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Characterization of candidate reference materials for bone lead via interlaboratory study and double isotope dilution mass spectrometry

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Summary

Four candidate ground bone reference materials (NYS RMs 05-01 through 04), were produced from lead-dosed bovine and caprine sources, and characterized by interlaboratory study. The consensus value (X) and expanded standard uncertainty (U_X) were determined from the robust average and standard deviation of the participants' data for each NYS RM 05-01 through 04. The values were 1.08 ±0.04, 15.3 ±0.5, 12.4 ±0.5, and 29.9 ±1.1 μg g^{-1} Pb, respectively. Youden plots of *z*-scores showed a statistically significant correlation between the results for pairs of NYS RM 05-02 through 04, indicating common sources of between-laboratory variation affecting reproducibility. NYS RM 05-01 exhibited more random variability affecting repeatability at low concentration. Some participants using electrothermal atomic absorption spectrometry (ETAAS) exhibited a negative bias compared to the all-method consensus value. Other methods used included inductively coupled plasma mass spectrometry (ICP-MS), isotope dilution (ID-) ICP-MS, and ICP atomic (optical) emission spectroscopy (-OES). The NYS RMs 05-01 through 04 were subsequently re-analyzed in house using double ID-ICP-MS to assign certified reference values (C) and expanded uncertainty (U_C) of 1.09 ± 0.03, 16.1 ± 0.3, 13.2 ± 0.3 and 31.5 ± 0.7, respectively, indicating a low bias in the interlaboratory data. SRM 1486 Bone Meal was analyzed for measurement quality assessment obtaining results in agreement with the certified values within the stated uncertainty. Analysis using a primary reference method based on ID-ICP-MS with full quantification of uncertainty calculated according to ISO guidelines provided traceability to SI units.

1. Introduction

Analytical measurement of lead in bone is currently important in clinical and in environmental epidemiological studies, as well as in archaeology, agriculture and materials science[1-7]. This wide interest reflects both the well-known toxicity of lead and its affinity for accumulating in bone [8]. The residence time of lead in bone varies from years to decades, depending on the bone type, subject age, and other factors[9]. Bone lead is thus perceived as a proxy for long-term or historical exposure to lead, in contrast to blood or urine lead that reflect more recent exposure[10,11]. Lead stored in bone can be remobilized as the body utilizes stored calcium during pregnancy, or lactation, or as a result of osteoporosis, thereby creating an internal exposure risk[12]. Lead can also reduce bone density[13].

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In clinical or epidemiological human studies, K-shell x-ray fluorescence spectrometry (KXRF) technology has been employed for non-invasive, *in vivo* determination of lead in tibia or other bone sites such as calcaneous[14,15]. Although the related method of L-shell (L)XRF has also been applied to *in vivo* bone lead measurement, KXRF is the dominant technique[15]. In situations where bone specimens are available for analysis (e.g., post-mortem cases, biopsy samples), analytical methods such as electrothermal atomic absorption spectrometry (ETAAS)[16], inductively coupled plasma – mass spectrometry (ICPMS) including isotope dilution (ID-) ICP-MS[17-19], and ICP - optical emission spectrometry (-OES)[20] have been employed. Bone is generally considered a challenging matrix to analyze by such solution-based methods as digests have high total dissolved solids, mostly calcium.

The National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) currently provide two bone-based Standard Reference Materials (SRMs) with certified lead concentrations, SRM 1486 Bone Meal and SRM 1400 Bone Ash. SRM 1486 Bone Meal has a certified lead concentration of 1.335 ±0.014 $\mu g\ g^{-1}$ and SRM 1400 Bone Ash has a certified lead content of 9.07 ±0.12 $\mu g\ g^{-1}$. The certified values were established by thermal ionization isotope dilution mass spectrometry (TI-IDMS). However, these two SRMs differ strongly in appearance and composition. According to the certificate, SRM 1486 Bone Meal was produced from material obtained from the Espoma Company, Millville, NJ. They describe their bone meal product as "… a by-product from meat (rendering factories). It consists of ground animal bones that have been cooked and sterilized, and then ground into a meal" (http://www.espoma.com/). It has a brown color reflecting a high organic content, including collagen (which is an intrinsic part of the bone matrix), fat, and possibly residual tissue. SRM 1400 Bone Ash was also obtained from commercial sources (Monsanto Co., St. Louis, MO) but was dry-ashed at high temperature, resulting in a white color and negligible (<1%) organic content.

In experimental studies of bone, the samples are rarely cooked or sterilized. Dry-ashing of bone samples is used in preparation for analysis, but digestion by mineral acids is more convenient and reduces the potential for contamination. In any case, it would be preferable to dry-ash an un-ashed reference material to test the procedure. Typical bone lead concentrations in humans range from about 1 to 50 $\mu g\ g^{-1}$ dry weight or greater in cases of chronic exposure to high lead levels. It would thus be valuable to produce additional bone reference materials to validate analytical methods for lead in bone that have similar composition, and multiple levels of lead content that span a wider concentration range.

Since the 1970s, the New York State Department of Health (NYS DOH) has maintained a herd of animals including goats (caprines) and cows (bovines), that have been routinely dosed with small quantities of lead acetate, to produce blood pools for laboratory proficiency testing exercises[20,21]. Since 1994, long bones from these animals have been harvested post-mortem, creating a repository of bones that are physiologically enriched in lead. As of 2005, a total of 50 animals have provided such bones for the repository. A number of these bones were used to produce four pools of ground bone candidate reference materials (RMs).

Production of this material presented significant procedural and technological challenges that are detailed elsewhere[22]. The primary intended use of the candidate RMs is the validation of analytical methods such as ETAAS, ICP-MS, and ICP-OES for the determination of lead in bone. There is also a pressing need for bone materials endogenously enriched in lead that span a significant range in lead concentration that can be used for routine quality control-purposes and/or for calibration of solid sampling techniques such as XRF, *in vivo* XRF[23] and laser ablation ICP-MS[24].

The principal aim of this work was to characterize the lead content of the candidate RMs. First, we conducted an interlaboratory study to establish consensus values for the RMs, an approach frequently employed in proficiency testing schemes. The study also allowed us to assess agreement among laboratories, and among different analytical methods, for the measurement of lead in bone. Given that methodological bias could affect the consensus values, we also analyzed the RMs in house using a double isotope dilution (ID)-ICP-MS method with full quantification of uncertainty. The latter became the basis for assigning certified values to the RMs, in a manner similar to that used by NIST to assign reference values for Certified Reference Materials (CRMs), which is different from the IAEA and former-BCR approach.

