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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

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Abstract Radiochemical neutron activation analysis (RNAA) was used to measure arsenic at four levels in standard reference material (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 1×10^{14} cm^{-2} s^{-1}. After sample dissolution in perchloric and nitric acids, arsenic was separated from the matrix either by retention on hydrated manganese dioxide (urine) or by extraction into zinc diethyldithiocarbamate in chloroform (blood). ^{76}As was 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 values from National Institute of Standards and Technology and collaborating laboratories to provide certified values of 10.81 ± 0.54 and 213.1 ± 0.73 μg/L for SRM 2668 Levels I and II, and certified values of 21.66 ± 0.73, 52.7 ± 1.1, and 78.8 ± 4.9 μg/L for SRM 955c Levels II–IV, respectively. Because of discrepancies between values obtained by different methods for SRM 955c Level I, an information value of <5 μg/L was assigned for this material.

Keywords Neutron activation analysis · Arsenic · Toxic elements · Biologicals · Radiochemistry · Standard reference materials

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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 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 (NIST) 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 SRMs. Arsenic is quantified by counting of the principal ^{76}As gamma-ray line (559 keV) emitted from ^{76}As, $t_{1/2} = 1.09379 \pm 0.00045$ days, 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 NAA (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 $\approx$100 μg/kg is often hampered by the presence of significant amounts of ^{24}Na ($t_{1/2} = 15$ h), ^{82}Br ($t_{1/2} = 35.3$ h), or ^{32}P ($t_{1/2} = 14.3$ days) resulting in high count rates, high dead time, and elevated spectrum baseline because of 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 NAA (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 (HMD) 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 $\approx$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. (A reference value represents the best estimate of the true value, but because NIST has not fully investigated all known or suspected sources of bias, the value does not qualify as a certified value [6].) In this investigation, RNAA was used 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. Level I is composed of base blood from undosed adult goats and contains endogenous (trace) levels of metals. Levels II–IV 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 II–IV were spiked to contain approximately 20, 50, and 80 μg/L of arsenic, respectively. Homogeneity assessment of the arsenic in Levels I and III 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. Each unit of the SRM consists of five vials of frozen urine of each of two levels: Level I, with approximately 10 μg/L of arsenic, is similar to the US population geometric mean ($\approx$50–95th percentile distribution) and Level II, with approximately 200 μg/L of arsenic, represents an elevated urine concentration (>95th percentile) based on National Report on Human Exposure to Environmental Chemicals [8] (2005–2006 National Health and Nutrition Examination Survey).

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 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 I blood and six samples of Level III blood were analyzed, each vial again representing one sample. Since no previous homogeneity testing was performed on the Levels II and IV 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.

Samples were prepared by transferring the contents of the vials to acid washed bags of linear polyethylene, seated inside Teflon bottles. The bottles were weighed before and after transference to determine the mass of each sample. The capped bottles containing the sample-filled bags were frozen at −80 °C overnight, then freeze-dried at −40 °C for 14 days. Freeze drying was performed 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). After freeze drying, bags were inspected for signs of leakage during the freeze-drying process; no leaks were detected. Bags were then sealed twice more into bags of polyethylene to provide triple containment.

Standards were prepared by deposition of 54.37 ± 0.31 μg/g of arsenic solution (prepared by gravimetric dilution of SRM 3103a Arsenic Standard Solution) onto Whatman 41 filter paper followed by drying in a clean hood. 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. Control materials and standards 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). Rabbits were 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.

Digestion of samples, standards, and controls

Approximately 3 days after irradiation, samples, standards, and controls were removed from the rabbits. Prior to sample dissolution, a digestion beaker was prepared for each sample, standard, or control by adding 0.1 mL of an arsenic carrier solution (approximately 1 mg/mL) and 1 mL of an ^{77}As ($t_{1/2} = 1.6179$ days [9]) tracer solution to a Teflon beaker. The ^{77}As tracer solution was used 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, and was prepared by irradiation and dissolution in KOH of 100 mg of high purity (>99.9 % based on measurement of impurities) GeO_2. Details of the tracer preparation are described elsewhere [7, 10]. 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 for samples. Each sample bag was transferred to a digestion beaker and approximately 10 mL of water (sufficient to completely cover the encapsulated sample) were added. A razor blade knife was 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 150–200 °C for approximately 4–5 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 h. 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 and the solution evaporated to near dryness. Next 10 mL of HNO_3 and 10 mL of $HClO_4$ were added to each beaker, the beaker heated (covered) at 150–250 °C for approximately 12 h to digest the sample, and the solution then evaporated to near dryness (1–2 mL). After repeating the $HNO_3/HClO_4$ digestion, with evaporation of solution to 1–2 mL, each digest was then set aside for further processing.

