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Research Article

Matrix-matched calibrators are necessary for robust and high-quality dried blood spots lead screening assays by inductively coupled plasma-mass spectrometry

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ABSTRACT

Background and aims: Reliable lead screening methods are necessary to support early identification of lead exposure in children. Sample collection using dried blood spots (DBS) offers advantages compared to traditional venipuncture and capillary collection. Here, we describe and compare three lead DBS inductively coupled plasma-mass spectrometry (ICP-MS) methods for lead screening.

Materials and methods: Lead was extracted from Whatman 903 protein saver cards punches and analyzed by ICP-MS across three independent clinical laboratories. Each laboratory evaluated the performance of aqueous and matrix-matched DBS calibrators using external quality control samples (WI State of Laboratory of Hygiene Program). Leftover patient samples (n = 39) were used for an interlaboratory comparison of lead DBS. Lead DBS results were compared to whole blood methods.

Results: The DBS ICP-MS methods using matrix-matched DBS calibrators had superior performance to the aqueous calibrations. There was a strong correlation between lead measured in DBS (matrix-matched) and whole blood for the three methods evaluated.

Conclusion: Lead can be measured accurately by ICP-MS in DBS samples when matrix-matched calibrators are used. External quality control programs are valuable to assess the performance of DBS methods. DBS lead ICP-MS methods are a robust analytical option for lead screening even though the limitations of DBS are well recognized.

1. Introduction

There is no safe blood lead concentration in children. Even low concentrations of lead are associated with behavioral, developmental, and physical impairment [1]. The sources of lead exposure in children are widespread but are most often attributed to residing in houses built before 1968 (e.g., with lead-based paint) or living in a highly industrialized area [2]. The lifelong effects of neurological damage due to lead exposure cost billions of dollars nationally, accounting for increases in healthcare, crime, special education, and an overall decline in lifetime earnings [3]. Despite significant reductions in blood lead levels (BLL) in U.S. children, disparities persist, with Non-Hispanic Black children or children living within households below the federal income poverty levels at greater risk of lead exposure [4]. Blood lead screening (BLS) programs support early exposure detection and help alleviate long-term outcomes.

Abbreviations: BLL, blood lead levels; BLRV, blood lead reference value; BLS, blood lead screening; CDC, Centers for Disease Control and Prevention; CMS, Centers for Medicare & Medicaid Services; CLIA, Clinical Laboratory Improvement Amendments; DBS, dried blood spots; EDTA, ethylenediaminetetraacetic acid; GF-AAS, graphite furnace-atomic absorption spectrophotometry; ICP-MS, inductively coupled plasma-mass spectrometry; NIST, National Institute of Standards and Technology; POC, point-of-care; PT, proficiency testing; TEa, total allowable error; WB, whole blood; WSLH, Wisconsin State Laboratory of Hygiene.

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In 2021, the Centers for Disease Control and Prevention (CDC) lowered the blood lead reference value (BLRV) from 5 μg/dL to 3.5 μg/dL based on the 97.5th percentile of the blood lead distribution in U.S. children aged 1 to 5 years, derived from the National Health and Nutrition Examination Survey [4]. The Centers for Medicare & Medicaid Services (CMS) require children enrolled in Medicaid to receive BLS at 12 and 24 months of age via a capillary blood draw (i.e., fingerstick) or venipuncture. A capillary BLL above the BLRV requires follow-up with a confirmatory venipuncture using a gold standard method to quantify BLL, preferably inductively coupled plasma-mass spectrometry (ICP-MS) or graphite furnace-atomic absorption spectrophotometry (GF-AAS). The CDC and the American Academy of Pediatrics Bright Futures guidelines mirror Medicaid guidelines for BLS and use environmental and risk assessment questionnaires to identify children who need BLS [5]. However, nearly half of Medicaid-enrolled children eligible for a screening test do not receive it [6]. Additionally, screening rates plummeted during the COVID-19 pandemic-related restrictions [7,8]. Several barriers contribute to the low BLS rates, including declined testing, aversion to phlebotomy, and loss of follow-up for sample collection, especially since it may require traveling to another location [6]. As a result, the U.S. Office of Inspector General recommends monitoring national screening rates and requests action plans from low-performing states, which may include launching point-of-care (POC) testing initiatives [6].

