

# An interlaboratory comparison of bone lead measurements via K-shell x-ray fluorescence

P. J. Parsons,<sup>1,2\*</sup> D. J. Bellis,<sup>1</sup> K. M. Hetter,<sup>2</sup> C. Geraghty,<sup>1</sup> N. A. Berglind,<sup>3</sup> N. R. Ginde,<sup>3</sup> P. Mata<sup>3</sup> and A. C. Todd<sup>3</sup>

<sup>1</sup> Trace Elements Laboratory, Wadsworth Center, New York State Department of Health, Albany, NY, USA

<sup>2</sup> Department of Environmental Health Sciences, School of Public Health, The University at Albany, Albany, New York, NY, USA

<sup>3</sup> Department of Community and Preventive Medicine, Mount Sinai School of Medicine, New York, NY, USA

Bone lead measurements assess chronic exposure and can be obtained via a noninvasive analytical technique based on K-shell x-ray fluorescence (KXRF) spectrometry. While KXRF has been practiced by a number of laboratories around the world, this technique has not, until now, undergone an assessment of interlaboratory agreement. The study described here provides such an assessment via circulation of nine bare tibiae selected from a repository of bones that were derived from lead-dosed goats. Fifteen KXRF systems were used by ten participating laboratories, to make five measurements on each of the nine caprine tibiae. Reported bone lead concentrations ranged from the KXRF detection limit to  $63 \mu\text{g g}^{-1}$  bone mineral. None of the data from the ten participant laboratories was found to be a statistical outlier. However for each of the nine tibiae, differences between the 15 reported bone lead concentrations were statistically significant. The standard deviations of the five replicate KXRF-measured concentrations ranged, across all laboratories and all tibiae, from 0.5 to  $6.5 \mu\text{g g}^{-1}$ , bone mineral. Copyright © 2007 John Wiley & Sons, Ltd.

## INTRODUCTION

The adverse effects of lead on human health are well known. In fact, lead is an increasing concern for public health due to accumulation of evidence of effects at levels of exposure previously considered to be 'safe'; recognition of new effects;<sup>1</sup> and lead-associated overall mortality.<sup>2</sup> Lead exposure is most commonly assessed via blood lead measurement(s), but blood lead reflects only recent exposure.<sup>3</sup> Bone lead, in contrast, reflects cumulative exposure<sup>4–12</sup> because the residence time of lead in bone is long.<sup>8,13–18</sup> The concentration of lead in bone can be determined via a number of established analytical methods, such as atomic absorption spectrometry (AAS) or inductively coupled plasma-mass spectrometry (ICP-MS), but the sample is destroyed during the analytical procedure. Nondestructive techniques for the determination of lead are possible using methods based on energy dispersive x-ray fluorescence (XRF) spectrometry. Both destructive and nondestructive methods have advantages and disadvantages.

For the analysis of human tissues, analytical methods based on AAS and ICP-MS are limited to cadaver or biopsy samples. Typically, tissue samples are either dry-ashed in a muffle furnace or wet-ashed via digestion in mineral acids before analyte determination is made. In contrast, nondestructive methods of analysis preserve the sample in its original state for further analysis. For analysis *in vivo*, XRF's noninvasive character renders it more acceptable for human subject studies.

The first *in vivo* bone lead XRF measurements were reported some 30 years ago.<sup>19</sup> Since then, three techniques

have been used to measure lead in bone *in vivo*: the <sup>57</sup>Co technique that fluoresces K-shell lead x-rays,<sup>19</sup> techniques that fluoresce L-shell x-rays,<sup>20–24</sup> and the <sup>109</sup>Cd-based technique that fluoresces K-shell x-rays (<sup>109</sup>Cd KXRF). The <sup>109</sup>Cd-based technique has become the most widely used; approximately 20 laboratories worldwide have practiced or are currently practicing this technique.

Although all <sup>109</sup>Cd KXRF methods use the same isotope as the fluorescing source, not all use the same hardware, analysis software, calibration protocols, or quality control protocols. Furthermore, there are no primary or secondary reference materials available for bone lead K-shell x-ray fluorescence (KXRF) measurements. Consequently, interlaboratory agreement among bone lead KXRF laboratories has not been established. By preparing and circulating a set of tibiae derived from lead-dosed goats, we have sought to assess the degree of interlaboratory agreement among those laboratories that perform KXRF bone lead measurements.

