HHS Public Access

Author manuscript

Environ Sci Technol. Author manuscript; available in PMC 2023 October 30.

Published in final edited form as:

Environ Sci Technol. 2021 April 20; 55(8): 5050–5055. doi:10.1021/acs.est.0c06622.

Feasibility of lead exposure assessment in blood spots using energy dispersive x-ray fluorescence

Aaron J Specht[1,*], **John F. Obrycki**[2], **Maitreyi Mazumdar**[1,2], **Marc G. Weisskopf**[1]

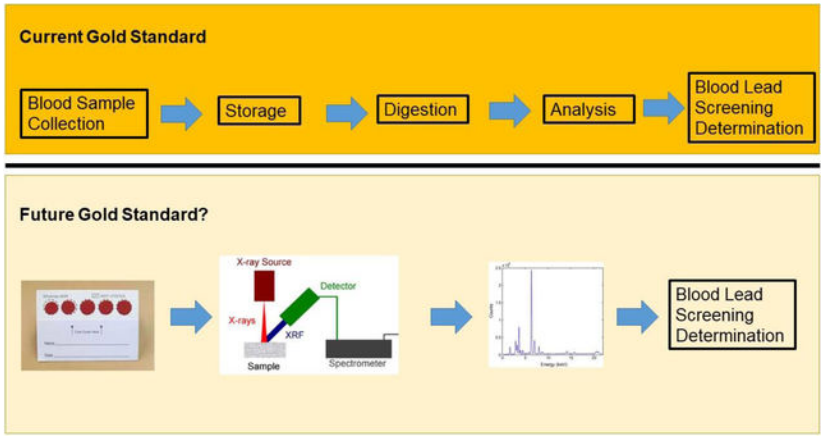
[1]Harvard T.H. Chan School of Public Health, Boston, MA

[2]Department of Neurology, Boston Children's Hospital, Boston, MA

Abstract

Collecting blood spots from newborns is a common procedure used to diagnose multiple health conditions. Fingerstick blood samples are routinely collected from children to diagnose elevated blood lead levels. In our study, we wanted to test the feasibility of using a high-powered energy dispersive x-ray fluorescence (EDXRF) device to accurately measure the concentration of lead in blood spots. We created spotted standards of known concentration of lead on filter paper at different volumes and concentrations. We determined the detection limit for lead through repeated measurements of our standards and calibration line slopes. We also tested the variability of measured lead concentration across procedures and spotted blood volume and found no significant additions to uncertainty in measurements. Finally, we compared blood lead concentrations measured by EDXRF and atomic absorption spectroscopy (AAS) and found EDXRF to be a significant predictor of blood lead (n=22 R=0.98 p-value <0.001) with an average detection limit of 1.7 ug/dL of blood lead. This detection limit is similar to atomic absorption spectrometer techniques, which are commonly used in clinical testing laboratories for blood lead surveillance. These findings indicate proof-of-concept that blood spots measured via EDXRF may be used as a surveillance tool for lead exposure, even at anticipated blood lead levels of 2–3 ug/dL.

Graphical Abstract



*Corresponding Author: aspecht@hsph.harvard.edu, 655 Huntington Ave Building 1 Rm 1402 Boston, MA 02115.

1. Introduction.

Accurately measuring metals in biological tissues is an essential part of environmental and occupational monitoring efforts and health studies. Blood samples are a primary biomarker of exposure. Levels of metals in blood in children have been widely measured since the 1970s with increasing emphasis beginning in the 1990s across the United States as part of standard lead surveillance.(1) The gold standard for evaluating children's blood lead levels is a venipuncture sample.(2) After collection, this sample is analyzed using some form of spectroscopy, such as an atomic absorption (AA) or inductively coupled plasma-based method (ICP).

Graphite furnace atomic absorption spectroscopy (AAS) was for decades the standard for metal measurements in blood, but AAS can only assess one metal at a time and has a practical limit of detection of about 1–2 ug/dL.(3) Inductively coupled plasma mass spectrometry (ICP-MS) has largely replaced AAS for blood metal measurements as it has a consistently lower detection limit, in the part per trillion range with current instrumentation, and multi-element capabilities.(4) AAS and ICPMS techniques take a substantial amount of time with typical sample read times (~3–5 minutes) only accounting for a small portion of the total time for sample preparation, calibration standards (run between samples), and quality control steps all adding a significant amount of time to the process that can take days in total. In addition, both AAS and ICP-MS require substantial laboratory instrumentation for blood collection, storage, and analysis.

