Challenges of Blood Spot Heavy Metal Testing – Using Blood Spot Area to Correct for Abnormal Sample Spread when Sample Matrix is Unknown

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ABSTRACT

Background:
Dried blood spot (DBS) samples consist of whole blood dropped onto laboratory grade filter paper from a finger prick or pipette. It is well known that hematocrit can influence blood spot size, effectively diluting or concentrating the sample if the whole spot is not extracted. In a similar way, whole blood element proficiency samples from agencies such as the Centers for Disease Control and Prevention (CDC) and College of American Pathologists (CAP) with an unknown matrix may spread differently when placed on filter paper than a 100% human blood sample. The purpose of this study is to show that by pipetting a set volume of whole blood, a simple area correction can be applied to account for matrix-related sample dilution or concentration for cadmium, lead, and mercury.

Materials and Methods:
The methodology for our DBS element method was presented as a poster at the 2017 AACC conference [Abstract A-402: Dried Urine and Blood Spot Analysis of Essential and Toxic Elements by ICP-DRC-MS]. Whole blood external controls from ClinChek, SeroNorm, and BioRad with known values for cadmium, lead, and mercury were pipetted at 60 µL on filter paper. Blood spot diameter was measured using a caliper to determine area, and two 6-mm punches from each spot were used for analysis. Results were compared before and after area correction. Punches from the inner and outer parts of each blood spot were also tested for homogeneity.

Results:
The addition of an area correction significantly improved the accuracy of DBS heavy metal testing when sample matrix is unknown. It was also determined that there was an even spread of analytes across the blood spot.

Conclusions:
The use of blood spot area correction for whole blood samples with an unknown matrix pipetted on filter paper can significantly improve the accuracy of results.

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Introduction

Dried blood spot (DBS) testing has been used for over a century to successfully measure hundreds of analytes, yet challenges related to sample collection and analysis still exist. DBS has many advantages over liquid samples, such as stability without preservatives, ease of collection and transport, and small storage footprint, making it ideal for remote collections and large-scale studies. To create and analyze DBS, whole blood from a venous blood draw or finger stick is applied to filter paper and dried, transported to the lab, and extracted for analysis. Our laboratory validated and commercialized a DBS method in 2016 for cadmium, lead and mercury, which are among the top 10 threats to human health according to the CDC's Agency for Toxic Substances and Disease Registry (ATSDR) 2017 Substance Priority List.[1] Unfortunately, element proficiency samples and external controls are only available as liquid whole blood, which must be transferred to filter paper so they can be treated identically to patient samples. Contrived liquid whole blood may contain preservatives or other diluents, causing it to behave differently than a true human matrix; it may spread differently on filter paper than human blood, diluting (larger blood spot) or concentrating (smaller blood spot) the sample if a set sample punch size is taken. To try and solve this problem, experiments were performed using external whole blood controls from ClinChek, BioRad and SeroNorm with manufacturer expected values for cadmium, lead and mercury to see if blood spot area from a set volume of blood can be used to correct for abnormal sample spread.

Methods/Procedures/Results

Blood Spot Creation and Measurements

Lyophilized whole blood external controls from 3 levels of ClinChek, BioRad and SeroNorm were reconstituted according to manufacturer instructions, and applied as 60 µL aliquots to Whatman 903 filter paper using a 200 µL pipette (Figure 1). Blood spots in 40, 80 and 100 µL aliquots were also created. Samples were left to dry for 4 h and stored at room temperature. A digital caliper [Huskey – Accuracy ± 0.02 mm] was used to measure the diameter of the blood spots to determine area (A=πr^2). We assumed that whole blood spreads evenly in all directions when pipetted onto a horizontal surface, which was confirmed with measurements.

Blood Spot Element Analysis

Two 6 mm punches [Perkin Elmer DBS Puncher] were taken from each blood spot. Samples were extracted and analyzed using methodology presented at the 69th AACC Conference in 2017.[2]

Blood Spot Volume vs. Area

The blood spot area of 40, 60, 80 and 100 µL aliquots of 3 levels of ClinChek, BioRad and SeroNorm were averaged and plotted against volume. Results were found to be linear, showing a predictable spread on filter paper as volume increases (Figure 2).