The New York State Department of Health's (NYSDOH) Wadsworth Center maintains a population of approximately 30 adult goats that are periodically dosed with lead acetate in order to generate large volumes of whole blood containing lead that is physiologically bound to erythrocytes. These blood pools are produced primarily for use in the NYSDOH's proficiency testing (PT) program for blood lead and erythrocyte protoporphyrin (EP). Complete details of the NYSDOH's PT program for blood lead have been described elsewhere.<sup>25,26</sup> Briefly, lead is administered orally as lead acetate trihydrate, packed in gelatin capsules. Five to six goats are dosed for several days prior to each PT event, with three events per year scheduled for blood lead PT and three events per year for EP PT. Blood lead concentrations are routinely measured in each goat to monitor its exposure to

\*Correspondence to: P. J. Parsons, Trace Elements Laboratory, Wadsworth Center, New York State Department of Health, Albany, NY, USA. E-mail: pparsons@wadsworth.org

lead. Routine measurements of EP and hematocrit are also made, to monitor for long-term toxic effects of lead on the hemopoietic system.

These animals are maintained in a farm environment under an active protocol (No. 04–096) that is approved by the Wadsworth Center's Institutional Animal Use and Care Committee. The protocol is renewed annually, and a full review occurs every 3 years. Under the protocol, the health of all animals is continually assessed and reviewed annually by the facility veterinarian who is responsible for their care and welfare. Those animals that are judged by the facility veterinarian to be too old and/or too weak to survive the following winter season are euthanized via lethal injection with sodium pentobarbital using standard veterinary protocols. Following euthanasia, an autopsy is conducted in which long bones (described more fully in the next paragraph) and critical organs are harvested for research purposes as approved under the active protocol. Typically, two or three goats are euthanized each year and the herd is replenished with younger animals. Thus, the goats were not euthanized solely for the purposes of this study.

Since 1989, long bones (e.g. tibiae) and samples of other bones have been collected for research purposes. Many of these bones were carefully cleaned and scraped to remove adhering tissues. Some of the long bones have undergone analysis by AAS to assess their lead concentration(s), and a number of bare bones have been stored, frozen at  $-80^{\circ}\text{C}$ , pending future analysis.

Preliminary work suggested that the caprine tibiae could be a suitable model for the validation and standardization of XRF measurements of bone lead.<sup>27</sup> The caprine tibiae were judged suitable for the following reasons: (1) a good correlation has been observed between the cumulative lead dose administered to the goats and bone lead concentrations measured by AAS, (2) higher lead concentrations have been found at trabecular bone sites such as the patella and calcaneus than at cortical bone sites; this distribution pattern resembles that reported elsewhere for human subjects, (3) the caprine tibia surface appears to be enriched in lead compared to the tibia core (unpublished data); again, this is consistent with observations reported for human tibiae,<sup>28</sup> (4) the caprine tibia lead concentrations, as measured by AAS, spanned a range from the reported method detection limit for AAS ( $0.6\ \mu\text{g g}^{-1}$  dry weight) to as much as  $60\text{--}80\ \mu\text{g g}^{-1}$  dry weight, (5) the caprine bones were available for destructive analysis, (6) the lead doses received by the goats were known, allowing selection of suitably dosed subjects, (7) since caprine bones are of animal rather than human origin, there was a reduced risk of potential exposure of the analysts to human-borne infectious diseases. We noted that exposure to rabies could also be discounted for this material because the goats received vaccination for rabies and were routinely tested for rabies at autopsy, (8) the dimensions of an adult caprine tibia are similar to those of a human adolescent tibia; (9) finally, because these caprine bone materials were readily available as part of an existing program, financial outlay could be substantially minimized.

## METHODS

Nine caprine tibiae were selected from a potential pool of 18 that form part of a larger collection of long bones in the NYSDOH bone lead repository. The tibiae were harvested postmortem from goats that were autopsied from 1997 to 2003 as described in the Background section. The tibiae were physically cleaned of adherent tissues and the marrow components were removed, leaving the calcified matrix intact. Next, the tibiae were treated with hydrogen peroxide to remove remaining blood and tissue fragments. After soaking in diethyl ether to remove fat, they were freeze-dried to remove remaining water content and were then stored in a freezer at  $-80^{\circ}\text{C}$ .

Ideally, the selection of the nine tibiae for the interlaboratory study would have been on the basis of on definitive lead determinations obtained via AAS or ICP-MS; however, since those methods result in sample destruction, this was not possible. As an alternative, we used data from several other sources based on (1) AAS analyses of the opposing (i.e. right) tibia for six of the bones, (2) the lead content of 11 of the 18 candidate tibiae estimated via KXRF on the days the animals were euthanized, and (3) the known administered cumulative lead dose. In addition, KXRF-measured lead concentration estimates, obtained as part of an independent experiment, were available for all of the cleaned, bare tibiae. Although knowledge of the right tibia lead concentration was not an ideal substitute for a definitive left tibia measurement, previous work had suggested that there was considerable symmetry in the lead concentration of caprine bones (unpublished data).