For processing of standards and controls, pellets were removed from the polyethylene bags and each added to a digestion beaker, previously prepared as described above. 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 10 mL concentrated HNO_3 and 10 mL H_2O were added, and each beaker heated (covered) at 150–200 °C for 4–5 h, followed by evaporation of the solution 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 from SRM 2668 urine samples was separated by absorption on HMD [7, 11, 12], since the

solvent extraction procedure was still under development. Ten milliliters of 1 mol/L HNO_3 was added to each digest, and the beaker covered and heated at 100–150 °C for approximately 30 min to ensure dissolution of residue. After cooling, each digest was added to a column containing about 5 mL of HMD conditioned in 1 mol/L HNO_3, with a flow rate of approximately 0.25–0.5 mL/min. When the digest had passed into the HMD column, an additional 5 mL of 1 mol/L HNO_3 was used to rinse the beaker and the rinse was added to the column. After that 5 mL had passed into the HMD column two 15 mL additions of 1 mol/L HNO_3 were added to each column sequentially. The HMD from each column was transferred to a plastic scintillation vial by inverting the column and rinsing with 1 mol/L HNO_3. Each vial was centrifuged to separate the HMD from the excess 1 mol/L HNO_3 and the liquid was discarded.

Arsenic in the digests from SRM 955c blood samples was separated using the more recently developed solvent extraction procedure [7, 13], which has been shown to yield a factor of three better detection limits than separation using HMD [7]. Five milliliters of concentrated H_2SO_4 were added to the digest in each beaker. The beakers were heated uncovered at a temperature of 250–275 °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+}–As^{3+}. Beakers were again covered and heated to boiling for 10–15 min. Lids were removed, 1.5 mL of 0.8 mol/L ascorbic acid added to complete the conversion of As–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 ($t_{1/2} = 1.09379 \pm 0.00045$ days; [14]). 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 of the 239 keV peak for each sample or control with the average count rate of the same peak for 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 ± 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 with an expanded uncertainty <0.001 g/mL, with 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 mass.

Results and discussion

SRM 2668 toxic elements in frozen human urine

Table 1 gives mass concentration values for As measured by RNAA in the Levels I and 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_c), which was then multiplied by a coverage factor of 2 to obtain the expanded uncertainty [15]. The following sources of uncertainty were evaluated, with associated relative standard 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, 4). (2) Uncertainties associated with measurement replication for standards, calculated as $1s/\sqrt{n}$ (0.5–1 %). (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, 0.1–0.7 % for others). (5) Pileup correction uncertainties, estimated from the uncertainty in the pileup calibration constant ($\leq$0.2 %). (6) Yield/counting geometry correction uncertainty for As, estimated from the average counting statistics given by the 239 keV ^{77}As peak and the uncertainty in the delivery volume of the 1 mL pipet used to transfer the ^{77}As solution to the digestion beakers ($\approx$0.5 %). (7) Uncertainties due to incomplete digestion of As in the bag, higher uncertainties for lower arsenic levels (0.2–2 %). 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 versus 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).

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

Replicate sample numbers	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)	[a]
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)

Uncertainties from counting statistics are given in parentheses for each measurement

[a] A sixth vial of the Level II urine was prepared for analysis, but sample was lost during chemical processing

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 [16, 17]. 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 because of loss during freeze-drying, irradiation, or dissolution (see "Discussion of SRM 955c Toxic Elements in Caprine Blood" section below). It is also unlikely that the ICP-MS values are biased high because of polyatomic interferences from ^{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 Levels I and II urine contained approximately same amount of chlorine (2.73 ± 0.12 and 2.62 ± 0.10 mg/L), and presumably equal amounts of calcium as well, since no calcium was added to the Level II urine,[1] if ^{40}Ar^{35}Cl and ^{40}Ca^{35}Cl are

[1] 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.