Blood Spot Interior vs. Exterior Punch Comparison

Interior and exterior punches of 60 µL blood spots (Figure 3) from 3 levels of ClinChek, BioRad and SeroNorm were analyzed to confirm a homogenous spread of blood on filter paper for all elements analyzed (Figure 4). Results showed no noticeable concentration difference based on punch location.

Blood Spot Area Correction

Three levels of ClinChek, BioRad and SeroNorm 60 µL blood spots were analyzed. The area of each blood spot was compared to the area of 60 µL blood spot calibrators we use for our element assay. The area difference between the 3 levels of ClinChek, BioRad and SeroNorm and our assay calibrators (Area Correction Factor = Experimental Blood Spot Area/Calibrator Blood Spot Area) was used to "area correct" results, and compare them to manufacturer expected values for cadmium, lead and mercury. Results with and without area correction can be seen in Figure 5. Area correction of blood spots significantly improved BioRad and SeroNorm results, while ClinChek results were practically unchanged due to their similar area to our blood spot assay calibrators.

Figure 1.

Figure 2.

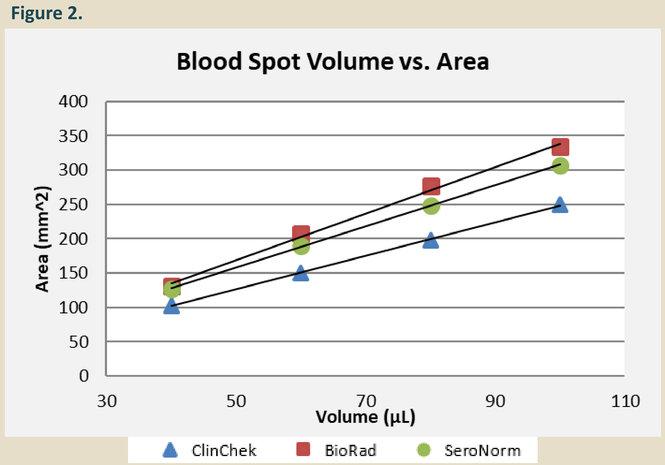


Figure 3.

Figure 4.