Encapsulation of the defatted and freeze-dried tibiae was necessary to protect them from physical damage and inadvertent contamination; it would also allow each sample to be clearly identified without physically altering the bone. The intended KXRF measurement site was indicated with a cross mark at the approximate midpoint of the tibia shaft using the type of water-soluble marker pen that is used on human subjects who undergo bone lead measurements *in vivo*. Various approaches to the encapsulation of the intact bones were explored, including (1) encapsulation in either an epoxy resin or a polymeric sheath, (2) simple wrapping in household polyethylene film (e.g. Saran wrap), and (3) use of a home vacuum packaging system. Ultimately, the latter approach proved most feasible, and it provided the necessary protection during sample shipment and subsequent KXRF measurement by each participating laboratory. Each bone was therefore vacuum-sealed in a polyethylene/nylon bag (Quart Bags, 946 ml capacity,  $20.3\ \text{cm} \times 27.9\ \text{cm}$  dimensions) using a FoodSaver Model V1205 packaging system (Jarden Consumer Solutions, San Francisco, CA). Several custom-designed holders were manufactured from Plexiglas acrylic resin, to secure the encapsulated tibiae for presentation to KXRF instrumentation.

The vacuum-sealed tibiae were packaged and shipped as per guidelines established by the International Air Transport Association (e.g. tertiary containment and leak-proof container) and were shipped on dry ice overnight (domestically) or via 'International Priority' courier service. Included in the package were the measurement protocol and

a sample holder. The nine tibiae were shipped as a set to each participating laboratory.

Prior to the circulation of the tibiae, a draft measurement protocol was provided to each KXRF laboratory that had previously indicated an interest in participating in the study, and comments/responses were solicited. The final protocol that accompanied the tibiae incorporated the feedback received from participating laboratories to the extent possible. Each laboratory was asked to make five consecutive half-hour measurements (real/clock-time or live-time not specified), according to its local standard operating procedure, at the proximal–distal midpoint of each tibia (marked, as noted above, by a water-soluble pen mark). The proximal–distal midpoint of the tibia shaft was selected as the measurement site because our previous studies have shown a variation in lead concentrations obtained via both KXRF and AAS for differing sites on the tibiae;<sup>28,29</sup> the selection of standardization at the midpoint should minimize this effect.<sup>30</sup> The KXRF laboratories were asked to follow their current calibration and quality control practices for these measurements. They were asked to provide results for bone lead according to their normal protocol, i.e. units of micrograms Pb per gram bone mineral, and the statistical uncertainty in their estimates of the tibia lead concentration values.

Technical information on the KXRF measurement system(s) used in each laboratory for this study was requested in order to enable us to determine potential sources of disagreement. The following details were requested: hardware manufacturer, model numbers and settings (e.g. pulse processing times), source dimensions and activity, dead time, calibration protocol (number of phantoms, concentration range, encapsulation), quality control protocol(s), and the method for calculating concentration (matrix conversion factor applied, handling of calibration line intercept).

Each participating laboratory was asked to limit the time spent analyzing these samples to 3 weeks, and the Trace Elements Laboratory at the NYSDOH coordinated the schedule, to try to maximize the efficiency of the circulation. When the tibiae were returned from a participant laboratory, they were inspected for any damage and, if necessary, resealed in a fresh bag before being shipped to the next laboratory. It was estimated that the circulation of the tibiae would take 12 months.

Participant laboratory identities were coded, to ensure confidentiality consistent with standard practices in such studies, but were known to the coordinating laboratory. Participants communicated their KXRF data to the Trace Elements Laboratory, either when they returned the bones, or later. Laboratory names were stripped before the data were forwarded to Mount Sinai for statistical analyses.

## STATISTICAL ANALYSES

For the statistical analyses, each KXRF measurement system was considered to be a separate entity even when two systems in the same laboratory utilized components of a method (e.g. peak-analysis program). The existence of outliers was assessed via Grubbs' iterative test, both for

the average concentration of all the bones and for each bone separately. Levene's test with the median as the central measure was used to assess the homogeneity of variance. Outlier and homogeneity of variance tests were adjusted for multiplicity with the Bonferroni correction. Comparisons between the measurement systems were performed for the mean lead concentration of all nine bones as well as for the mean lead concentration of each individual bone. For the overall comparison of the nine bones, a linear mixed-effects model was applied to the data, with 'system' as a fixed effect, and both 'bone' and the interaction between 'system' and 'bone' as random effects. This approach ensured that the model assumptions with regard to independence among observations were met. The model permitted correlation between measurements within the same bone within the same system and within the same bone across different systems, but it did not permit correlation between measurements of different bones on different systems. For the comparison of the individual bones, a one-way ANOVA was used. Multiple comparisons of the mean values for each system were conducted using Tukey's correction for multiplicity. Comparisons of the average lead concentrations between two systems at the same laboratory were performed via a linear mixed-effects model, with 'bone' as a random effect. When the statistical analyses were complete, a report of the findings was prepared and circulated to the participating laboratories.