To help overcome some of the sample collection limitations, focus has been placed on capillary sampling, which is a minimally invasive and well-accepted alternative for lead testing. Thus, BLS by capillary POC testing increased screening rates during routine healthy children checks [9,10]. Lead POC testing is performed on the LeadCare II device (Meridian Bioscience, Cincinnati, OH), a Clinical Laboratory Improvement Amendments (CLIA) waived POC system that measures lead in whole blood (WB) capillary samples by anodic stripping voltammetry. The LeadCare II device is widely used as evidenced by participation in lead proficiency testing (PT) programs [13,14]. However, it has undergone several recalls due to falsely low results initially for venipuncture samples and more recently for capillary samples [11,12], limiting its utility to clinically improve BLS, albeit improving screening rates when reagents are available. The limitations of the Lead Care II device highlight the need for superior BLS alternatives particularly at remote screening locations where phlebotomy capabilities are absent. An attractive alternative is coupling capillary sample collection with ICP-MS or GF-AAS testing.

There is growing interest in the use of capillary dried blood spots (DBS) for clinical elemental analysis [15], which currently can only be achieved with ICP-MS or GF-AAS methodologies. Relative to standard capillary sample collection, a DBS is easier to collect, store, and transport. Yet, testing with DBS samples has additional pre-analytical challenges associated with the collection media, including sample heterogeneity, influence of hematocrit, and the potential for contamination during collection and/or storage. Additional challenges are related to the performance of quantitative methods for microsample analysis and uncertain regulatory requirements. To this end, a PT program for DBS lead was developed in the 1990s [16]. The pilot indicated variable performance across six participating laboratories, with approximately half performing sub-optimally at the CLIA total allowable error (TEa) limit of 4 μg/dL or 10 %. They hypothesized that under-recovery by some laboratories was attributed to the use of aqueous calibrators (versus WB) or inefficient extraction. Since DBS lead is typically extracted using an aqueous extraction buffer, a calibration derived from spiking lead directly into buffer is practical and logistically easier than preparing a DBS calibration curve. Although suggested, this hypothesis was not confirmed. The original PT program was terminated in 2011 due to the limited number of participants and discontinuation of federal funding. Following renewed interest in DBS testing, the PT program was re-established in 2021 with support from the CDC (personal communication). On re-initiation of the PT program, the distribution of results from participating laboratories, ours included, indicated that test performance for many groups did not consistently meet acceptability criteria.

In this study, we describe accurate and precise DBS lead measurement by ICP-MS independently developed by three high-complexity clinical laboratories. These laboratories confirm that matrix-matched calibrators are required for accurate results. This study also highlights the benefit of PT programs on the quality of laboratory results.

2. Materials and methods

2.1. Samples

Residual, deidentified venous WB samples were collected in royal blue ethylenediaminetetraacetic acid (EDTA) metal-free vacutainer tubes and submitted to the laboratory for lead screening. In addition, previously graded Wisconsin DBS PT Program samples (Wisconsin State Laboratory of Hygiene (WSLH), University of Wisconsin, Madison, WI, USA) were obtained. More information about the PT samples can be found in section 2.3. As only fully anonymized patient samples were used that were not obtained specifically for use in this study through an interaction or intervention with living individuals, neither informed consent nor IRB review were required.