2. Materials and Methods

2.1 Material preparation

Adult cows and goats were dosed with lead under an active protocol (number 01-096) approved by the Wadsworth Center's IACUC (Institutional Animal Care and Use Committee). Post mortem, soft tissues were removed from the long bones using tantalum-based tools, and each bone thoroughly cleaned with hydrogen peroxide solution, and defatted with ether.

The first pool of bones selected to create a base material (level 1) consisted of the major long bones (i.e., humerus, femur etc) collected from a single bovine that had not been previously dosed with Pb. The second pool (level 2) consisted of long bones from a single bovine dosed with a total of 12.7 g Pb during its lifetime (cumulative dose). The third pool (level 3) was produced by combining long bones from nine caprines that had received a cumulative lifetime dose of between 10 and 20 g Pb. The fourth pool (level 4) consisted of the long bones from 14 caprines that had received a cumulative lifetime dose of between 40 and 55 g Pb.

Full details of the optimal procedures for grinding and homogenizing these long bones are given elsewhere[22]. Briefly, the pooled bones were freeze-dried over 24 h and refrozen to −80°C. They were processed into a fine, homogeneous powder in three stages, using (1) a Retsch SM 2000 knife mill, (2) a Retsch ZM 200 ultra-centrifugal mill, and (3) a WAB T2F Turbula homogenizer (all supplied by Glen Mills, Clifton, NJ). The bulk materials were sample-scooped into plastic bottles to produce the RMs. Five hundred-vials containing approximately 5 g of material were produced at each level. The materials were labeled NYS RM 05-01 Lead in Bovine Bone (level 1), NYS RM 05-02 Lead in Bovine Bone (level 2), NYS RM 05-03 Lead in Caprine Bone (level 3) and NYS RM 05-04 Lead in Caprine Bone (level 4) (hereafter NYS RMs 05-01 through 04).

2.2 Inter-laboratory study

Formal invitations to participate in an inter-laboratory study of the lead in ground bone reference materials were distributed in July 2005, and an open invitation was posted on the PlasmaChem Web server (http:listserv.syr.edu/archives/plasmachem-l.html). All positive respondents, numbering 39 laboratories worldwide, were sent packages containing randomly selected vials of NYS RMs 05-01 through 04. The approximate lead concentration of the NYS RMs 05-01 through 04 was not specified in advance to the participants. However, an aliquot of NIST SRM 1486 Bone Meal was provided as a known control for the dual purpose of providing a quality control material that the participants might not have had access to, and to provide an indication of analytical performance. Results were received from 29 external laboratories from 13 countries, and the study was closed in March, 2006. Our laboratory also participated in the study.

Laboratories were supplied with the following recommended measurement protocol:

- Dry the samples using either an oven for 2 hrs at 105°C or a desiccator for 24 hrs
- Remove 3 sub-samples, i.e., triplicate analysis, of at least 150 mg from each vial for the digestion procedure
- Determine the lead content of the triplicate sub-samples using an instrumental method(s) of your choice on two separate days; and
- Calculate the mean and standard deviation of *n* measurements for each sub sample measured, and provide details of the analytical method(s) employed.

Participants were also invited to submit results for additional elements, as well as data on lead isotope ratios, where available. The additional data are not considered here.

2.3 In house double ID-ICP-MS

NYS RMs 05-01 to 04 were analyzed at the Wadsworth Center by ICP-MS for determination of the natural Pb isotope ratio. ID-ICP-MS was employed for both homogeneity assessment and for assigning certified reference values.

2.3.1 Sample preparation—Samples were handled in a Class 100 clean room when appropriate. High-purity, analytical grade concentrated nitric acid was used for digestions and solution acidification (Veritas Double Distilled; GFS Chemicals, Columbus, OH). Double deionized water (MilliQ 18.2 MΩcm; Millipore, Billerica, MA) was used for all dilutions. Twenty-four vials of each material were selected on a random basis from stratified groups according to the vial number representing the fill sequence.

The bone materials were stored 24 h in a desiccator over anhydrous calcium sulfate (Drierite, W.A. Hammond Drierite Company Ltd, Xenia, OH). Twenty-four sample vials were analyzed to assess the homogeneity of the material and to determine the reference value of NYS RMs 05-01 through 04. A single 0.2 g aliquot was taken from each vial for analysis. This relatively small sample mass was sufficient given the limited vial size and the absence of substantial within-vial variation. The aliquots were weighed directly into a 50-ml polypropylene tube (Sarstedt, Newton, NC) using an electronic analytical balance (Analytical Plus; Ohaus Corporation, Pine Brook, NJ). The balance had a readability of ±0.01 mg and was calibrated by standard weights (Fisher Scientific Permas Class S; Denver Instrument Company, Denver, CO). Ten vials were analyzed for the natural Pb isotope ratio determination.

2.3.2 Isotopic spiking—Preliminary lead concentrations for the NYS RMs 05-01 through 04 were determined by ETAAS.[16] A spike of SRM 991 Lead-206 Spike Assay and Isotopic (National Institute of Standards and Technology, Gaithersburg, MD), sufficient to create a blend with a $^{206}Pb/^{208}Pb$ ratio of 2 was added to NYS RM 05-01, to improve counting statistics and limit uncertainty propagation. However, a blend ratio of 1 was used for each of NYS RMs 05-02 through 04, to achieve better accuracy of the isotope ratio measurement and to limit consumption of the expensive spiking material. The spike was delivered using an appropriate calibrated pipette. The mass of spike delivered was measured with the same analytical balance employed for sample weighing. SRM 1400 Bone Ash was used as the primary standard for reverse spike calibration, or double ID-ICP-MS. SRM 1400 Bone Ash has a relatively high uncertainty in its certified lead content (1.3%) compared to other potential standards but it was selected based on the following rationale. The aim of accurately determining the concentration of the spike delivered to individual samples of the NYS RMs 05-01 through 04 was best achieved through replicating, as near as possible, the spiking and measurement procedure used for those bone samples. SRM 1400 Bone Ash had

comparable lead content, physical form and matrix to the samples that allowed it to be analyzed in a similar fashion. SRM 1400 Bone Ash was dried by simple desiccation. Subsequently, the dry weight was re-calculated by determining a correction factor after drying the sample for four hours at 105 °C as instructed by the certificate. The difference between simple desiccation and thermal drying methods was negligible for SRM 1400.

2.3.3 Sample digestion—NYS RMs 05-01 through 04 and SRM 1400 Bone Ash were digested at room temperature in closed 50-ml polypropylene tubes (Sarstedt) with 5 ml concentrated nitric acid for at least 12 h, resulting in a clear, particle-free solution. The mean blank concentration was subtracted from the concentration for sample plus blank obtained by ID-ICP-MS. All digests were initially diluted to 25 ml. A further 10-fold dilution was performed prior to ID-ICP-MS measurement. Blank samples were prepared by adding concentrated nitric acid (5 ml) to tubes without sample and subsequently diluted in the same manner described above. The blanks were analyzed separately by ICP-MS using an external calibration method with 4 standard solutions diluted from commercially available lead standards and with ^{209}Bi as an internal standard.