## RESULTS

The nine bones selected for this interlaboratory comparison were left tibiae from goats that had received cumulative lifetime lead doses ranging from 5.5 to 50.5 g Pb. Existing data from a single KXRF system located at Mount Sinai indicated that the tibiae had lead concentrations in the range of 10–60  $\mu\text{g g}^{-1}$  bone mineral. The ages of the animals when euthanized ranged from 6 to 15 years. The lengths of the tibiae ranged from 14.5 to 19.5 cm. The diameter and bone thickness at the midpoint of the tibia shaft, while varying, were  $\sim 2$  cm and  $\sim 5$  mm respectively.

After investigation of various approaches to encapsulation, we adopted a method in which each bone was vacuum-sealed in a polyethylene/nylon bag. One tibia that was thus encapsulated was sent to Mount Sinai and underwent measurement there to confirm that this method had not produced any gross interference in the KXRF spectrum.

Thirteen KXRF laboratories were sent the bone set but only ten returned measurement data within the allotted time frame. Two laboratories were unable to analyze the bones for technical reasons, and another returned results too late to be included in this analysis. Five of the ten laboratories that reported results measured the bones on two different <sup>109</sup>Cd-based KXRF measurement systems, giving a total of 15 sets of concentration values as the study dataset.

There were no outliers in the concentration data reported from any of the individual bones. The data for all bones from ten laboratories are presented in Table 1. The values for the tibiae with the lowest, median, and highest lead concentrations (consensus values from the data from all the

**Table 1.** Mean lead concentrations ( $\mu\text{g g}^{-1}$  bone mineral) and standard deviations (SD) for five repeated KXRF measurements of each of nine caprine tibiae made with 15 bone lead measurement systems in ten laboratories. KXRF bone lead measurement systems are identified by the convention 8NN-n, where NN is the laboratory number and n is the system designation for laboratories with more than one KXRF instrument

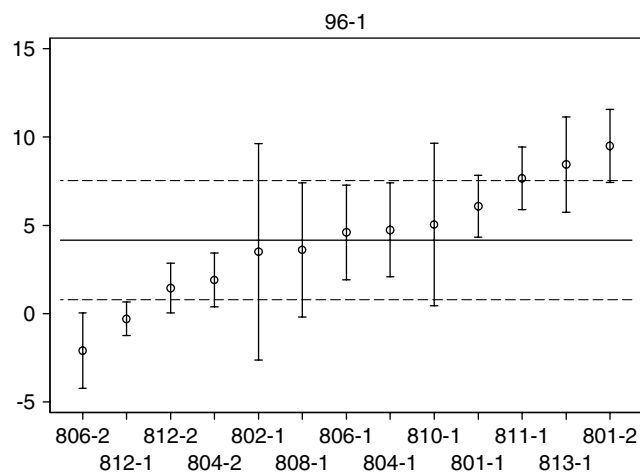
Tibia ID		System (8NN-n)														
		801-1	801-2	802-1	803-1	803-2	804-1	804-2	806-1	806-2	808-1	810-1	811-1	812-1	812-2	813-1
82-17	Mean	58.5	58.3	60.1	55.0	50.7	51.6	47.6	52.9	53.7	57.0	59.0	63.5	53.3	51.5	62.9
	SD	2.3	3.1	4.6	3.1	3.8	1.2	1.0	0.9	4.5	1.6	2.4	3.1	0.6	2.6	2.6
86-6	Mean	34.0	33.0	33.6	26.0	27.9	23.4	25.6	28.7	19.1	29.5	31.3	37.1	25.4	25.3	35.7
	SD	1.5	1.7	5.9	1.2	1.5	2.1	1.3	5.1	4.7	1.1	3.2	0.7	1.0	1.0	1.3
89-4	Mean	52.9	53.9	56.4	46.9	43.4	42.5	37.9	42.7	27.6	50.0	51.7	56.9	46.2	45.4	60.0
	SD	0.9	0.8	3.6	3.9	3.6	3.3	1.0	2.1	5.8	2.0	2.2	1.9	1.1	1.7	2.2
89-14	Mean	25.4	29.2	27.7	22.7	22.5	19.7	19.3	25.7	20.0	21.7	25.2	31.2	19.6	21.8	31.1
	SD	1.4	1.1	4.5	1.2	2.0	3.0	1.1	1.8	4.6	1.6	1.9	2.9	1.1	1.2	2.7
89-15	Mean	25.9	30.8	27.2	19.8	23.3	19.8	18.5	20.7	12.9	20.9	24.5	31.4	20.3	20.8	28.3
	SD	1.0	2.0	3.0	3.2	3.7	2.4	1.2	3.6	3.1	2.9	1.5	1.6	1.3	1.3	4.5
93-2	Mean	15.6	21.6	2.0	11.3	11.9	11.0	10.5	12.5	6.6	11.3	14.0	21.1	9.8	10.3	17.2
	SD	0.8	2.5	2.6	3.3	5.1	3.3	0.5	3.8	2.6	1.8	0.8	2.7	1.6	1.7	3.6
93-6	Mean	27.1	26.4	23.5	21.4	19.4	18.4	16.6	19.7	20.9	21.9	22.7	29.9	18.6	18.7	26.7
	SD	1.5	1.0	1.8	2.9	3.3	2.0	0.9	1.4	1.3	2.6	3.4	1.5	1.4	1.1	5.0
95-4	Mean	14.5	19.3	11.3	10.1	10.4	8.9	7.9	12.8	9.5	11.5	11.5	18.1	7.4	8.5	18.6
	SD	1.7	1.1	6.5	2.1	1.6	2.8	0.9	3.5	1.5	3.1	2.3	4.3	1.2	1.4	2.9
96-1	Mean	6.1	9.5	3.5	5.0	1.4	4.7	1.9	4.6	-2.1	3.6	5.0	7.7	-0.3	1.4	8.4
	SD	1.7	2.1	6.1	2.2	5.1	2.6	1.5	2.7	2.1	3.8	4.6	1.8	0.9	1.4	2.7