2.2. ICP-MS methods

Laboratories A-C have each developed previously unpublished methods to measure lead from DBS sampled from Whatman 903® protein saver cards. The assays were tested using either a liquid calibrator in extraction buffer or a matrix-matched calibrator, as follows:

Laboratory A (LGC) DBS Method: Lead from a 6 mm punch was extracted into 2 mL of a water-based diluent containing 0.2 mg/mL EDTA, 0.1 mg/mL ammonium pyrrolidine dithiocarbamate, 0.25 % tetramethylammonium hydroxide, 0.05 % Triton X-100, 1 % ethanol, and 2 ppb iridium as internal standard. Lead was detected using an Agilent7800 ICP-MS (Santa Clara, CA) ran in no-gas mode coupled with the Agilent SPS4 autosampler and ISIS-3 introduction system.

To prepare the matrix-matched calibrators, a 6-point calibration curve was made using custom UTAK (Valencia, CA) WB lead controls and a diluent blank (target concentrations: 0, 1.0, 7.0, 14.0, 60.0 and 100.0 μg/dL). The concentrations of the WB calibrators were confirmed using a previously validated lead WB method. Once the WB calibration concentrations were verified, 40 μL of each calibrator was spotted and allowed to dry for at least 24 h before punching and extracting.

Liquid calibrators were prepared by diluting 20 μL of 3 % hydrochloric acid spiked with Agilent Lead Standard (Santa Clara, CA) and 20 μL of UTAK blank WB (Valencia, CA) in 2 mL of the diluent listed above. The calibrators included a matrix blank that was prepared by diluting 20 μL of water and 20 μL of UTAK blank WB (to account for the UTAK blank WB added into the calibrators). The calibration set consisted of 8-points (target concentrations: 0, 1.0, 2.5, 5.0, 10, 50, 100, 200 μg/dL). The 6 mm punches were extracted using the procedure described above with the addition of 20 μL water to account for the acid volume in the liquid calibrators. A water blank was prepared by diluting 40 μL of water into diluent to account for the water added to the 6 mm punches.

Laboratory B (NCH) DBS Method: DBS were sampled into a 48-well plate using two 3.2-mm punches per well. Lead was extracted using a water-based diluent (3 mL) containing 0.01 % ammonium pyrrolidine dithiocarbamate, 0.5 % tetramethylammonium hydroxide, 0.05 % Triton X-100, 2 % methanol, and 0.3 μg/dL terbium (internal standard). The well plates were sealed after the addition of diluent and shaken for 5 min before centrifuging at 142 × g for 2 min. Lead was detected using a NexION 2000C ICP-MS (PerkinElmer, Shelton, CT) coupled with an Elemental Scientific 4DX FAST Autosampler with SampleSense valve (ESI, Omaha, NE, USA).

To prepare the matrix-matched calibrators, a 5-point calibration

curve was prepared using pooled EDTA WB. The spiked lead concentrations for the calibrators were as follows: 0, 3.0, 12.5, 25.0, and 100.0 μg/dL. The stock solution (1000 μg/mL) used for this purpose was National Institute of Standards and Technology (NIST) traceable and obtained from SPEX (CLPB2-2 M). The concentrations of the WB calibrators were confirmed using a previously validated lead WB method. After verifying the concentrations of the WB calibrators, each calibrator (50 μL) was spotted onto Whatman 903 protein saver cards without blood and left to dry for at least 24 h before being punched and extracted. Whatman 903 protein saver cards without blood were used as calibration blanks. For liquid calibrators, a 6-point calibration curve was prepared using saline spiked with lead at concentrations of 0.0, 3.5, 10.0, 20.0, 50.0, and 100.0 μg/dL.

Laboratory C (CCF) DBS Method: Lead from a 6 mm punch was extracted using 5 mL of a water-based diluent containing 5 mM EDTA and 0.05 % Triton X-100. The sample was vortexed at 915 × g for 1 min, incubated at room temperature for 30 min and centrifuged. Lead was detected using a Thermo Fisher iCAP RQ and TQ (Thermo Fisher Scientific, Waltham, MA, USA) in kinetic energy discrimination mode, coupled with an Elemental Scientific SC-FAST autosampler. The internal standard, bismuth, was introduced via loop injection.