Table 2 Arsenic mass concentration values in SRM 2668 measured by RNAA and CCT–ICP-qMS at NIST and values contributed by outside laboratories along with expanded uncertainties (*U*)

Levels	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	10.53 ± 0.33	10.61 ± 0.18	10.81 ± 0.54
	($n = 6$)		11.6 ± 2.5	
			11.49 ± 0.61	
			14.6 ± 5.7	
II	209.6 ± 4.8	220.8 ± 5.3	213.405 ± 3.06	213.1 ± 4.4
	($n = 5$)		207.877 ± 5.75	
			218.132 ± 13.51	
			210.662 ± 16.59	

Uncertainties for collaborating laboratories are based on sample replication only, whereas NIST uncertainties represent the true expanded uncertainties. Certified values are weighted means of the results

contributing to the bias, one would expect the same amount of bias at Level I. Yet ICP-MS and RNAA agree for Level I 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 RNAA 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 [18, 19]. The estimation procedures were supplemented by the parametric bootstrap for uncertainty propagation [20–22]. The certified values for both levels are given in Table 2 along with expanded uncertainties.

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 I blood and 6 vials of Level III blood. Table 4 gives arsenic values measured for eight paired samples of each of Levels II and IV, with each vial of blood providing two 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 Levels I and III 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 H_2 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 I–IV were distributed for analysis in addition to regularly scheduled proficiency testing samples as part of a special education event. Arsenic results for this study were provided by 9–10 reference laboratories with 8–9 laboratories employing ICP-MS and 1 laboratory employing HR-ICP-MS [16]. 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, 21.9 ± 1.7, 54.9 ± 3.4, and 77.5 ± 4.5 μg/L for arsenic mass concentrations of Levels I–IV, respectively. Reference values for Levels II and IV were based solely on data from the non-NIST reference laboratories, while reference values for the Levels I and III 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. Table 5 shows that mean values obtained by CCT–ICP-qMS from NIST and the non-NIST reference laboratories for the Levels I and III blood agree within expanded uncertainties, although the relative expanded uncertainty for the Level I blood measurements for NYSDOH laboratories is 76 %, due to the As content being close to the LOD for most ICP-MS laboratories.

The RNAA value measured here for Level III is in agreement with both NIST and non-NIST reference

Table 3 RNAA results for determination of As in SRM 955c Toxic Elements in Caprine Blood, Levels I and III, with uncertainties

Sample designations	As (μg/L) Level I	As (μg/L) Level III
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 %)[a]	–
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)

Relative 1*s* uncertainties from counting statistics for each sample are given in parenthesis for Level I

[a] 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 II and IV, with uncertainties, showing paired samples (a and b) from the same vial

Sample designations	As (μg/L) Level II	As (μg/L) Level IV
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[a]	77.8
4b	22.4	74.9[a]
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)

[a] Bag leaked during irradiation, value not included in mean or final value

laboratory ICP-MS values, and RNAA values for Levels II and IV are in agreement with the mean value obtained from the reference laboratories within the expanded uncertainties (Table 5). No heterogeneity was observed in the Level II material by RNAA, but analyses of the Level IV 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 (Table 5). With the exception of one outlying value, individual RNAA values ranged from about 0.15 to 0.4 μg/L. Neglecting the outlier, a weighted mean and standard deviation (1*s*) 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 contamination 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 11 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 because of a 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 II–IV bloods, as indicated by agreement between RNAA and ICP-MS values. However, those materials were spiked with As(III), while arsenic may

Table 5 Arsenic mass concentrations (in μg/L) measured in SRM 955c

Levels	CCT–ICP-qMS NIST	ICP-MS reference laboratory	RNAA NIST	HR-ICP-MS NIST	Previously reported reference value from ICP-MS data	Certified value from all data
I	2.07 ± 0.63	2.8 ± 2.1	0.28 ± 0.06 ($n = 11$)	0.79 ± 0.24 ($n = 6$)	2.07 ± 0.63[a]	<5[c]
II	No data	21.9 ± 1.7	21.6 ± 0.8 ($n = 7$)	No data	21.9 ± 1.7[b]	21.66 ± 0.73
III	53.9 ± 3.4	51.6 ± 2.4	52.9 ± 1.5 ($n = 5$)	No data	53.9 ± 3.4[a]	52.7 ± 1.1
IV	No data	77.5 ± 4.5	80.1 ± 2.6 ($n = 7$)	No data	77.5 ± 4.5[b]	78.8 ± 4.9

The previous reference value (determined using only NIST CCT–ICP-qMS and reference laboratory ICP-MS data) and the new certified value are given. All uncertainties are expanded uncertainties (U)