The above digestion procedure used for NYS RMs 05-01 through 04 and SRM Bone Ash was not sufficient, however, to digest SRM 1486 Bone Meal fully. Thus, SRM 1486 Bone Meal was digested using a closed-vessel (HP500; CEM, Matthews, NC) high-pressure microwave assisted digestion system (MARS 5, CEM, Matthews, NC). The samples were subjected to a maximum 170°C temperature and 180 psi pressure for 30 min, resulting in clear, particle-free homogenous solutions. The digestate was transferred to a 50-ml tube. Procedural blanks were also prepared in a similar manner.

2.3.4 Isotopic standards—Isotopic standards for calculating the mass bias factor were prepared from NIST SRM 981 Natural Lead (isotopic) and NIST SRM 982 Equal Atom Lead (Isotopic). About 0.25 g, accurately weighed, of each material were dissolved in 20 ml concentrated nitric acid and 20 ml high purity water in a 50-ml tube, equipped with a vented cap, and moderately heated in a Model 2100 microwave digestion system (CEM, Matthews, NC). The solution was quantitatively transferred to a 200-ml Teflon volumetric flask and diluted to volume. For the analysis, the solutions were diluted to about 10 $\mu g\ l^{-1}$ Pb, similar to the bone digests.

2.3.5 ICP-MS measurements—We used the ELAN Model DRC Plus quadrupole ICP-MS instrument (Perkin Elmer, Shelton, CT) fitted with a Meinhard nebulizer (WE024371) and cyclonic spray chamber (WE025221) supplied by Meinhard Glass Products, (Golden, CO). The instrument was optimized prior to each run, following the manufacturer's recommended protocols. An individual run consisted of analyzing a single RM/SRM for natural isotope ratio, or single RM/SRM plus primary lead standards and blanks for spiked ratio determination. The mass bias calibration solutions of either SRM 981 Natural Lead (Isotopic) or SRM 982 Equal Atom Lead (Isotopic) were analyzed at the start of the run and subsequently at every 3 samples. A mass bias correction factor was calculated for each standard. The mass bias factor applied to each sample was calculated from the neighboring standards using a linear model.

Natural isotopic abundances of ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb for the RMs were recorded by acquisition of counts at *m/z* 204 (corrected for Hg), 206, 207, and 208, using 10-ms dwell times, 500 sweeps and 10 replicates. Mass bias was determined using SRM 981 Natural Lead Isotopic. The values of the natural ^{206}Pb/^{208}Pb ratios were 0.520 to 0.525 for NYS RMs 05-01 through 04, 0.522 for SRM 1486 Bone Meal, and 0.524 for SRM 1400 Bone Ash, indicating that the materials had similar Pb isotopic abundance. For ID measurements, spiked ^{206}Pb/^{208}Pb ratios were recorded by acquisition of counts at *m/z* 206 and 208 for

1000 sweeps with 10 replicates. Mass bias was determined using NIST SRM 982 Equal Atom Lead (Isotopic).

3. Results and Discussion

3.1 Interlaboratory study

Laboratories that participated in the inter-laboratory study are identified in Table 1. Each laboratory was assigned an arbitrary identification number (Lab ID), to preserve confidentiality. No restrictions were placed on the methods that could be employed by participants so as to (1) maximize participation and (2) ensure that a wide range of methods were employed. Results of the interlaboratory measurements of Pb in the RMs are given in Table 2. In proficiency testing schemes, it is common practice to assign target values for the materials from participant data. This is often based on a sub-group of participants referred to as the expert or reference laboratory group.[25] The criteria for defining an expert group are not well established, and are based mostly on past performance. In this study, it was not possible to judge past (proven) performance for bone lead and, therefore, any definition of an expert group would have been highly subjective.

An aliquot of NIST SRM 1486 Bone Meal was provided to each participant as a known (unblinded) CRM, thereby allowing participants to check the quality of their data prior to submission. The availability of participants' results for SRM 1486 Bone Meal might be considered useful for assessing the quality of data provided for NYS RMs 05-01 through 04. The NYS RMs were thoroughly cleaned and defatted but, unlike SRM 1486, they were not heated or sterilized and, unlike SRM 1400, they were not dry ashed.

Consensus values for the NYS RMs 05-01 through 04 were determined from the data submitted by all participants. The consensus values and their standard uncertainty were established using a robust analysis to calculate the robust average and robust standard deviation (i.e., the estimate of the population mean and population standard deviation calculated using a robust algorithm, since robustness is a property of the algorithm and not of the estimate), as described in ISO 13528, 2005.[25]

Individual results were ranked in increasing order:

$$\left(x_1, x_2, \ldots, x_i, \ldots, x_p\right)$$

Initial values of the robust average x^* and robust standard deviation s^* were calculated as:

$$x^* = \text{median } x_i \ (i = 1, 2, \ldots, p) \quad (1)$$

$$s^* = \text{median}|x_i - x^*| \ (i = 1, 2, \ldots, p) \quad (2).$$

The initial values x^* and s^* were updated by calculating:

$$\delta = 1.5 s^* \quad (3).$$

For each x_i, x_i^* was calculated where:

$$\begin{aligned} &\text{if } x_i < x^* - \delta, \ x_i^* = x^* - \delta \\ &\text{if } x_i > x^* - \delta, \ x_i^* = x^* + \delta \\ &\text{otherwise}, \ x_i^* = x_i \end{aligned} \quad (4).$$

New values for x^* and s^* were calculated as:

$$x^* = \sum x_i^* / p \quad (5)$$

$$s^* = 1.134 \sqrt{\sum (x_i - x^*)^2 / (p - 1)} \quad (6).$$

The robust estimates of x^* and s^* were calculated by iteration by updating the values of x^* and s^* until they converged to the third significant figure.

The consensus value X was thus defined as $X = x^*$.

The standard uncertainty of X was calculated as:

$$u_X = 1.25 s^* / \sqrt{p} \quad (7).$$

The consensus values and expanded standard uncertainty ($U_X = 2u_X$) are given in Table 3A.

Figure 1 shows Youden plots of the z-scores for each NYS RM 05-01 through 04 (z_A) plotted against a different NYS RM 05-01 through 04 (z_B) with a 95% confidence ellipse[26]. Using the above consensus values (X) and standard uncertainties (u_X), z-scores were calculated for the data (xi), where

$$z = \frac{x_i - X}{u_X} \quad (8).$$

In the Youden plots, the data show a strong linear trend, indicating that individual laboratories reported results that were consistently lower or higher compared to the consensus value. This trend, and thus the axis of the confidence ellipse, fell along the line $zA = zB$.