To prepare the matrix-matched calibrators, a 5-point calibration curve was prepared using pooled EDTA WB and spiked lead certified reference material traceable to NIST SRM 3128 (VHG labs, Manchester, NH, USA) at concentrations 0.0, 1.0, 5.0, 10.0, 25.0, 50.0 and 100.0 μg/dL. The concentrations of the calibrators were established using a previously validated lead WB method. Once the WB calibration concentrations were verified, 50 μL of each calibrator was spotted in the filter paper and allowed to dry for at least 24 h before analysis. The liquid calibration curve was prepared using the water-based diluent spiked with lead at concentrations 0.0, 1.0, 5.0, 10.0, 25.0, 50.0 and 100.0 μg/dL.

2.3. External quality assessment

Laboratories A-C measured lead levels in fifteen pre-spotted DBS samples obtained from the Wisconsin DBS PT Program. These laboratories used their DBS methods calibrated with either liquid or DBS standards. The results were compared to the PT target values and a TEa limit of 4 μg/dL or 10 % (CLIA), whichever was greater. Alternatively, the more stringent TEa recommended by the Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) of 2 μg/dL was used [17].

The WSLH PT samples consisted of bovine EDTA WB collected from lead-dosed animals and applied to filter paper. The target values for these samples were determined from more than ten referee laboratories using liquid blood aliquots in CLIA-validated ICP-MS blood lead methods. Samples that had results below the AMR of the referee laboratories' methods were assigned a target value of 0 μg/dL. The target for the 15 challenges ranged from 0.0 to 38.02 μg/dL.

Some samples were not run by Laboratories A (5 out of 15, using liquid calibrator) and B (3 out of 15, using both liquid and DBS calibrators). This was either due to not having the assays with the liquid and DBS calibrators available simultaneously or not receiving all the samples, respectively.

2.4. Inter-laboratory comparison

Whatman 903 protein saver cards were spotted with 50 μL of residual venous samples (n = 39) collected into royal blue EDTA metal-free vacutainer tubes. The expected concentrations were derived from a WB method conducted by Laboratories B (n = 14) or C (n = 25). The cards were then distributed to laboratories A-C for lead measurement using their respective DBS methods. The DBS results were compared to the concentration measured in WB.

2.5. Statistical analysis

Statistical analyses and graphing were performed using GraphPad Prism version 9.0, GraphPad Software (San Diego, CA, USA) and EP Evaluator (Data Innovations, Colchester, VT). Multiply by 0.048 to convert lead from μg/dL to μmol/L.

3. Results

3.1. Performance of the lead DBS method using aqueous or DBS matrix-matched calibrators

Table 1 presents the DBS lead concentrations in samples from the WSLH PT program using three ICP-MS assays, with either an aqueous calibrator or a matrix-matched DBS calibrator. For samples with low results (peer mean ≤ 0.7 μg/dL), Laboratories A-C reported results below the AMR using both calibrator approaches (liquid and DBS). All these results were acceptable, using a TEa of ± 2 μg/dL or ± 4 μg/dL. For samples with lead results ranging from 5.6 − 38.0 ug/dL, 37.5–83.3 % of samples had acceptable results within ± 4 μg/dL or 10 %, whichever is greater, in the methods calibrated using a liquid standard (Table 1 and Fig. 1). Using the same acceptability criteria, the DBS matrix matched calibrator recovered within acceptability criteria in all samples, and in 83.3—100.0 % for the tighter TEa of ± 2 μg/dL or 10 %, whichever is greater. The bias in the liquid calibrator method was larger in samples with concentrations > 20.0 ug/dL (Fig. 1A). The use of a DBS calibrator corrected recovery and minimized bias across the range of concentrations measured (Fig. 1B).