Methods that simplify analysis of samples collected in the field do exist. For example, anodic stripping voltammetry (ASV) can be done in the field on capillary blood,(5) but this has a detection limit of 2–3 ug/dL (20–30 ppb) with read times of about 5 minutes.(3) However, the equipment cannot determine levels above 65 ug/dL and are less accurate for levels >8 ug/dL.(3) Also, portable lead testing kits are known to have higher readings compared to venipuncture tests.(6–8)

An alternative field-friendly approach for blood metal analysis is to collect capillary blood spots using filter paper. This has been used in lead screening as an alternative approach to measuring venous blood lead levels.(9–12) The benefit of using filter paper is the samples can be collected in the field, stored at ambient temperatures, and then analyzed at a later date. However, the most frequently used analysis methods require some form of digestion of a small punch from the blood spot or laser ablation to quantify the metal in a small part of the blood spot.(11, 13, 14) These sampling techniques face challenges, such as hematocrit level influencing blood wicking and drying and therefore potentially causing variation in metal concentration depending on where in the spot is sampled.(15, 16)

Energy dispersive x-ray fluorescence (EDXRF) provides a non-destructive approach for quantifying metals in samples and is routinely used for assessment of metals on air filters in air monitoring and exposure assessment (17). EDXRF has also been used in the past to look at biologic tissues including whole blood, serum, water, liver, and other tissue samples although typically with rather high detection limits.(18–23) EDXRF has been utilized to analyze metals in blood spots, but has primarily not focused on trace metals like Pb because

of the high detection limits, and instead considered metals such as Ca, Cu, and Zn that are found at much higher concentrations.(24)

Advances in technology, however, have substantially reduced the detection limits for EDXRF. Thus, EDXRF has the potential to improve on many of the analytic problems of other approaches for the analysis of metals in blood spots, which could then make analysis of metals in blood spots far more useful for screening and research purposes. EDXRF has many advantages over other approaches, including ease of sample preparation and analysis, which reduces the need for complicated equipment expertise, reduces the consumable costs, and reduces the time needed for analyses. Importantly, EDXRF offers the tremendous benefit of a non-destructive analysis that can measure over a wide area, such as an entire blood spot, which reduces the potential biases from hematocrit induced variation in metal concentrations across the blood spot. Furthermore, EDXRF has steadily improved both in size and functionality due to drastic improvements in detector and x-ray generator capabilities.

Given these improvements in current EDXRF technology, we aimed to assess the capabilities of the current generation of EDXRF systems to assess metals in blood. To do this, we used EDXRF to measure blood Pb concentrations on blood spots made from blood samples that had been previously measured by other standard approaches and focused on four analytical outcomes: (i) determining optimal measurement parameters, (ii) comparing measured concentrations with standard approaches, (iii) establishing detection limits, and (iv) assessing the effect of blood spot volume on measured concentrations. A feasible EDXRF approach for metal measurements in blood would provide a new assessment opportunity to a larger research community, and offer potential advantages to field studies, freeing them from many collection, storage, and shipping requirements typical of blood samples.

2. Methods

2.1 Epsilon 3XLE EDXRF Spectrometer

In this study, we used the PANalytical (Westborough, MA) Epsilon 4 measurement system. The system uses a silver anode x-ray tube up to 50 kV max energy with a max power output of 15 watts. Each instrument is equipped with a silicon drift detector with a resolution of about 145 eV. Measurements were made for varying times from 5 to 30 minutes. Measurements of dried blood spots were completed by cutting out the entire spot using a cleaned 20mm arc punch and placing the spot face down in a standard 32mm XRF cup (Premier Lab Supply SC4131) with a 2.5 um Mylar film (SPEX SamplePrep 3518).

2.2 Blood Spot Calibration

We made simulated blood spot standards using de-ionized (DI) water doped with different concentrations of lead. We used the same spotting procedures and materials for the water standards as was used for blood (see sections 2.3 and 2.4 below). We spotted 150uL of ultra-pure type 1 DI water spiked with 0, 5, 10, 50, and 100 ug/dL of lead (Fisher Chemical, certified reference standard solution) on standard blood spot cards inside a clean room

facility. We used Whatman 903 protein saver cards (GE Healthcare for Life Sciences) for spotting the standards and blood in this study. These standards were used to develop a calibration line under ideal conditions and to quantify results from other blood spot measurements. We also calculated the limit of detection from the calibration line (see section 2.4).