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

[b] Value based on data from reference laboratories

[c] Information only value

be dominant in more volatile forms in the natural matrix material. Arsenic measured at natural levels in the Level I urine 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 (Figs. 1 and 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, CCT 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 I blood were performed at NIST using HR-ICP-MS. This method is capable of higher mass resolution with capabilities exceeding those of ICP-qMS. Arsenic concentrations measured at NIST in six samples using this method ranged from 0.64 to 0.91 μg/L with a mean value and expanded uncertainty of 0.79 ± 0.24 μg/L, significantly lower than the mean value obtained by CCT–ICP-qMS, but not as low as the RNAA value (Table 5). The limits of detection for measurement of As by RNAA and ICP-MS are relatively comparable in the absence of interferences: a detection limit of 0.1 μg/L was calculated for ICP-MS as three 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

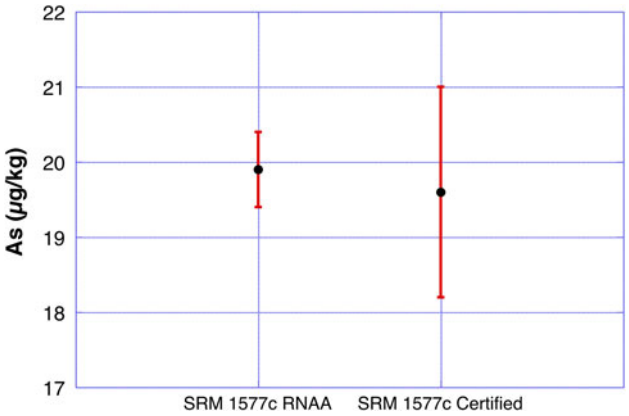

Fig. 1 Arsenic measured in SRM 1577c Bovine Liver by RNAA, compared with certified values. *Error bars* represent expanded uncertainties (U)

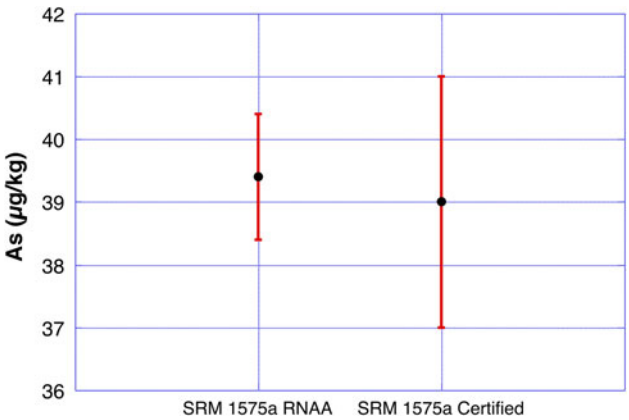

Fig. 2 Arsenic measured in SRM 1575a Pine Needles by RNAA, compared with certified values. *Error bars* represent expanded uncertainties (U)

Certificate of Analysis [23]. Table 6 summarizes the statistical evaluation of the certified values. Certified values have been assigned for Levels II–IV. No evidence of material heterogeneity was observed for the Levels II and

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

Levels	Final value (μg/L)	u_c (μg/L)	df	k	U (μg/L)	Standard uncertainty for heterogeneity (μg/L)	df heterogeneity uncertainty	Suggested value types
I	<5	–	–	–	–	None	na	Information
II	21.66	0.368	≥60	2	0.73	None	na	Certified
III	52.7	0.53	≥60	2	1.1	None	na	Certified
IV	78.8	2.65	≥60	2	4.9	2.342	3	Certified

III bloods. An additional standard uncertainty of 2.342 μg/L was added to the Level IV 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 I blood, no certified value was assigned for this material. Instead, data from all methods were combined to provide an information value of <5 μg/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, RNAA enabled assignment of certified values for arsenic mass fractions at two levels in SRM 2668 Toxic Elements in Frozen Human Urine and at three of four levels in SRM 955c Toxic Elements in Caprine Blood. Furthermore, RNAA separation using solvent extraction, with detection limits down to 0.1 μg/kg, allowed determination of arsenic in the Level I blood at levels <1 μg/L. These measurements represent the first time the solvent extraction procedure has been used in the certification of As in a NIST SRM. This method has also been used to value assign arsenic mass fractions in food SRMs at levels below 10 μg/kg levels and should continue to prove valuable for arsenic certification of future reference materials.

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