3.2. Interlaboratory performance of the methods using DBS matrix-matched calibrators

The performance characteristics of the ICP-MS DBS methods using matrix-matched calibrators, established independently by each clinical laboratory, are summarized in Table 2. The assays in Laboratories A and C exhibit linearity from 1.0 to 100.0 μg/dL (A: y = 1.01x-1.6, R = 0.9990; C: y = 1.02x-0.59, R = 0.9985), while Laboratory B shows linearity from 2.0 to 100.0 μg/dL (y = 0.964x-0.4, R = 0.9930). The total precision was found to be less than 9.5 %. Paired WB EDTA and DBS samples (n = 39) were analyzed across three laboratories, and the results from all laboratories correlated with the WB method: A: y = 1.066x + 0.23, R = 0.9886; B: y = 1.088x + 0.34, R = 0.9889; C: y = 0.957x + 0.49, R = 0.9894 (Fig. 2). On average, the DBS to WB bias was 1.1 μg/dL (8.3 %) in Laboratory A, 1.5 μg/dL (11.4 %) in Laboratory B, and −0.1

Table 1
. Number of PT samples with acceptable results measured using Lead DBS methods calibrated with liquid or matrix-matched (DBS) standards.

		Lab A		Lab B		Lab C	
		Liquid n = 10	DBS n = 15	Liquid n = 12	DBS n = 12	Liquid n = 15	DBS n = 15
TEa = 4 μg/dL or 10 %	Results < AMR	2/2 (100 %)	3/3 (100 %)	2/2 (100 %)	2/2 (100 %)	3/3 (100 %)	3/3 (100 %)
	Numeric results	3/8 (37.5 %)	12/12 (100 %)	6/10 (60 %)	10/10 (100 %)	10/12 (83.3 %)	12/12 (100 %)
TEa = 2 μg/dL or 10 %	Results < AMR	2/2 (100 %)	3/3 (100 %)	2/2 (100 %)	2/2 (100 %)	3/3 (100 %)	3/3 (100 %)
	Numeric results	1/8 (12.5 %)	10/12 (83.3 %)	5/10 (50 %)	10/10 (100 %)	6/12 (50 %)	12/12 (100 %)

TEa, total allowable error; AMR, analytical measurement range.

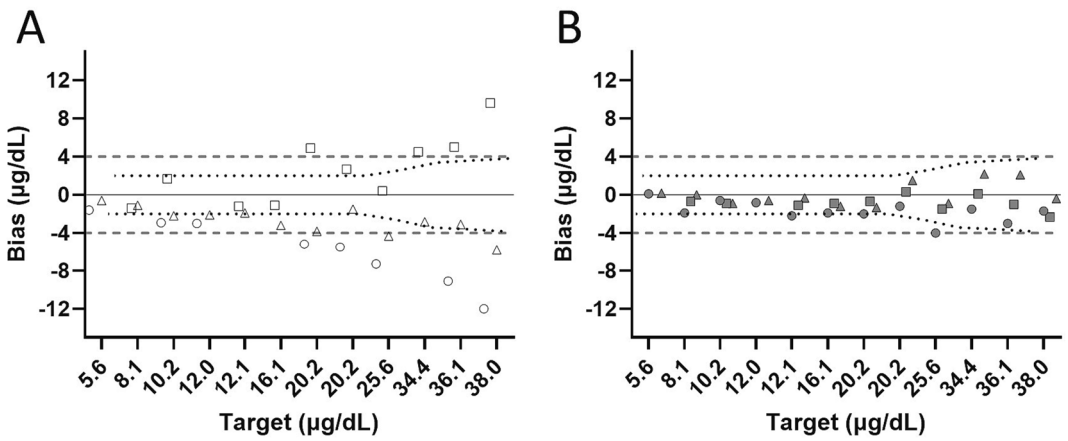

Fig. 1. Bias of PT samples measured using Lead DBS methods calibrated with a liquid or DBS standard Bias of DBS samples obtained from the WSLH PT program relative to peer target values, measured in Laboratories A (circle), B (square) and C (triangle) using a (A) liquid calibration curve (open symbols) and a (B) matrix-matched DBS calibration curve (filled symbols). The grey dashed lines indicate acceptability limits for TEa ± 4 µg/dL or 10 % and the dotted black lines denote acceptability limits for the TEa ± 2 µg/dL or 10 %. Samples with target results < AMR are excluded from this figure.