We measured the standards using the air filtration analysis setting in the Epsilon 4 Spectrometer software for an initial measurement of 5-minutes each. The standard air filtration analysis uses traditional peak fitting methods to extract the counts for individual elemental peaks. This method utilizes Gaussian elemental peak fits for each of the elements in the energy range and a background fit for any residual spectral components not accounted for from the elemental peaks themselves. Based on the fitted net counts from the Pb peak area and known concentrations we produced the calibration line. For all subsequent results, this calibration line was used to determine concentration from the fitted Pb peak net counts for the blood measurements, which were obtained from a deconvolution of the spectral components that comes standard on the instrument.

2.3 Blood Samples

2.3.1 Normative Aging Study—In order to determine effects of card loading volume, detection limits, and procedural reproducibility of the EDXRF approach, we used blood samples from participants in the Normative Aging Study, a cohort of men in the Greater Boston area.(25) ICP-MS measurements on the blood samples were done using a Perkin Elmer Elan DRCII. On average 1.02 g of blood was digested using 1 mL of trace metal grade nitric acid and 1 mL of hydrogen peroxide and then diluted to a final volume of 10 mL. The detection limit for Pb determined by 10 replicate quality control standards was <0.01 ppb and percent recovery was between 100 and 105% for all quality control standards. An initial calibration was done each day using 8 standards ranging in concentration between 0 and 5 ug/dL.

For analysis with the EDXRF the NAS blood samples were spotted onto Whatman 903 protein saver cards in June, dried in a fume hood, and stored in individual bags with desiccant for about two months prior to XRF analysis. The detection limit was calculated as twice the standard deviation of repeated measurements of the same blood spot or standard, which is routinely used as a standard in XRF for detection capabilities.(26–30) For procedural reproducibility testing the same blood sample was spotted on multiple cards and we compared results of EDXRF analyses of those cards.

2.3.2 Boston Children's Hospital—To have blood samples with relatively high blood Pb concentrations, we obtained 22 blood samples from children who were tested for blood Pb at Boston Children's Hospital. The whole blood samples were analyzed using graphite furnace AAS as part of the lead testing protocol of the Hospital and we collected left over blood for EDXRF analyses. Sample concentrations measured by AAS ranged from 3 μg/dL to 40 μg/dL and were used to test the analytical range of the EDXRF. For EDXRF analyses, we spotted the blood samples at a volume of 150 uL on Whatman 903 protein saver cards in June (the same batch of cards from the NAS blood), dried them in a fume hood, stored them

in individual bags with desiccant, and stored them for 6 months prior to measurement (due to instrument availability).

2.4 Statistical Analysis

We determined a Minimum Detection Limit (MDL) from the measurements of the standards using the equation

$$MDL = \frac{2\sqrt{C_{BKG}}}{Slope} \approx \sigma$$

, where σ is the uncertainty associated with the measurement, C_{BKG} is the background counts (from the EDXRF) estimated using the 0 ug/dL standard and Slope is the beta from the calibration line. To estimate EDXRF measurement detection limits at other measurement times than the 5-minute measurements used to create the calibration line, we used the equation

$$\sigma_{unknown} = \frac{\sigma_{5min}}{\sqrt{\frac{t}{5_{min}}}}$$

where t is the new EDXRF measurement time, $\sigma_{unknown}$, is the new uncertainty, and σ_{5min} is the uncertainty as estimated by the calibration line slope. We additionally estimated empirical detection limits from repeated measurements of our 10 ug/dL standard and actual blood spots. In this case the MDL was calculated as twice the standard error of the repeated measurements.

We used a repeated measure ANOVA and correlations to determine whether spotted sample volume affected measurements. We used linear regression and a Pearson correlation coefficient to examine the agreement between measurements made with AAS and EDXRF. Statistical significance was set at a 95% level of confidence. We used R version 3.4.2 for all analyses.

3. Results

3.1 EDXRF Reproducibility for Blood Spot Measurement

We tested the influence of the volume of blood used for spotting on EDXRF measurements by spotting the same blood sample on different cards using different volumes. We spotted two blood samples of 7.1 and 8.8 ug/dL Pb as measured by ICP-MS using 75 (2 cards), 150 (7 cards), and 300 (5 cards) uL of blood. The correlation between EDXRF quantified results as a percentage of known ICP-MS concentrations and the known volume of spotted sample showed no significant relationship between volume and measured signal from XRF (p-value=0.47 R=0.21). Similarly, a repeated measures ANOVA showed no differences by volume (p-value=0.40 F-value=3.309) with sphericity and normality not violated in this data.