The trend in the data indicates common sources of systematic errors and between-laboratory variation that affects the reproducibility of results from one lab to the next. For the pairs of RMs involving NYS RM 05-02 through 04 exclusively, the data had greater linearity than for pairs including NYS RM 05-01. Rank correlation coefficients showed that the correlation between the data for NYS RMs 05-01 and 02, 05-01 and 03, and 05-01 and 04 was statistically significant at the 95% confidence level. NYS RMs 05-02 and 03, 05-02 and 04, and 05-03 and 04 were significantly correlated at the 99% confidence level. Possible common sources of between-laboratory variations include the calibration strategies, specifically the comparability of standards and samples given the composition of the sample matrix. A further common source of uncertainty is the effectiveness of the drying procedure. It was not possible, however, to determine the cause of the variability from the details participants provided on analytical method. The lower linearity of the data including NYS RM 05-01 indicates further sources of random variation affecting repeatability, presumably resulting from the relatively low concentration of Pb in NYS RM 05-01.

Those measurements that are well separated from the rest of the results indicate outlying data. Lab 410, Lab 419 and Lab 424 returned outlying data for all NYS RMs 05-01 through 04. Grubbs' Tests also identified the data reported by these Laboratories as outliers (Table 2). Surprisingly, one of those laboratories (410) reported using ID-ICP-MS for the analysis. For the pairs of RMs involving NYS RM 05-02 through 04 exclusively, these outlying results were close to the axis of the ellipse indicating systematic bias affecting the reproducibility of the results between different laboratories. For the pairs of RMs involving

NYS RM 05-01 the outlying results were removed from the axis of the ellipse indicating poor within laboratory repeatability.

The Pb concentration in SRM 1486 Bone Meal was known to participants, thus limiting its use as an unbiased measure of laboratory performance. Nonetheless, six laboratories reported outlying results for SRM 1486 Bone Meal. Lab 419 was an outlier for SRM 1486 Bone Meal and for NYS RM 05-01 through 04. Labs 426, 430, 434, 436 and 438, however, reported outlying results for SRM 1486 Bone Meal, but satisfactory results for NYS RM 05-01 through 04. Conversely, Lab 410 and 424 were satisfactory for SRM 1486 Bone Meal but outlying for NYS RM 05-01 through 04. These data show that a satisfactory result for SRM 1486 Bone Meal does not necessarily guarantee the quality of results for experimental bone samples with a different Pb concentration, and *vice versa*.

Figure 2 shows the data (mean ±s) reported by participants for each NYS RM 05-01 through 04, ranked from lowest to highest bias (relative to the consensus value). The data from the three consistently outlying Laboratories identified above are excluded. Data are categorized into four general method groups: ICP-MS including methods using external calibration or standard additions procedures (●, n=22); ETAAS (○, n=5); ID-ICP-MS (–, n=3); and ICPOES (×, n=1). Despite this simple categorization, it should be noted that some techniques are implemented quite differently from one laboratory to another in terms of sample preparation, data acquisition methods, instrumental hardware, and method optimization.

Most participants used some form of acid digestion procedure, however, two employed a dry-ashing step. The majority of participants used ICP-MS and aqueous external calibration standards, with internal standard correction for drift, though a small number employed matrix matched standards, or standard additions. ETAAS techniques included conventional instruments equipped with Zeeman background correction, high-resolution continuum source AAS instrumentation, and a transverse heated filter atomizer instrument. In general, results based on ETAAS methods exhibited a low bias, relative to both the consensus value and compared to other instrumental methods. The low bias with ETAAS was most marked with NYS RMs 03 and 04. ETAAS low bias was not apparent with NYS RM 05-01 and not as marked with NYS RM 05-02. ETAAS bias was not associated with lead concentration, as the lead content of NYS RM 05-03 is lower than the lead content of NYS RM 05-02.

The ID-ICP-MS methods used by participants included conventional quadrupole-based instrumentation, high-resolution (HR-) ICP-MS, and multi-collector (MC-) ICP-MS methodologies. Lab 110-2 and 429 indicated that they used double ID-ICP-MS procedures. The ID-ICP-MS laboratories showed a consistent relative rank in their reported results, Lab 420 being the lowest, followed by Lab 110, and Lab 429 (being the highest). Lab 429 was in closest agreement with the consensus value for NYS RM 05-01, whilst Lab 110 and Lab 420 were in closer agreement with the consensus value for NYS RM 05-02 through 04. The ID-ICP-MS methods typically had a low standard deviation of measurements compared to other methods. The sole ICP-OES method in the study gave results consistent with consensus value for all samples.

The interlaboratory study revealed generally good agreement in the determination of lead in bone by atomic spectrometric techniques. The Youden plots provided evidence of common sources of between-laboratory variation. However, the consensus values for NYS RMs 05-01 to 04 are highly dependent on the quality of the analytical methods used in the interlaboratory study. Those methods may be subject to systematic bias and may not provide an accurate estimation of the ‘true’ value (which is not known). Also, the uncertainty estimation, which is based on the variation of the interlaboratory data, may not fully account

for all possible sources of uncertainty as is required to be consistent with the ISO Guidelines for the Expression of Uncertainty in Measurement (GUM)[27]. It was not thought practical in the context of this interlaboratory study to ask participants for a full uncertainty estimation of their method.

3.2 In-house double ID-ICP-MS

ID-ICP-MS is considered a potential primary method of analysis, and is thus used by national metrological laboratories to assign certified values to reference materials.[28] It has a number of benefits for the analysis of bone; notably, since the ratio measurement is independent of the instrument sensitivity, matrix issues such as signal suppression are insignificant. The method is also free from dilution errors, since spiking is performed at the earliest possible stage after sample weighing. Further improvements in accuracy have been achieved using double isotope dilution (also called reverse spike calibration),[29] where the spike is added to a well-known primary standard allowing the concentration of the spike to be cancelled from the measurement equation (or calculated). The reference value (C) for NYS RMs 05-01 through 04 was determined as the mean of the results (C_X) of n sample aliquots analyzed by a double ID-ICP-MS procedure for lead based on addition of an enriched ^{206}Pb spike similar to procedures reported by other workers[30-32]. We used the following equation derived from the (single) isotope dilution equation given by Fasset[33] for the concentration of analyte in the sample (C_x)

$$C_x = \frac{W_y}{W'_s C_y}\left(\frac{K'_b R'_b B_y - A_y}{A_s - K'_b R'_b B_s}\right)\frac{W_s}{W_x}\left(\frac{A_s - K_b R_b B_s}{K_b R_b B_x - A_x}\right) \quad (9),$$

the terms used in Equation 9 are defined in Table 4.