Table 2
. Performance characteristics of DBS methods using matrix-matched calibrators.

Characteristic	Lab A	Lab B	Lab C
Precision			
Means (µg/dL)Inter-	6.8/20.7/35.8	3.6/8.9/32.9	5.4/18.5
assay	<7.6 %	<6.6 %	<5.8 %
(n = 25)	<8.1 %	<6.6 %	<8.0 %
Total			
Linearity			
AMR (µg/dL)	–100.0	2.0–100.0	–100.0
y = mx + b	y = 1.01x – 1.6	y = 0.964x – 0.4	y = 1.02x – 0.59
R	0.9990	0.9930	0.9985

AMR, analytical measurement range; R, correlation coefficient.

µg/dL (-0.6 %) in Laboratory C. The DBS results across the laboratories and the WB result are not statistically different (p = 0.9479, non-parametric Kruskal-Wallis test).

4. Discussion

Our objective was to develop robust methods for screening lead in DBS samples using ICP-MS. Compared to traditional WB assays, there are still relatively few validated assays for quantifying lead in DBS samples in clinical laboratories. This may be due, in part, to the challenges associated with DBS testing such as contamination risk, spot heterogeneity, and hematocrit effect. Additionally, in order to measure DBS lead using ICP-MS, it is crucial to effectively extract lead from the filter paper. It is important to develop extraction protocols that minimize background contamination and optimize accuracy and precision [15]. In this study, we present data on the performance of three independently developed and validated DBS methods. Initially, each method used liquid calibrators where lead standards were added to an aqueous extraction buffer. This approach was appealing due to the simplicity of standard preparation. However, none of the three methods accurately recovered lead when liquid calibrators were used, despite attempts at optimization through modifications to the extraction buffer, longer extraction times, different assumptions about blood DBS volume, and correction factors, among other strategies. The issue of choosing a calibration method, including matrix matching, has long been recognized in elemental analysis as a way to deal with interferences (reviewed in [18]). In our experience, the use of matrix-matched DBS calibrators was essential for accurate quantification of lead. We avoided relying on correction factors from liquid calibrators because they were proven unreliable in previous iterations of our methods; the bias appears proportional and significant at higher lead concentrations leading to both under- and over-recovery of results. Our method improvements were

