± 4.9

[†]Value determined from NIST analyses with corroboration for reference laboratories

[‡]Value based on data from reference laboratories

[i]Information only value

Table 6

Statistical evaluation of certified values for SRM 955c, where u_c is the combined standard uncertainty, df is degrees of freedom, k is the 95 % coverage factor, and U is the expanded uncertainty

Level	Final Value µg/L	u_c µg/L	df	k	U µg/L	Standard uncertainty for heterogeneity µg/L	df Heterogeneity Uncertainty	Suggested Value Type
1	< 5	-	-	-	-	none	na	Information
2	21.66	0.368	183	1.973	0.73	none	na	Certified
3	52.7	0.53	161	1.975	1.1	none	na	Certified
4	78.8	2.65	Inf	1.855	4.9	2.342	3	Certified