laboratories) are also presented in Figs 1-3, respectively. In addition to determining whether any laboratory's data were outliers with regard to reported concentrations, we were also interested in assessing whether any of the laboratories reported greater variability in their concentrations than their peers. Levene's test was failed for only one tibia, suggesting that variability in concentrations was fairly uniform overall. Levene's test was also passed by each laboratory/system dataset of five replicate measurements on each of the nine bones.

The overall mean lead concentrations for the set of all nine bones ranged from 18.7 to 33.0  $\mu\text{g g}^{-1}$  bone mineral, with the values from the 15 systems fairly evenly distributed across that range. Table 2 shows the overall mean lead concentrations of all nine bones for the 15 systems, and the results of the multiple comparison tests. The smallest measured difference that was statistically significant was 3.6  $\mu\text{g g}^{-1}$ , between systems 803-1 and 804-2.

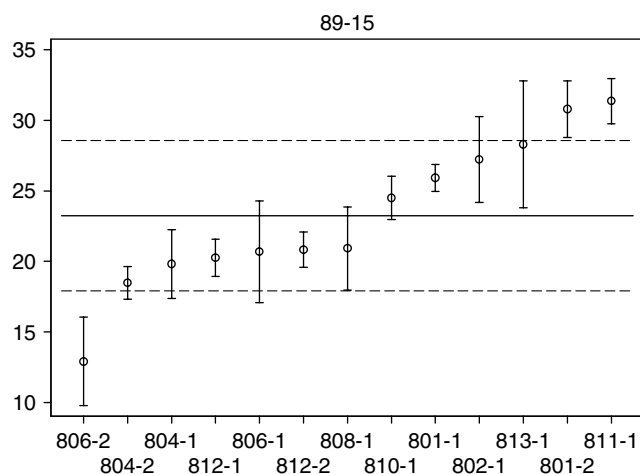
The rank order of laboratories shown in Table 2 was not maintained across all bones. Tables 3-5 show the result of multiple comparisons for three representative tibiae selected from the set of nine: 96-1 with the lowest lead concentration (Fig. 1); 86-15 with the median lead concentration (Fig. 2); and 82-17 with the highest lead concentration (Fig. 3). Tibia 96-1's smallest statistically significant difference was 6.7  $\mu\text{g g}^{-1}$  (between systems 806-1 and 806-2). Tibia 89-15's smallest statistically significant difference was 6.0  $\mu\text{g g}^{-1}$  (between systems 810-1 and 812-1). Tibia 82-17's smallest statistically significant difference was 5.2  $\mu\text{g g}^{-1}$  (between systems 811-1 and 801-2).