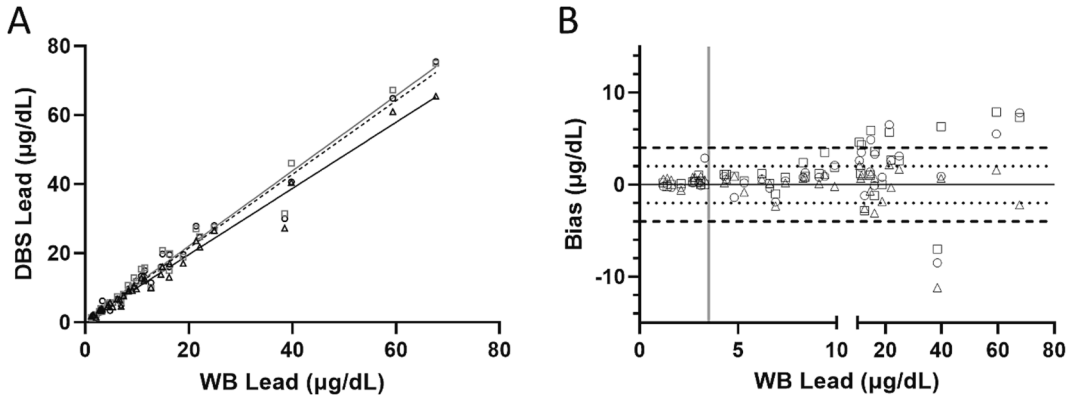

Fig. 2. Interlaboratory comparison of DBS methods using matrix-matched (DBS) Calibrators Linear correlation (A) of DBS samples measured in Laboratories A (circle, dotted line), B (square, solid gray line) and C (triangle, solid black line) by ICP-MS using matrix-matched DBS calibration curves, relative to lead measured in whole blood (WB). Samples (n = 39) were measured in whole blood (WB) in Laboratories B (n = 14) and C (n = 25) and DBS (n = 39) in Laboratories A-C. (B) Bias plot of the DBS and WB results. The dashed horizontal line indicates acceptability limits for TEa ± 4 µg/dL and the dotted horizontal line denotes acceptability limits for the TEa ± 2 µg/dL. Note that 50 % of the x-axis represents concentrations up 10.0 µg/dL, and the right 50 % includes samples with concentrations > 10.0 µg/dL. The recommended BLRV for lead, 3.5 µg/dL, is represented with the vertical solid grey line.

initiated after sub-optimal PT results. Thus, the availability of external quality assurance samples through WSHL PT program was critical as it provided an objective and matrix-matched system for evaluating our methods. This exemplifies the value of PT programs in improving the quality of testing, particularly for laboratory developed tests and tests using alternative matrices that cannot be easily replicated in the laboratory.

Our study addresses several gaps related to DBS methods for BLS. The data shows a strong inter-laboratory correlation between the concentration of lead in WB and matched DBS filter paper extracts for the three evaluated methods. These methods were developed independently and use different extraction buffers, DBS size, calibrators, internal standards, and ICP-MS instruments. To our knowledge, this is the first inter-laboratory comparison of lead by DBS and ICP-MS. Importantly, the methods used in this study demonstrate the necessary analytical reliability for BLS and suggest the feasibility of using DBS for capillary collection at the POC.

Testing capillary samples by ICP-MS under controlled quality assurance programs is superior to current POC platforms [13,14]. The limitations of DBS for elemental analysis were recently reviewed by Parsons et al. [19], and the CDC has warned about the use of filter paper for BLS due to contamination concerns and uneven blood distribution [20]. However, there are several approaches that can be used to prevent or investigate lead contamination of filter paper used for sample collection and transportation. The CDC provides guidance on capillary collection best practices [21], which is considered a screening method with follow-up confirmatory testing on a venous sample if capillary results exceed the CDC's BLRV [22]. Although burdensome, laboratories and government agencies may choose to pre-screen filter paper lots, test a blank spot to rule out contamination, or use pre-cleaned filter cards [23]. Although ideal, certified metal-free filter paper is not commercially available as far as we know. Newer technology such as microfluidic systems with volume control show promise in overcoming the limitations of blood distribution in DBS [24 –26].

There are various limitations to this study. The DBS samples used for inter-method comparison were spotted from WB samples collected in certified metal-free EDTA tubes. This may not account for capillary collection variables and introduces EDTA into the spotted samples. Despite these limitations, this approach was practical and scientifically sound. First, the venous samples used reflect in vivo conditions of lead (versus artificially spiking lead). Second, the correlation between venous and capillary blood, and DBS has been extensively demonstrated [16–18,27–30]. Finally, increasing EDTA concentrations in extraction buffers did not affect lead extraction efficiency (data not shown). It's worth noting that our laboratories did not focus on challenging the sensitivity of the methods below 1–2 μg/dL, since the current recommended BLRV is 3.5 μg/dL [4]. If the BLRV continues to decrease in the U.S., further characterization of our methods at lower concentrations will be necessary.

5. Conclusions

Elevated blood lead levels are associated with serious health and neurodevelopmental outcomes. BLS is necessary for detecting, intervening, and remedying exposure to lead. Although there is clear guidance on which populations are at a higher risk of exposure, screening rates remain lower than necessary, and disparities in exposure levels persist. Lead testing presents various challenges, including high pre-analytical and analytical complexity. While DBS testing has limitations, combining DBS with ICP-MS provides a robust alternative for BLS. The use of matrix-matched calibrators and the availability of an external quality control program were crucial in developing accurate and precise DBS lead methods for concentrations ranging from 1.0 to 100 μg/dL.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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