3.3 Sample drying procedures

A comparison between various drying methods conducted in our laboratory indicated that simple desiccation over anhydrous calcium sulfate (Drierite, W.A. Hammond Drierite Company Ltd, Xenia, OH) in a storage cabinet was not sufficient to dry the caprine and bovine sourced material (NYS RMs 05-01 through 04) fully, but it was effective for drying SRM 1486 Bone Meal. A more detailed study that compared simple desiccation to vacuum desiccation (Scienceware, Space Saver vacuum desiccator) over anhydrous calcium sulfate (Drierite, W.A. Hammond Drierite Company Ltd, Xenia, OH) for 24 h yielded dry weights that were 3.8% lower for the latter, whilst thermal drying at 105 °C for 2 h (Precision gravity convection oven Model 14EG) yielded dry weights that were 4% lower than simple desiccation. Thus, there was a small difference of 0.2% between vacuum desiccation and thermal drying methods. Therefore, different drying procedures were a likely source of uncertainty in the results obtained in the interlaboratory study. Subsequent studies in our laboratory showed that the rate of atmospheric water vapor adsorption, following oven drying, was approximately 0.004% min^{-1} during the first hour, depending on the relative humidity, but appeared to stabilize after 24 h. After simple desiccation, the dry weight was relatively stable over the first hour. Thus, it is recommended that, for future work with the NYS materials, dry weight should be established using oven drying at 105 °C for 2 hours.

For our in house ID-ICP-MS data that are reported here, a dry weight correction factor of 0.961 ±0.001 was applied, since we used a simple desiccation method. The uncertainty in the dry weight based on simple desiccation (u_{Wx}) was included in the estimate of combined measurement uncertainty (u_c) as described below.

The mean, standard deviation, and expanded uncertainty of the double ID-ICP-MS determinations of NYS RMs 05-01 through 04 and NIST SRM 1486 are shown in Table 3B.

These values determined by ID-ICP-MS in our laboratory are the 'certified values' subsequently assigned to NYS RMs 05-01 through 04. Only 1 or 2 outliers were found during the homogeneity measurements of the 24 aliquots of NYS RMs 05-01 through 04 based on the Grubbs' tests. These data were all positive outliers suggesting either a contamination error or, less likely, an error in spike delivery. These outliers were omitted from the calculation of the certified values. The ID-ICP-MS value found for NIST SRM 1486 (1.33 ±0.03) was in good agreement with the NIST certified value of 1.335 ±0.014.

Uncertainty in the certified value—Uncertainty in the certified values was calculated in a manner consistent with the ISO Guidelines for the Expression of Uncertainty in Measurement (GUM)[27]. The procedure used was developed from the guidelines provided by NIST,[34] Eurachem[35], and from examples given by Ellison et al.[36], Botha et al.[37] and Sturgeon et al.[32] The uncertainty for our analysis of SRM 1486 Bone Meal is also given; it was approximately twice the uncertainty as stated by NIST on the certificate.

The expanded uncertainty U_C was calculated as

$$U_C = k u_c \quad (10),$$

where k is the coverage factor and u_c the combined measurement uncertainty. We used a coverage factor of 2, to provide a 95% confidence interval. The combined measurement uncertainty u_c was calculated as

$$u_c = \sqrt{u^2_{C_X} + u^2_{\text{hom}} + y^2_{blk} + u^2_{dig}} \quad (11),$$

where u_{C_X} is the uncertainty associated with the isotope dilution calculation (Eq. 9), u_{hom} is the uncertainty associated with material homogeneity, u_{blk} is the uncertainty associated with the blank, and u_{dig} is the uncertainty associated with the digestion process (the isotopic homogenization of sample and spike). Values for each of these terms are given in Table 5. In each case, the u_{Cx} term was the largest component.

The component uC_x was calculated from the uncertainty of the 15 individual components of Equation 9 using the Kragten spreadsheet method[38]. The relative contribution of each component in the isotope dilution equation (Eq. 9) to the uncertainty of the u_{CX} term is given in Table 6. The uncertainty of the certified value of SRM 1400 Bone Ash (u_{Cy}), was the largest source of uncertainty, accounting for approx. 40 % of the total uncertainty in u_{Cx} for each NYS RM 05-01 through 04. The isotope ratio measurements for the blends of sample and internal standard, and for the calculation of the mass bias coefficient (K) also contributed to the uncertainty. This is consistent with the findings of Encinar et al.[39] who showed that the precision of the isotope ratio measurements was a large source of uncertainty for quadrupole based ICP-MS. According to these workers, this source of uncertainty was substantially reduced using double focusing or multi-collector (MC-) ICP-MS, since these methodologies have better measurement precision.[39]

The uncertainty associated with the (in-)homogeneity of the materials u_{hom} was calculated from the equation

$$u_{\text{hom}} = \sqrt{u^2_{measured} - u^2_{method}/10} \quad (12),$$

where $u_{measured}$ is the standard deviation from measurement of $\sqrt[3]{3n}$ (=24) vials[36] of the RM that were selected randomly on a stratified basis according to the fill sequence, and u_{method} is the uncertainty of the analytical method, calculated as the standard deviation of identical sample analyses (repeatability). As recommended[40], identified and explained gross outliers

were not included in the homogeneity term. The u_{blk} term was determined experimentally from 10 replicate blanks using ICP-MS with external aqueous calibration. A value of 0.2% relative standard deviation was used for u_{dig}.[37]

The certified values determined using the double ID-ICP-MS method (Table 3B) for NYS RM 05-01 were similar to the consensus values calculated from the interlaboratory data (Table 3A). However, the consensus values for NYS RMs 02 through 04 were lower than the certified values by about 5-6% (Table 3). The estimated uncertainties in the certified values (Table 3B) were smaller than the corresponding uncertainties in the consensus values (Table 3A), but there was some overlap between the two.

Some participants exhibited a low bias relative to the certified values (Figure 2). However, this may be due to the hygroscopic nature of the RMs and the different procedures used to dry the RMs as explained above. As noted earlier from our own observations, desiccation alone is inadequate to dry the RMs, and residual moisture adsorption can result in a measurable gain in mass depending on the relative humidity in the laboratory. The low bias exhibited by some participants using ETAAS methods for the analysis of NYS RMs 05-02 through 04 also contributed to the low bias of consensus values compared to the certified values, but this effect alone could not fully account for the discrepancy. It is notable that the data reported by Lab 429, that was based on a double isotope dilution procedure with full uncertainty estimation were consistently in good agreement with the certified values determined in our laboratory by double ID-ICP-MS. It is also surprising that one participant using ID-ICP-MS reported results that were identified as a gross outlier.

SRM 1486 Bone Meal was analyzed for measurement quality assessment in our laboratory obtaining results in good agreement with the NIST certified values within the stated uncertainty. Application of the primary reference ratio method of ID-ICP-MS with full uncertainty statements calculated according to ISO guidelines provided traceability to SI units.