The relative order of the lead concentrations for each tibia was similar across almost all systems; Table 6 shows a

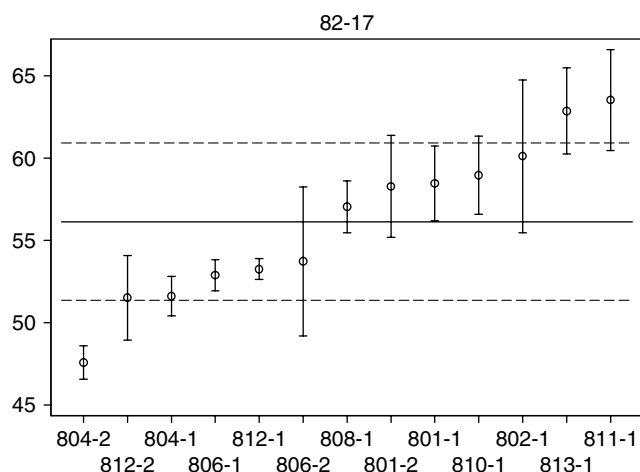


**Figure 1.** Average of lead concentrations ( $\mu\text{g g}^{-1}$  bone mineral) from replicate measurements, obtained by the participant laboratories, of caprine tibia 96-1, arranged in increasing order of the average. Each error bar represents the standard deviation of five replicate measurements obtained by the laboratory. The solid line represents the average of the lead concentrations reported by all of the laboratories, and the dashed lines represent the between-laboratory standard deviation.

comparison of how the lead concentration (i.e. the average of replicate measurements of the concentration) was ranked for each tibia, both overall and for each system. All 15 systems ranked 82-17 as the bone with the highest lead concentration, and only one system failed to rank 96-1 as the bone with the lowest lead concentration. Agreement in ranking the fourth,



**Figure 2.** Average of lead concentrations ( $\mu\text{g g}^{-1}$  bone mineral) from replicate measurements, obtained by the participant laboratories, of caprine tibia 89–15, arranged in increasing order of the average. Each error bar represents the standard deviation of the five replicate measurements obtained by the laboratory. The solid line represents the average of the lead concentrations reported by all the laboratories, and the dashed lines represent the between-laboratory standard deviation.



**Figure 3.** Average of lead concentrations ( $\mu\text{g g}^{-1}$  bone mineral) from replicate measurements, obtained by the participant laboratories, of caprine tibia 82–17, arranged in increasing order of the average. Each error bar represents the standard deviation of the five replicate measurements obtained by the laboratory. The solid line represents the average of the lead concentrations reported by all the laboratories, and the dashed lines represent the between-laboratory standard deviation.

fifth, and sixth bones was poorer, but the lead concentrations in these bones spanned a range of only  $2 \mu\text{g g}^{-1}$  bone mineral.

Three laboratories measured the tibiae on each of two systems that used a 'spot' source located at the center of the detector face. One of these laboratories obtained significantly different ( $-2.46 \mu\text{g g}^{-1}$ ; standard error SE,  $0.44 \mu\text{g g}^{-1}$ ;  $p < 0.001$ ) concentrations from its two measurement systems. There was no significant difference between either of the two pair sets of concentrations reported by the other two laboratories.

**Table 2.** Multiple comparisons of average lead concentration ([Pb]) from the set of nine caprine tibiae obtained with 15 bone lead measurement systems in ten laboratories. Average concentrations that are not significantly statistically different from one another have the same 'Result of Multiple Comparison Test' letter. KXRF bone lead measurement systems are identified by the convention 8NN-*n*, where NN is the laboratory number and *n* is the system designation for laboratories with more than one KXRF instrument

System	Average [Pb]	Result of multiple comparison test
806-2	18.70	A
804-2	20.64	A B
804-1	22.23	A B C
812-1	22.25	A B C
812-2	22.65	A B C
803-2	23.42	B C D
803-1	24.23	C D E
806-1	24.48	C D E F
808-1	25.26	C D E F
802-1	27.25	D E F G
810-1	28.52	E F G H
801-1	28.88	F G H I
801-2	31.35	G H I
813-1	32.09	H I
811-1	33.00	I

**Table 3.** Multiple comparisons of average lead concentration ([Pb]) from caprine tibia 96-1, obtained by 15 bone lead measurement systems in ten laboratories. Average concentrations that are not significantly statistically different from one another have the same 'Result of Multiple Comparison Test' letter. KXRF bone lead measurement systems are identified by the convention 8NN-*n*, where NN is the laboratory number and *n* is the system designation for laboratories with more than one KXRF instrument

System	Average [Pb]	Result of multiple comparison test
806-2	-2.1	A
812-1	-0.3	A B
803-2	1.35	A B C
812-2	1.44	A B C
804-2	1.90	A B C D
802-1	3.50	A B C D E
808-1	3.60	A B C D E
806-1	4.60	B C D E
804-1	4.74	B C D E
803-1	5.02	B C D E
801-1	6.08	B C D E
810-1	7.20	C D E
811-1	7.66	C D E
813-1	8.44	D E
801-2	9.50	E