3.2 Calibration Line and Detection Limit

Figure 1 shows the calibration line for Pb measurements of our 0, 5, 10, 50, and 100 ug/dL DI water standards. Using the calibration line we estimated a detection limit of 7.2 ug/dL for a 5-minute measurement or 2.9 ug/dL for a 30-minute measurement. We also calculated an empirical detection limit from repeated measures of the same spot made from a 10 ug/dL lead standard and two NAS blood samples, with Pb concentrations of 7.1 and 5.5 ug/dL as measured by ICP-MS. The distribution of those repeated measurements is shown in Table 1, from which we calculated MDLs of 1.0, 1.0 and 3.2 ug/dL.

3.3 Procedural Repeatability

To test for the error inherent in the whole procedure involved in EDXRF measurements we used a 7.1 ug/dL blood sample from the NAS to make 4 separate blood spots with 300 uL of blood and a 8.8 ug/dL blood sample from the NAS to make 6 blood spots with 150 uL of blood. The distribution of 30-minute EDXRF measurements made on the separate spots from the same blood sample are shown in Table 2. The intraclass correlation coefficient for this data was 0.49 (p-value=0.003), indicating relative agreement with repeated blood spot cards.

3.4 Comparison Between AAS and EDXRF Pb Measurements

The Boston Children's blood samples ranged from 3 to 40 ug/dL with an average of 21±12 ug/dL as measured by AAS. We then compared these to measurements done with the EDXRF on blood spots made from the whole blood samples. The correlation between AAS measurements and 30-minute EDXRF measurements was 0.99 (95% CI 0.97, 1.00) and in a linear regression the beta term was 1.00 (SD=0.04) indicating that the two measurement approaches gave nearly identical readings on average (Figure 2).

4. Discussion

In this study, we (i) found optimal parameters and procedures for measurement of lead in blood spots using EDXRF, (ii) found excellent agreement between AAS measurements of whole blood and EDXRF measurements made of blood spots from that whole blood (R=0.99 95% CI [0.97,1.00], beta=1.00±0.04), (iii) determined the MDL for a 30-minute EDXRF measurement to be 2.9ug/dL from our calibration line and empirical estimates were in as low as 1ug/dL from repeated blood spot measurements, and (iv) identified no significant effect of spotting sample volume on EDXRF measurements ranging from 75–300 uL (ANOVA p-value=0.4), although presumably if the volume is insufficient to create a spot at least the diameter of the EDXRF beam (~8 mm) this would likely affect measurements as the beam would capture areas without any blood. Finally, repeated measures of different spots made from the same blood sample also showed excellent agreement (coefficient of variation =0.16), indicating good reliability of the EDXRF blood spot measurement procedure.

We created the calibration line using DI water standards, which is not a perfect phantom for human blood. For our purposes of quantification of blood spots and identification of the Pb detection limit, however, we believe this was a suitable alternative to doping human

blood or using blood standard reference materials. The analysis quantification performed exceptionally well against AAS results, which, again, shows the promise of using EDXRF as a versatile method in the future.

In comparison to other studies using EDXRF for biological tissue or water analysis, the detection limit was much lower in our study. Past studies typically had detection limits for most metals closer to a level of 100 ug/dL.(21, 23) One study using EDXRF claimed a detection limit of 0.4 ppb (0.04 ug/dL), but this study used a 500-watt x-ray tube, which is much larger than the system used in the analysis presented in this study, and would likely need an entire room dedicated to operation.(31)

The detection limit we calculated ranged from 1.0 to 3.0 ug/dL for the various tests we performed. The detection limit calculated for EDXRF is on the order of what was previously used for blood analysis using GF-AAS (MDL~1–2ug/dL) and is enough to capture exposure levels in many settings, and certainly at the CDC reference level for elevated lead levels in children of 5 ug/dL. Of note, the detection limit we measured can be modified using different systems and measurement times to decrease the detection limit. The detection limit using XRF counting statistics is proportional to the square root of the factor increase in time and power from the device. The system in these measurements had a 15 watt output, but utilizing a higher output should give a reduction proportional to the square root of the increase in power.(32) For example, a 50-watt system would then lower the detection limit by a factor of 1.8, and 50 watt systems are readily available in the same form factor as the device used here. Similarly, a doubling of the time would reduce the detection limit by a factor of 1.41.