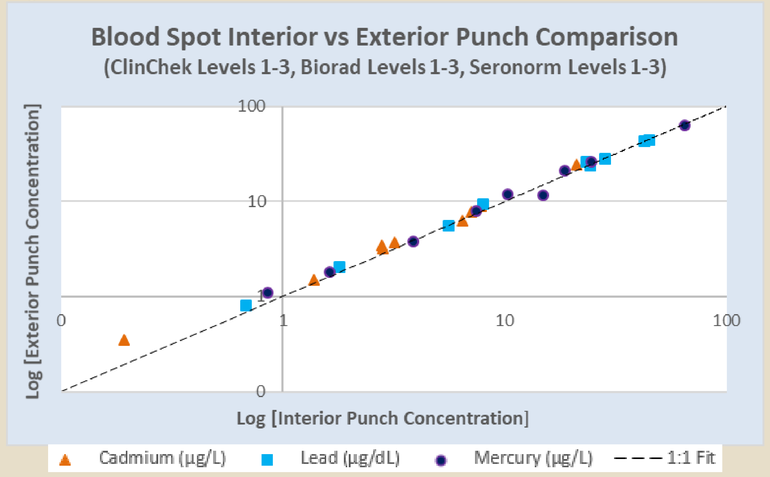
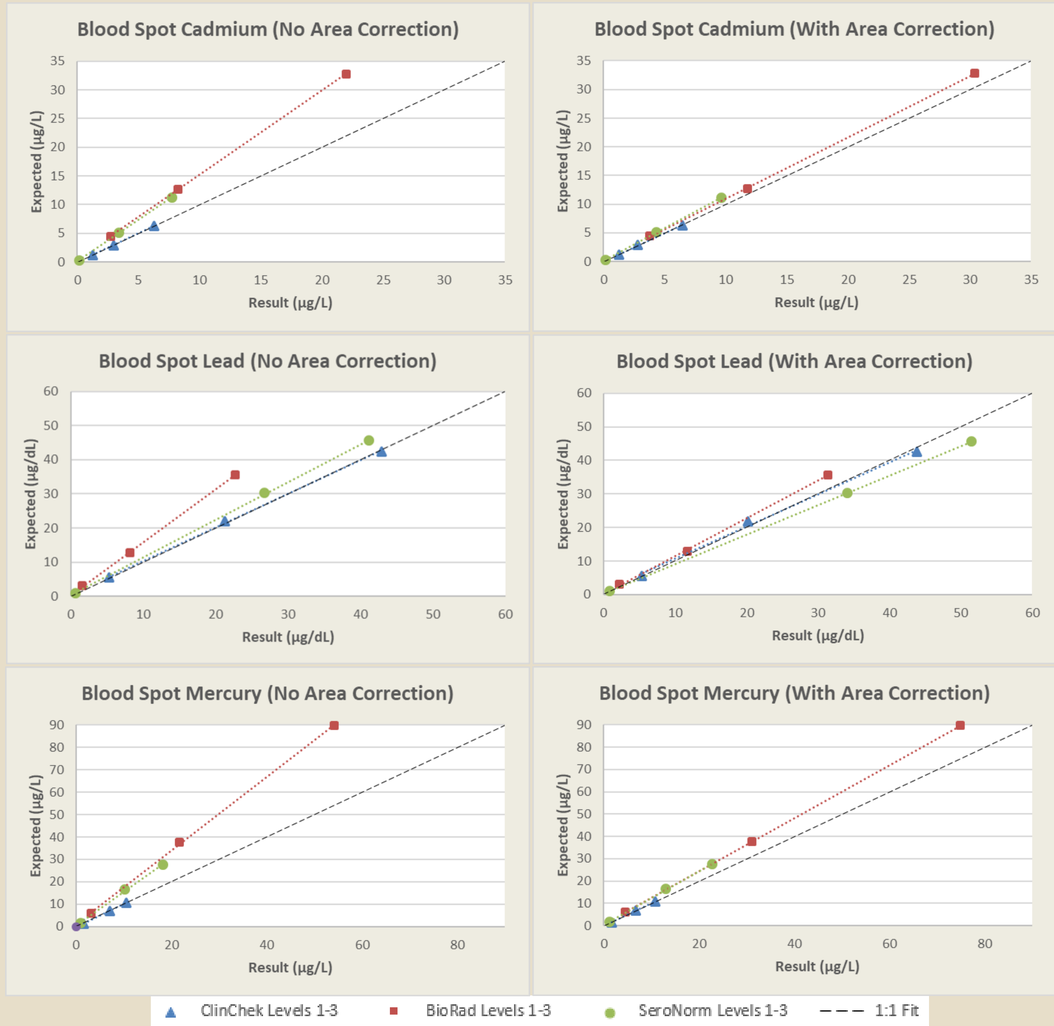


Figure 5.



Discussion/Conclusion

DBS element analysis is challenging because no DBS controls/calibrators/proficiency samples are available for purchase, only liquid/lyophilized whole blood samples that must be dried on filter paper. Our work shows that if unknown samples and blood spot assay calibrators are pipetted at a set volume, an accurate caliper can be used to determine area and a factor can be applied to the unknown samples to correct results. This is possible because the area of the blood spot increases in a linear fashion when volume increases and because there is equal spread of analytes within a blood spot. Alternative solutions such as analyzing the whole blood spot or using hematocrit markers are currently not practical for DBS testing, but collection using Volumetric Absorptive Microsampling (VAMS) technology [Mitra Device - Neoteryx] has shown promising results.[3] While blood spot area correction is not required for samples with a 100% human blood matrix, being able to accurately analyze unknowns with abnormal sample spread using area correction solves a challenge of blood spot heavy metal testing.

References

1) Agency for Toxic Substances and Disease Registry (2017). ATSDR 2017 Substance Priority List. Available at: https://www.atsdr.cdc.gov/spl/index.html#2017spl Accessed 10 Jan 2019.
2) Abstract A-402: Dried Urine and Blood Spot Analysis of Essential and Toxic Elements by ICP-DRC-MS. https://www.zrtlab.com/media/1469/aacc-poster-final-version.pdf Accessed 10 Jan 2019.
3) Velghe S, Delahaye L, Stove CP. Is the hematocrit still an issue in quantitative dried blood spot analysis? J Pharm Biomed Anal. 2019 Jan 30;163:188-196.