In addition, two laboratories measured the tibiae with both a single-detector system and a multidetector measurement system. For one of these two laboratories, the

**Table 4.** Multiple comparisons of average lead concentration ([Pb]) from caprine tibia 89–15, obtained with 15 bone lead measurement systems in ten laboratories. Average concentrations that are not significantly statistically different from each other have the same ‘Result of Multiple Comparison Test’ letter. KXRF bone lead measurement systems are identified by the convention 8NN-n, where NN is the laboratory number and n is the system designation for laboratories with more than one KXRF instrument

System	Average [Pb]	Result of multiple comparison test				
806–2	12.92	A				
804–2	18.48	A	B			
803–1	19.77	B				
804–1	19.81	B				
812–1	20.25	B	C			
806–1	20.68	B	C	D		
812–2	20.82	B	C	D		
808–1	20.92	B	C	D		
803–2	23.27	B	C	D	E	
801–1	25.92	C		D	E	F
810–1	26.24	D			E	F
802–1	27.22	E				F
813–1	28.30	E				F
801–2	30.80	F				
811–1	31.36	F				

**Table 5.** Multiple comparisons of average lead concentration ([Pb]) from caprine tibia 82–17 obtained by 15 bone lead measurement systems in ten laboratories. Average concentrations that are not significantly statistically different from one another have the same ‘Result of Multiple Comparison Test’ letter. KXRF bone lead measurement systems are identified by the convention 8NN-n, where NN is the laboratory number and n is the system designation for laboratories with more than one KXRF instrument

System	Average [Pb]	Result of multiple comparison test							
804–2	47.59	A							
803–2	50.69	A	B						
812–2	51.51	A	B	C					
804–1	51.60	A	B	C					
806–1	52.88	A	B	C	D				
812–1	53.26	A	B	C	D				
806–2	53.72	A	B	C	D	E			
803–1	54.95	B		C	D	E	F		
808–1	57.04	C			D	E	F	G	
801–2	58.28	C		D	E	F	G		
801–1	58.46	D			E	F	G	H	
810–1	59.66	E				F	G	H	
802–1	60.10	F					G	H	
813–1	62.86	G						H	
811–1	63.52	H							

multidetector system yielded significantly lower ( $1.59 \mu\text{g g}^{-1}$ , SE  $0.45 \mu\text{g g}^{-1}$ ,  $p < 0.001$ ) concentrations than their own

**Table 6.** Ranking of nine caprine tibiae lead concentrations ([Pb]) obtained by 15 bone lead measurement systems (in ten laboratories). The overall rank for an individual tibia is that of the average of all concentrations from all systems. The ‘Rank by system’ columns give the number of KXRF measurement systems that conferred to each tibia, a particular rank. Cohen’s kappa is a measure of agreement ranging from 0 (the amount of agreement expected by chance alone, i.e. none) to 1 (perfect agreement). Kappa for the average concentration of the nine tibiae was 0.63. All Kappa statistics were statistically significant on the 5% confidence level

Bone	Average [Pb]	Overall Rank	Rank by system									
			1	2	3	4	5	6	7	8	9	
96–1	4.18	1	14	1								
95–4	12.08	2	10		5							
93–2	12.50	3	1	4	10							
93–6	22.23	4					11	1	2	1		
89–15	23.12	5						3	7	5		
89–14	24.31	6							1	6	8	
86–6	29.09	7								1	14	
89–4	47.72	8									15	
82–17	55.74	9										

single-detector crystal system. In contrast, the multidetector system of the other laboratory yielded significantly higher ( $5.7 \mu\text{g g}^{-1}$ , SE  $0.86 \mu\text{g g}^{-1}$ ,  $p < 0.001$ ) concentrations than their own single-detector system. Since the difference between multidetector and single-detector systems was not consistent, we deemed it inappropriate to try to combine the data from the multidetector systems, for a group comparison to single-detector systems.

Temporal analysis conducted over a 9-month time frame revealed a statistically insignificant ( $p = 0.88$ ) decrease of  $0.013 \mu\text{g Pb g}^{-1}$  bone mineral per year in the average lead concentration of all nine bones. A repeat set of measurements, performed by the NYSDOH coordinating laboratory during a hiatus in the circulation of the tibiae, yielded concentrations that were not significantly different from those yielded by the same laboratory’s first set of measurements of the tibiae.