Of note, previous attempts at analyzing blood spots demonstrated significant issues in the quantification.(16, 33) This is mainly due to the sampling techniques utilized for collecting blood (34) and the use of small punches from the larger blood spot. This sampling method is subject to variability if metal concentrations vary across the blood spot as can occur because of the hematocrit effect, which relates to the drying pattern of blood on the card.(15, 16) While new approaches for blood spot collection and making punches could improve on this problem, (35–38) the EDXRF largely avoids this problem by measuring essentially the whole blood spot, and the beam diameter can be adjusted to further aid with this issue. Furthermore, EDXRF measurements have the great benefit of being non-destructive, so the blood spots can still be retained for other analyses.

Measurements of similar samples at different spotted volumes (70–300 uL) produced similar measurements from our XRF methodology. The quantification of lead did not appear to vary with large changes in volume, but further study is needed to definitively put this issue to rest. Thus, further study including more samples at higher and lower volumes will need to be completed to accurately determine whether the sample volume was indeed not having a significant impact on the outcome of the measurement. Lower sample volumes typical of neonatal finger sticks, may have more likelihood of impacting the results rather than the higher volumes used in the analysis included here. Standard blood will need to be used in order to test this effect to produce the number of spots necessary to make a conclusion either way.

The procedural repeatability found that laboratory methods did not have an impact beyond what we saw already with the detection limit of the device. Creating blood cards in the lab will always increase the level of uncertainty from measuring the samples directly, but the main finding here, demonstrated that this increase in uncertainty was lower than what we already identified with the detection limit. Thus, we can successfully create samples in the lab without harming the integrity of the measurement irreparably. In order to quantify the level of uncertainty introduced through laboratory preparation of the samples, a much larger controlled study of these effects would need to be undertaken.

However, more study on potential contamination in field settings should be completed to rule out other sources of variability that could be introduced during the measurement. Further study at lower levels of blood lead, less than 3 ug/dL, with more optimized settings could elucidate better detection settings and better indicate the device capabilities in real world settings. Most studies in the US currently have blood lead levels closer to 1 ug/dL, so this method would be most valuable in its current state as a screening tool for likely exposed populations rather than general population, but could potentially be improved in the future. This could also be an issue with previously collected blood spots. We potentially could get around this by measuring portions of the spotted paper free of blood and subtracting concentrations. This approach will need to be explored in further studies.

Measuring samples at increasingly lower levels of lead brings up a further question of contamination of the blood cards themselves. Previous studies have shown lead to be present in the blood cards at levels that would potentially influence results <1 ug/dL.(39, 40) The calibration used here has a non-zero lead signal from the blank source material and, thus, already takes this contamination into account. Utilization of a background subtraction method can be used more effectively in future measurements in tandem with corrections for contamination by collecting blanks from each card used during the collection process and identifying the zero point specific to each card. The variability of this would not have impacted the overall conclusions found here that EDXRF works well at quantifying blood spots with levels greater than 1–3 ug/dL. However, this is something that should be explored in the future to verify result integrity for spots collected on different lots of cards with potential for varying contamination inherent in the raw materials.

This technique could be used similarly to measure other metals in blood at levels comparable to what was shown with lead. XRF devices generate a spectra that encompasses a wide range of elemental excitations and any of those within a similar optimized range of lead could be identified and fitted likewise to what we have done with lead in this manuscript. However, adequate calibration and testing would need to be done to ensure disentanglement from surrounding elemental peaks, which is a possibility with some common trace elements that rely only on a single (alpha) peak rather than two peaks (alpha and beta).

In summary, the desktop EDXRF could be easily applied using a standard spotting procedure to the measurement of blood lead and similarly to multi-element quantification using an analysis of about 20–30 minutes to get a reasonable detection limit close to what has been used in the past with AAS (1–2 ug/dL), which would be viable for measurement

of large-scale studies to reduce costs and sample preparation burden. Future studies can evaluate blood spot standards collected in field conditions, for example global public health lead surveillance programs. Additional work on optimization, sample prep, and contamination correction could help explore the feasibility of using EDXRF for blood lead screening programs and how these financial, personnel, and equipment costs compare to the existing screening systems.

EDXRF effectively quantified blood Pb in spotted blood with a detection limit on the order of 1.0 to 3.0 ppb with good agreement between blood measured via AAS. The procedural stability was shown to be within the instrumentation uncertainty and the volume of spotted sample was shown to have little effect on the measurement using EDXRF. EDXRF would be ideal for quick and inexpensive population screening for metal exposures, including blood lead. Additional analyses could consider how sample collection location, such as within a clinic or collected in the field, affects sample quality and quantification.

Acknowledgements

The research described in this paper was supported by NIEHS ES-000002 and NIOSH 1K01 OH011648. The authors would also like to thank the many different research assistants and lab staff who helped prepare samples for measurements.