4. Conclusions

The interlaboratory study revealed generally good agreement among participants for the determination of lead in bone by spectrometric techniques. Consensus values were calculated for the NYS RMs 05-01 through 04 consistent with ISO recommendations. Youden plots, however, provided evidence of common sources of between-laboratory variation. In addition, some ETAAS methods showed a general low bias. Subsequently, certified values were established based on a double ID-ICP-MS procedure that was performed in the coordinating laboratory. There was good agreement between some laboratories and the certified values whilst others exhibited a low bias. Drying procedures based on thermal methods are recommended. The study showed the limitations of assigning target values based on participant data from interlaboratory studies. Investigators interested in obtaining the NYS RMs 05-01 through 04 should contact the Trace Elements Laboratory, Wadsworth Center, NYS DOH regarding availability.

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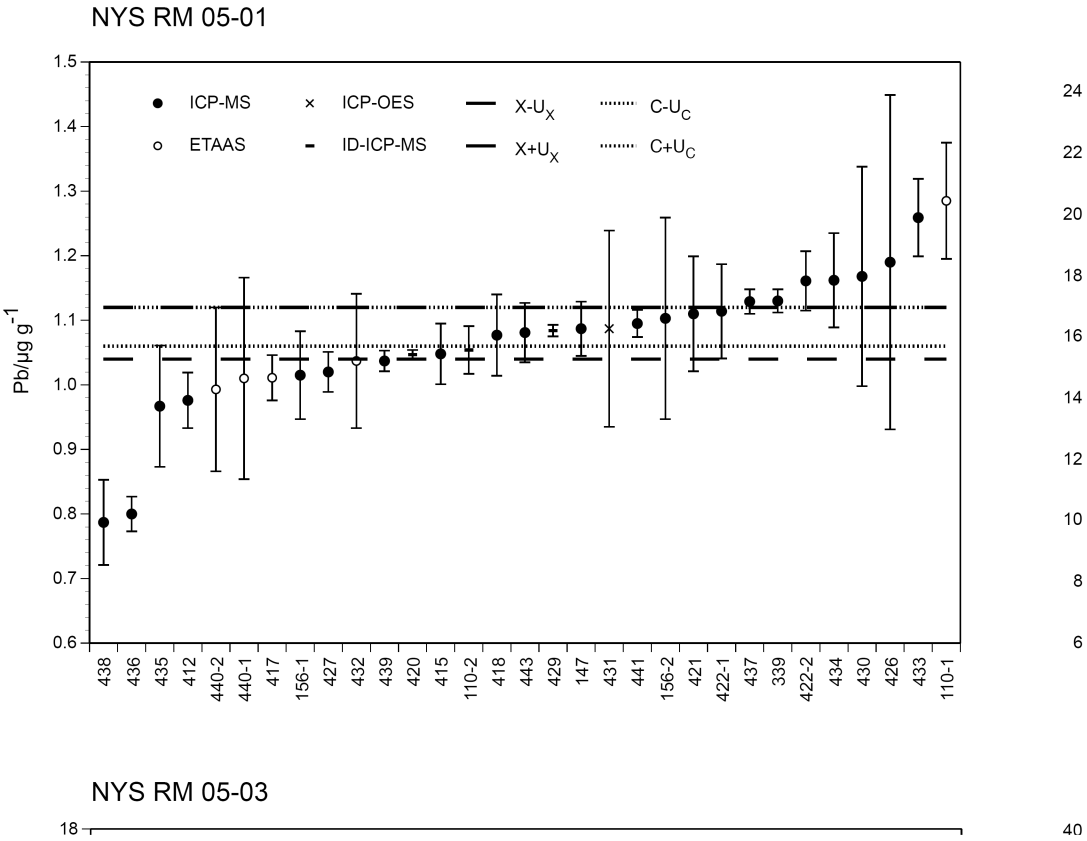

Fig. 1.

Youden plots comparing *z*-scores for the interlaboratory data on each NYS RM 05-01 through 04. Data symbols 1 through 34 indicate the Laboratory/methods when listed in order of ascending Lab ID as shown in Table 4.