## DISCUSSION

The goat bones were circulated from February 2005 to September 2006, far longer than the 12 months originally projected. During this period, they were preserved at  $-80^\circ\text{C}$  for upto 5 months in the coordinating laboratory between times until it was convenient for one of the participating laboratories to measure them; they ‘sat’ for a further 3 months in one laboratory that was ultimately unable to measure them.

One possible limitation of this study is that the KXRF measurement program required 45 half-hour measurements in addition to the calibration and quality control procedures that were routinely employed by the participating laboratory. Although none of the three nonparticipants stated that the time required was a reason for not participating, we cannot rule out that it played a role. The reasons stated

concerned technical difficulties and local radiation licensing status issues.

The practical limitations of time and effort, when balanced against an idealized design for the KXRF measurement protocol, prevented the inclusion of other desirable procedures, such as recalibration of the KXRF before each tibia measurement so as to remove the effect of a common calibration. Further, any system used for analytical determinations should be in a state of statistical control.<sup>31</sup> Whether and how each laboratory determines its KXRF measurement system to be in a state of statistical control may be addressed when we circulate a second set of caprine tibiae (see subsequent text).

It was our hope that the tibiae used for this study would provide a matrix-based reference material suitable for bone lead measurements by KXRF. When tibia reference materials are used to test and validate KXRF, each step in the measurement process should be probed in order that the entire analytical process may be assessed. In the study reported here, some steps that are normally part of the *in vivo* KXRF method were omitted (e.g. the tibiae were not repositioned between measurements, as would be the case when a human subject underwent bone lead measurement on two separate occasions). Furthermore, it is not common practice to perform repeated *in vivo* measurements on human subjects. Neither of these departures or deviations from the *in vivo* method is considered to be a major flaw in our study. Data (unpublished) from Dr Todd's laboratory suggest that repositioning of the sample between measurements need not introduce additional variability in KXRF-measured lead concentration, except in the case when an area of localized inhomogeneity is sampled for some KXRF measurements but not for others.

The lowest KXRF-measured lead values reported for these tibiae approached the detection limits of the measurement systems: the average concentrations reported for tibia 96–1 ranged from –2.1 to 9.5  $\mu\text{g g}^{-1}$  bone mineral. The level of agreement between the participating laboratories was variable; the range of average concentrations obtained from the tibia for which the results of multiple comparison tests yielded the fewest number (4) of concentration 'groups' (i.e. designated as letters in Tables 2–5) was 7.4–19.3  $\mu\text{g g}^{-1}$ ; for the two tibiae with the largest number (8) of concentration 'group' (letters), the ranges were 47.6–63.5  $\mu\text{g g}^{-1}$  (data for tibia 82–17 as shown in Table 5) and 27.6–60.0  $\mu\text{g g}^{-1}$  (tibia 89–4). Investigation into the sources of differences between laboratories will benefit from the bone lead data that will be obtained via a different analytical method. Discrepancies between KXRF systems that are operated in the same laboratory should also be investigated.

Future work principally includes the determination of lead in the tibiae reported here via atomic mass spectrometry, and the circulation of a second set of tibiae. A second reference set of nine tibiae will be circulated to the KXRF laboratories for the assessment of interlaboratory agreement and for a second indirect validation study. The second set of tibiae will, like the first set, be selected to cover an appropriate concentration range from a maximum of 70  $\mu\text{g g}^{-1}$  bone mineral down to 3.5, or possibly, <1.0  $\mu\text{g g}^{-1}$  bone mineral.

This second set of bones will be prepared and encapsulated as before, and will be returned to the Trace Elements Laboratory at the NYSDOH once the second interlaboratory study has been completed. Unlike the first set of reference tibiae, the second set will not be destroyed, so that they can serve as reference materials for future bone lead measurements by KXRF.

## CONCLUSIONS

A practical assessment of the interlaboratory agreement among bone lead KXRF laboratories yielded consensus concentrations that ranged, across nine caprine tibiae, from 4.0 to 55.7  $\mu\text{g g}^{-1}$  bone mineral. An average of five replicate measurement concentrations obtained by ten laboratories with 15 measurement systems was found to be distributed evenly over the range of Pb concentrations reported for the tibiae (4–56  $\mu\text{g g}^{-1}$ ), without any single KXRF system consistently reporting particularly low or high concentrations. The different systems were in reasonable agreement as to the relative rank order of the lead concentrations of the bones. Within-lab variability, measured as the bone lead standard deviation for a given caprine tibia, ranged from 0.5 to 6.5  $\mu\text{g g}^{-1}$  bone mineral across nine tibiae. An assessment of the sources of the variability in KXRF bone lead measurements will hopefully improve interlaboratory agreement in the subsequent circulation of a caprine tibiae set.

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