6. References

1. Ettinger AS, Leonard ML, Mason J. CDC's Lead Poisoning Prevention Program: A Long-standing Responsibility and Commitment to Protect Children From Lead Exposure. Journal of public health management and practice : JPHMP. 2019;25 Suppl 1, Lead Poisoning Prevention(Suppl 1 LEAD POISONING PREVENTION):S5–S12.
2. Campbell JR, Paris M, Schaffer SJ. A comparison of screening strategies for elevated blood lead levels. Archives of Pediatrics & Adolescent Medicine. 1996;150(11):1205–8. [PubMed: 8904864]
3. Organization WH. Brief guide to analytical methods for measuring lead in blood. Geneva: 2011.
4. Caldwell KL, Cheng P-Y, Vance KA, Makhmudov A, Jarrett JM, Caudill SP, Ho D-P, Jones RL. LAMP: A CDC Program to Ensure the Quality of Blood-Lead Laboratory Measurements. J Public Health Manag Pract. 2019;25:S23–S30. [PubMed: 30507766]
5. Morrell G, Giridhar G. RAPID MICROMETHOD FOR BLOOD LEAD ANALYSIS BY ANODIC-STRIPPING VOLTAMMETRY. Clinical Chemistry. 1976;22(2):221–3. [PubMed: 1248123]
6. Anderson MK, Amrich M, Decker KL, Mervis CA. Using state lead poisoning surveillance system data to assess false positive results of capillary testing. Matern Child Health J. 2007;11(6):603–10. [PubMed: 17340181]
7. Parsons R, Esernio-Jenssen. Screening children exposed to lead: an assessment of he capillary blood lead fingerstick test.
8. Schonfeld DJ, Rainey PM, Cullen MR, Showalter DR, Cicchetti DV. Screening for lead poisoning by fingerstick in suburban pediatric practices. Archives of pediatrics & adolescent medicine. 1995;149(4):447–50. [PubMed: 7704175]
9. Verebey K, Rosen JF, Schonfeld DJ, Carriero D, Eng YM, Deutsch J, Reimer S, Hogan J. Blood collection and analytical considerations in blood lead screening in children. Clinical Chemistry. 1995;41(3):469–70. [PubMed: 7882527]
10. Stanton NV, Maney JM, Jones R. Evaluation of filter paper blood lead methods: Results of a pilot Proficiency testing program. Clinical Chemistry. 1999;45(12):2229–35. [PubMed: 10585357]
11. Srivuthana K, Yee HY, Bhambhani K, Elton RM, Simpson PM, Kauffman RE. A new filter paper method to measure capillary blood lead level in children. Archives of Pediatrics & Adolescent Medicine. 1996;150(5):498–502.