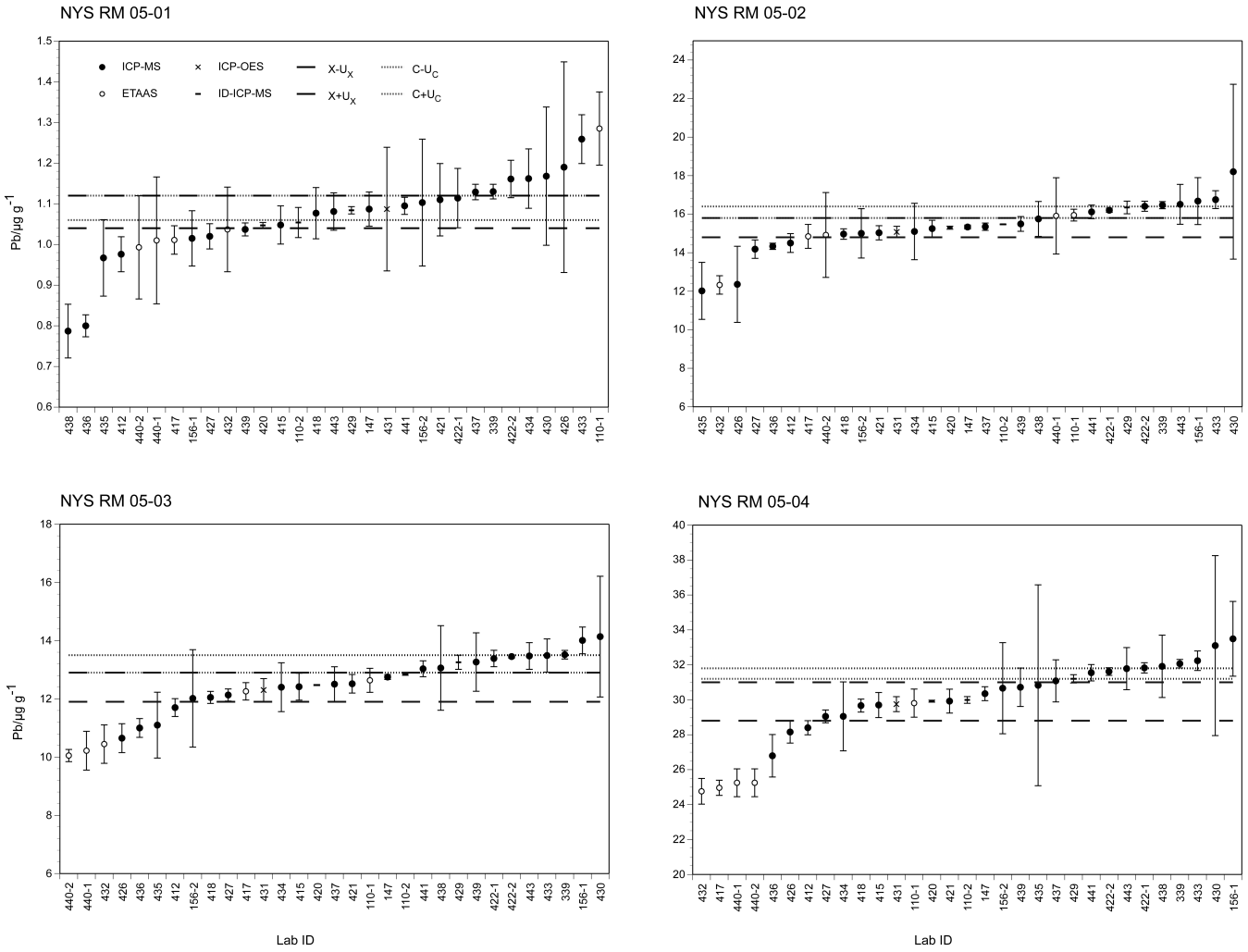

Fig. 2.

Results from the interlaboratory study of NYS RMs 05-01 through 05-04. Data points indicate the reported mean lead concentration (Pb μg g^{-1}) with error bars representing the standard deviation (s). For Lab 429 the error bars represent the expanded uncertainty. Laboratory data are ranked by ascending mean and symbols denote the analytical method used: ● = ICP-MS; ◯ = ETAAS; — = IDICP-MS; and × = ICP-OES. The dashed lines show the expanded uncertainty ($\pm U_X$) in the consensus value (X), while the dotted lines represent the expanded uncertainty ($\pm U_C$) in the certified value (C). Results from Labs 410, 419 and 424 are not shown, since they were consistent outliers.

Table 1

Participating Laboratories in the inter-laboratory study of NYS RMs 05-01 through 04

Participating Laboratory/Institute	Principal Scientist
ALS Technichm (M) Sdn BhD, Shah Alam, Malaysia	Stephen Pang
Analytica AB, Lulea, Sweden	Ilia Roduskin
Applied Speciation and Consulting, Tukwila, WA, USA	Hakan Gurleyik
Institute of Food Research, Norwich, UK	Jurian Hoogewerff
ARC Seibersdorf Research Gmbh, Seibersdorf, Austria	Peter Spindler
Brooks Rand LLC, Seattle, WA, USA	Frank McFarland
Butler University, Indianapolis, IN, USA	Olujide Akinbo
College of Veterinary Medicine, College Station, TX, USA	Robert Taylor
Elemental Research, North Vancouver, BC, Canada	David Gray
Embrapa Pecuaria Sudeste, Sao Carlos, SP, Brazil	Ana Rita Araujo Nogueria
Florida International University, Miami, FL, USA	Jose Almirall
Ghent University, Ghent, Belgium	Frank Vanhaeke
Harvard School of Public Health, Boston, MA, USA	Howard Hu
Centre de Toxicologie du Québec, INSPQ, Québec, Canada	Jean-Philippe Weber
National Institue of Occupational Health, Oslo, Norway	Yngvar Thomassen
National Medical Services Inc., Willow Grove, PA, USA	Robert Middleberg
North Creek Analytical, Beaverton, OR, USA	Lisa Domenighini
Northwest University, X'ian, P.R. China	Honglin Yuan
Politechnika Pozanska, Poznan, Poland	Henryk Matusiewicz
Technikon Pretoria, Pretoria, South Africa	Dimitri Katskov
The Natural History Museum, London, UK	Raquel Garcia-Sanchez
Universidade, Federal de Santa Catarina, Florianopolis, SP, Brazil	Berhard Welz
University Hygenic Laboratory, Ankeny, IA, USA	Brian Wels
University of Florida, Gainesville, FL, USA	George Kamenov
University of Natural Resources and Applied Life Siences, Vienna, Austria	Thomas Prohaska
University of Plymouth, Plymouth, UK	Mike Foulkes
University of Sheffield, Sheffield, UK	Cameron McLeod
University of Wisconsin, Madsion, WI, USA	James Burton
University of Wisconsin-Madison, Madison, WI, USA	Martin Shafer
New York State DOH, Wadsworth Center, Albany, NY, USA	Patrick Parsons

Table 2

Results of the interlaboratory study of NYS RMs 05-01 through 04. The lead content (in μg g^{-1}) is the mean ± standard deviation (unless indicated) of n replicates, quoted to the first uncertain digit.

Lab ID	n	NYS RM 05-01	NYS RM 05-02	NYS RM 05-03	NYS RM 05-04	SRM 1486 Bone Meal
110-1	6	1.29 ±0.09	16.0 ±0.3	12.6 ±0.4	29.8 ±0.8	1.3 ±0.2
110-2	6	1.05 ±0.04	15.47 ±0.02	12.84 ±0.03	30.0 ±0.2	1.329 ±0.009
147	6	1.09 ±0.04	15.3 ±0.1	12.75 ±0.08	30.4 ±0.4	1.31 ±0.02
156-1	6	1.02 ±0.07	17 ±1	14.0 ±0.5	33 ±2	1.2 ±0.1
156-2	3	1.1 ±0.2	15 ±1	12 ±2	31 ±3	1.3 ±0.2
339	6	1.13 ±0.02	16.5 ±0.2	13.5 ±0.1	32.1 ±0.2	1.36 ±0.03
410	6	*1.83 ±0.07	*6.2 ±0.1	*5.2 ±0.1	*11.9 ±0.2	1.35 ±0.01
412	3	0.98 ±0.04	14.5 ±0.5	11.7 ±0.3	28.4 ±0.4	1.32 ±0.07
415	6	1.05 ±0.05	15.3 ±0.4	12.4 ±0.5	29.7 ±0.7	1.41 ±0.03
417	6	1.01 ±0.04	14.8 ±0.6	12.3 ±0.3	25.0 ±0.4	1.33 ±0.04
418	6	1.08 ± 0.06	15.0 ±0.3	12.1 ±0.2	29.7 ±0.4	1.30 ±0.05
419[1]	1	*7.7	*21.0	*29.0	*47.8	*2.7
420	3	1.047 ±0.007	15.30 ±0.08	12.47 ±0.02	29.92 ±0.06	1.27 ±0.05
421	6	1.11 ±0.09	15.0 ±0.4	12.5 ±0.3	29.9 ±0.7	1.3 ±0.1
422-1	6	1.11 ±0.07	16.2 ±0.1	13.4 ±0.3	31.8 ±0.3	1.33 ±0.02
422-2	6	1.16 ±0.05	16.4 ±0.3	13.45 ±0.05	31.6 ±0.2	1.37 ±0.03
424	6	0.93 ±0.02	*5.0 ±0.5	*5.4 ±0.2	*7.9 ±0.5	1.3 ±0.2
426	6	1.2 ±0.3	12.4 ±2.0	10.7 ±0.5	28.2 ±0.6	*1.6 ±0.7
427	6	1.02 ±0.03	14.2 ±0.5	12.1 ±0.2	29.1 ±0.4	1.38 ±0.05
429[2]	6	1.084 ±0.009	16.3 ±0.3	13.3 ±0.2	31.2 ±0.2	1.32 ±0.02
430	6	1.2 ±0.2	18.2 ±4.5	14 ±2	33 ±5	*1.6 ±0.6
431	6	1.1 ±0.2	15.1 ±0.3	12.3 ±0.4	29.8 ±0.4	1.3 ±0.1
432	6	1.0 ±0.1	12.3 ±0.5	10.4 ±0.7	24.8 ±0.7	1.2 ±0.1
433	8	1.26 ±0.03	16.8 ±0.5	13.5 ±0.6	32.2 ±0.6	1.39 ±0.01
434	6	1.16 ±0.07	15.1 ±1.5	12.4 ±0.8	29 ±2	*1.6 ±0.4
435	6	0.97 ±0.09	12.0 ±1.5	11.1 ±1.1	31 ±6	1.3 ±0.2
436	6	0.80 ±0.03	14.3 ±0.2	11.0 ±0.3	27 ±1	*1.17 ±0.06
437	6	1.13 ±0.02	15.3 ±0.2	12.5 ±0.6	31 ±1	1.37 ±0.06
438	6	0.79 ±0.07	15.8 ±0.9	13 ±1	32 ±2	*0.96 ±0.08
439	6	1.04 ±0.02	15.5 ±0.4	13 ±1	31 ±1	1.30 ±0.07
440-1	3	1.0 ±0.2	16 ±2	10.2 ±0.7	25.3 ±0.8	1.4 ±0.3
440-2	3	1.0 ±0.1	15 ±2	10.1 ±0.2	25.3 ±0.8	1.3 ±0.3
441	6	1.10 ±0.02	16.1 ±0.4	13.0 ±0.3	31.6 ±0.5	1.35 ±0.04
443	6	1.08 ±0.05	17 ±1	13.5 ±0.5	32 ±1	1.3 ±0.1

[1] stated as reported

[2] stated ± error is expanded uncertainty calculated using GUM Workbench®

*Outlier identified by Grubbs' Tests

Table 3

Analytical results for NYS RMs 05-01 through 04, and NIST SRM 1486 Bone Meal: (A) Consensus values (Pb μg g^{-1}) and expanded uncertainty[†] calculated from the robust average and standard deviation of the interlaboratory data; and (B) in house ID-ICP-MS certified values and expanded uncertainty. The certified value for SRM 1486 Bone Meal is 1.335 ± 0.014

	A: interlaboratory consensus data	B: ID-ICP-MS certified data
	$X \pm U_X$ (n)	$C \pm U_C$ (n)
NYS RM 05-01	1.08 ± 0.04 (34)	1.09 ± 0.03 (22)
NYS RM 05-02	15.3 ± 0.5(34)	16.1 ± 0.3 (22)
NYS RM 05-03	12.4 ± 0.5 (34)	13.2 ± 0.3 (24)
NYS RM 05-04	29.9 ± 1.1 (34)	31.5 ± 0.7 (23)
SRM 1486 Bone Meal	1.33 ± 0.05 (34)	1.331 ± 0.03[*] (10)

[†] calculated from the standard uncertainty with a coverage factor of 2

[*] our data, not NIST certified value

Table 4

Description of the terms used in Equation 9 for Isotope Dilution ICP-MS.

Term	Description
C_x, C_y	concentration of sample (x), or primary standard (y) ($\gamma g\ g^{-1}$)
K_b, K'_b	mass bias factor for blend of sample or primary standard (')
R_b, R'_b	$^{208}Pb/^{206}Pb$ ratio measured in the blend of sample and spike or primary standard and spike (')
W_s, W'_s	weight of spike added to sample, or primary standard(') (g)
W_x, W_y	weight of sample (x), or primary standard (y) (g)
A_s, B_s	atomic fraction of isotopes A (^{206}Pb) and B (^{208}Pb) in spike
A_x, B_x	atomic fraction of isotopes A (^{206}Pb) and B (^{208}Pb) in sample
A_y, B_y	atomic fraction of isotopes A (^{206}Pb) and B (^{208}Pb) in primary standard

Table 5

Values of uncertainty components used to estimate the combined uncertainty *uC* from Eqn 5. (A) denotes Type A uncertainties (determined from the statistical analysis of a series of observations), (B) denotes or Type B uncertainties (other).

	u*Cx*	*u* hom (A)	*ublk* (A)	*udig* (B)
NYS RM05-01	0.011	0.008	0.0007	0.002
NYS RM05-02	0.12	0.075	0.0007	0.031
NYS RM05-03	0.12	0.078	0.0007	0.025
NYS RM05-04	0.33	0.140	0.0007	0.061

Table 6

Percentage contribution (to 1 decimal place) of individual terms to the uncertainty in C_X (Eq. 9). (A) denotes Type A uncertainties (determined from the statistical analysis of a series of observations), (B) denotes or Type B uncertainties (other).

	NYS RM 05-01	NYS RM 05-02	NYS RM 05-03	NYS RM 05-04
u_{W_x} (A)	1.5%	3.4%	1.7%	1.4%
u_{W_y} (A)	0.7%	1.6%	0.8%	0.7%
u_{C_y} (B)	36.1%	68.2%	45.8%	37.3%
u_{W_s} (A)	5.2%	1.6%	1.8%	0.2%
u'_{W_s} (A)	5.1%	1.6%	1.8%	0.2%
u_{K_x} (A)	3.1%	9.6%	7.4%	5.5%
u_{K_y} (A)	15.9%	7.1%	6.0%	3.1%
u_{R_x} (A)	8.1%	3.8%	2.2%	1.4%
u_{R_y} (A)	19.7%	1.4%	2.1%	0.2%
u_{A_s} (B)	0.0%	0.0%	0.0%	0.0%
u_{B_s} (B)	0.0%	0.0%	0.0%	0.0%
u_{A_x} (A)	0.3%	0.1%	29.1%	18.7%
u_{B_x} (A)	2.8%	1.3%	1.0%	31.1%
u_{A_y} (A)	1.1%	0.1%	0.1%	0.0%
u_{B_y} (A)	0.2%	0.1%	0.1%	0.0%