12. Resano M, Belarra MA, Garcia-Ruiz E, Aramendia M, Rello L. Dried matrix spots and clinical elemental analysis. Current status, difficulties, and opportunities. Trac-Trends in Analytical Chemistry. 2018;99:75–87.
13. Cizdziel JV. Determination of lead in blood by laser ablation ICP-TOF-MS analysis of blood spotted and dried on filter paper: a feasibility study. Anal Bioanal Chem. 2007;388(3):603–11. [PubMed: 17396247]
14. Wagner M, Tonoli D, Varesio E, Hopfgartner G. The use of mass spectrometry to analyze dried blood spots. Mass Spectrom Rev. 2016;35(3):361–438. [PubMed: 25252132]
15. Daousani C, Karalis V, Malenovic A, Dotsikas Y. Hematocrit effect on dried blood spots in adults: a computational study and theoretical considerations. Scandinavian journal of clinical and laboratory investigation. 2019;79(5):325–33. [PubMed: 31154908]
16. Hall EM, Flores SR, De Jesús VR. Influence of Hematocrit and Total-Spot Volume on Performance Characteristics of Dried Blood Spots for Newborn Screening. Int J Neonatal Screen. 2015;1(2):69–78. [PubMed: 28868497]
17. Prost J, Zinkl A, Ingerle D, Wobrauschek P, Streli C. Evaluation of a sample preparation procedure for total-reflection X-ray fluorescence analysis of directly collected airborne particulate matter samples. Spectrochimica Acta Part B-Atomic Spectroscopy. 2018;147:13–20.
18. Carvalho ML. X-ray Fluorescence spectrometry: applications in trace elements studies in human tissues from patients with cirrhosis. X-ray Spectrometry. 2001(30):397–402.
19. Cesareo R Biomedical applications of photon-induced X-ray fluorescence. Biol Trace Elem Res. 1987;13(1):371–82. [PubMed: 24254692]
20. Kinoshita H, Tanaka N, Jamal M, Kumihashi M, Okuzono R, Tsutsui K, Ameno K. Application of energy dispersive X-ray fluorescence spectrometry (EDX) in a case of methomyl ingestion. Forensic Sci Int. 2013;227(1–3):103–5. [PubMed: 22999231]
21. Stump IG, Carruthers J, D'Auria JM. Quantitative analysis of trace elements in human blood and plasma by energy dispersive X-ray fluorescence. Clin Biochem. 1977;10(3):127–32. [PubMed: 884840]
22. Takahashi M, Kinoshita H, Nishiguchi M, Nishio H. Bromide detection in blood using energy dispersive X-ray fluorescence; a chemical marker supportive of drowning in seawater. Leg Med (Tokyo). 2010;12(3):132–6. [PubMed: 20335061]
23. Watanabe H, Berman S, Russell DS. Determination of trace metals in water using x-ray fluorescence spectrometry. Talanta. 1972;19:1363–75. [PubMed: 18961191]
24. Margui E, Queralt I, Garcia-Ruiz E, Garcia-Gonzalez E, Rello L, Resano M. Energy dispersive X-ray fluorescence spectrometry for the direct multi-element analysis of dried blood spots. Spectrochimica Acta Part B-Atomic Spectroscopy. 2018;139:13–9.
25. Bell B, Rose C, Damen A. The Normative Aging Study: an interdisciplinary and longitudinal study of health and aging. Int J of Aging Hum Dev. 1972;3:4–17.
26. Anjos M, Barroso RC, Perez CA, Braz D, Moreira S, Dias KRHC, Lopez RT. Elemental mapping of teeth using μSRXRF. Nuclear Instruments and Methods in Physics Research Section B Beam Interactions with Materials and Atoms. 2004;213:569–73.
27. Fleming DE, Ware CS. Portable x-ray fluorescence for the analysis of chromium in nail and nail clippings. Appl Radiat Isot. 2017;121:91–5. [PubMed: 28040603]
28. Nie H, Chettle D, Luo L, O'Meara J. In vivo investigation of a new 109Cd gamma-ray induced K-XRF bone lead measurement system. Phys Med Biol. 2006;51(2):351–60. [PubMed: 16394343]
29. Specht AJ, Weisskopf M, Nie LH. Portable XRF technology to quantify Pb in bone in vivo. Journal of Biomarkers. 2014;2014:9.
30. Zhang X, Specht AJ, Weisskopf MG, Weuve J, Nie LH. Quantification of manganese and mercury in toenail in vivo using portable X-ray fluorescence (XRF). Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals. 2018;23(2):154–160:1–7. [PubMed: 28901783]
31. Sky-Peck HH, Joseph BJ. Determination of trace elements in human serum by energy dispersive X-ray fluorescence. Clin Biochem. 1981;14(3):126–31. [PubMed: 7296822]
32. Specht AJ, Kponee K, Nkpaa KW, Balcom PH, Weuve J, Nie LH, Weisskopf MG. Validation of x-ray fluorescence measurements of metals in toenail clippings against inductively coupled plasma

mass spectrometry in a Nigerian population(). Physiol Meas. 2018;39(8):085007. [PubMed: 30091720]

33. Lawson AJ, Bernstone L, Hall SK. Newborn screening blood spot analysis in the UK: influence of spot size, punch location and haematocrit. J Med Screen. 2016;23(1):7–16. [PubMed: 26113437]
34. George RS, Moat SJ. Effect of Dried Blood Spot Quality on Newborn Screening Analyte Concentrations and Recommendations for Minimum Acceptance Criteria for Sample Analysis. Clin Chem. 2016;62(3):466–75. [PubMed: 26647314]
35. Spooner N, Olatunji A, Webbley K. Investigation of the effect of blood hematocrit and lipid content on the blood volume deposited by a disposable dried blood spot collection device. J Pharm Biomed Anal. 2018;149:419–24. [PubMed: 29154197]
36. Neto R, Gooley A, Breadmore MC, Hilder EF, Lapierre F. Precise, accurate and user-independent blood collection system for dried blood spot sample preparation. Anal Bioanal Chem. 2018;410(14):3315–23. [PubMed: 29623384]
37. Panchal T, Spooner N, Barfield M. Ensuring the collection of high-quality dried blood spot samples across multisite clinical studies. Bioanalysis. 2017;9(2):209–13. [PubMed: 27960550]
38. Veenhof H, Koster RA, Brinkman R, Senturk E, Bakker SJL, Berger SP, Akkerman OW, Touw DJ, Alffenaar JC. Performance of a web-based application measuring spot quality in dried blood spot sampling. Clinical chemistry and laboratory medicine. 2019;57(12):1846–53. [PubMed: 31373896]
39. Chaudhuri SN, Butala SJ, Ball RW, Braniff CT, Rocky Mountain Biomonitoring C. Pilot study for utilization of dried blood spots for screening of lead, mercury and cadmium in newborns. J Expo Sci Environ Epidemiol. 2009;19(3):298–316. [PubMed: 18398444]
40. Langer EK, Johnson KJ, Shafer MM, Gorski P, Overdier J, Musselman J, Ross JA. Characterization of the elemental composition of newborn blood spots using sector-field inductively coupled plasma-mass spectrometry. J Expo Sci Environ Epidemiol. 2011;21(4):355–64. [PubMed: 20588324]

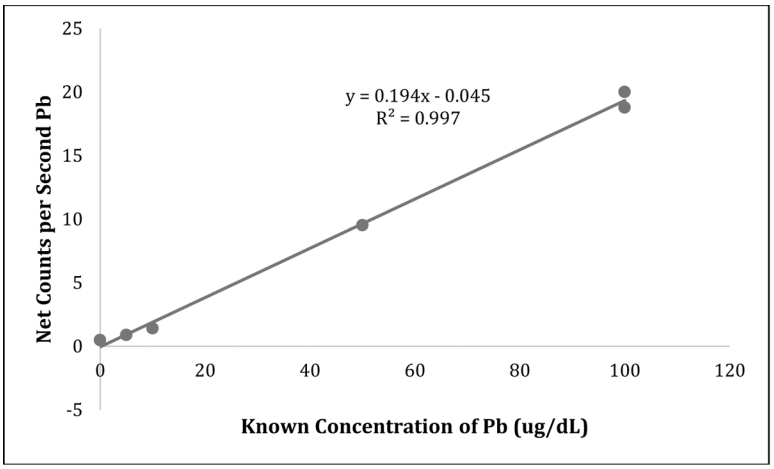


Figure 1.

Calibration line of lead counts versus known lead concentration in water using 5–minute measurements (N=6, error bars shown).

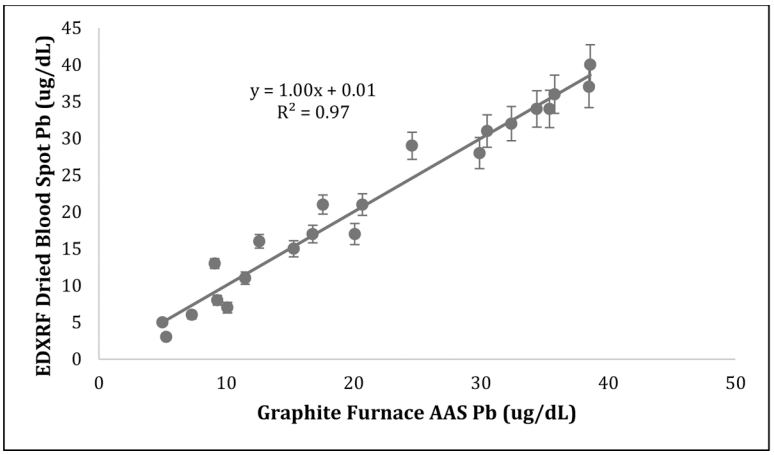


Figure 2.
Comparison of XRF measured blood spots to the same blood measured via AAS (N=22).

Table 1.

Distribution of repeated 30-minute measurements of blood spots.

Sample	Number of Measurements	Concentration* (ug/dL)	Coefficient of Variation	MDL (ug/dL)
Blood Spot Standard	22	10	0.05	1.0
Blood Spot 1 (150 uL)	20	5.5	0.09	1.0
Blood Spot 2 (300uL)	30	7.1	0.23	3.2

*As measured by ICP-MS.

Table 2.

Procedural repeatability tested making a number of blood spots using the same blood.

Sample	Number of Samples	ICP-MS (ug/dL)	Mean (ug/dL)	Coefficient of Variation	Standard Deviation (ug/dL)
Blood 1 (300 uL)	4	7.1	5.1	0.27	1.36
Blood 2 (150 uL)	6	8.8	9.1	0.08	0.74