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Evaluation of LeadCare Ultra® as an initial screen for elevated blood lead levels

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ABSTRACT

Objective: The LeadCare Ultra® (LCU) was compared to inductively coupled plasma mass spectrometry ICP-MS for use as a screening test for elevated blood lead levels (BLLs) in capillary samples from children.
Methods: During the validation, method comparisons between LCU and ICP-MS were analyzed to determine the bias above, near, and below the BLL cut-off of 5 μg/dL. Additionally, capillary samples that screened positive by LCU (above the 5 μg/dL cut-off) were compared to venous samples analyzed by ICP-MS for confirmatory testing.
Results: LCU had a positive bias (1.7 μg/dL) below the cut-off of BLL < 5 μg/dL, no bias near the cut-off from BLL 5–10 μg/dL, and a negative bias (−0.8 μg/dL) for BLL > 10 μg/dL compared to ICP-MS. Of the 59 capillary samples that screened positive by LCU between May of 2017 to April of 2018, 19 were confirmed positive by ICP-MS, 30 were confirmed negative by ICP-MS, and 10 did not have a confirmed result.
Conclusion: The LCU assay is an acceptable screen for capillary samples with the BLL cut-off of 5 μg/dL.

1. Introduction

In 2012, the CDC issued new guidelines for assessing children's blood lead levels (BLLs) based on the Advisory Committee on Childhood Lead Poisoning Prevention's (ACCLPP) recommendation that the reference value be population-based [1]. The data from the 2007–2010 National Health and Nutrition Examination Survey (NHANES) from children 1 to 5 years old defined a BLL reference value of 5 μg/dL based on the 97.5th percentile. Prior to these guidelines, children had a BLL "of concern" at or above 10 μg/dL. Given the current recommendations, it is critical that laboratory methods are accurate at the lower BLL of 5 μg/dL. Additionally, ACCLPP has suggested that the CDC update the reference value every four years [1]. Based on the 2011–2014 NHANES study, the BLL reference value should be lowered to 3.48 μg/dL, but this has yet to be accepted by the CDC [2].

Well-established methods for measuring BLLs include Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [3]. These methods are used by reference laboratories and are highly accurate and precise, with published coefficients of variation < 5% [4,5]. However, the instruments are very costly and require expertise to operate and maintain. GFAAS and ICP-MS are also classified as lab developed tests by the FDA, meaning that they can only be used in high complexity laboratories.

Another method employed to measure BLLs is anodic stripping voltammetry (ASV) [6,7], which is the technology used by LeadCare Systems® (Magellan Diagnostics). These FDA-approved instruments are cheaper and easier to maintain than GFAAS and ICP-MS. LeadCare Ultra (LCU) is a moderate complexity test that can be used as a screening assay for capillary whole blood samples; if the screen is positive for a high BLL, then a venous sample should be collected within two weeks to confirm by ICP-MS or GFAAS. Given that a majority of our patient samples had a BLL < 5 μg/dL, the LCU was an attractive screening alternative to sending out all samples to a reference laboratory. Since there have been no prior studies assessing the performance of the LCU at the BLL reference value of 5 μg/dL [8], our objective was to perform a method comparison study of the LCU with ICP-MS.

2. Methods

The samples were treated per the manufacturer's instructions [9]. Briefly, 50 μL of whole blood from an EDTA or heparinized capillary tube was transferred into a vial with 250 μL of the LeadCare reagent to lyse the red blood cells and release lead into the solution. The solution was analyzed by placing a few drops on an electrochemical sensor, and the concentration was recorded. The LCU was calibrated according to the manufacturer's instructions and can measure BLLs in the range of 1.9–65 μg/dL.

Patient capillary samples were screened for BLL ≥ 5 μg/dL with the

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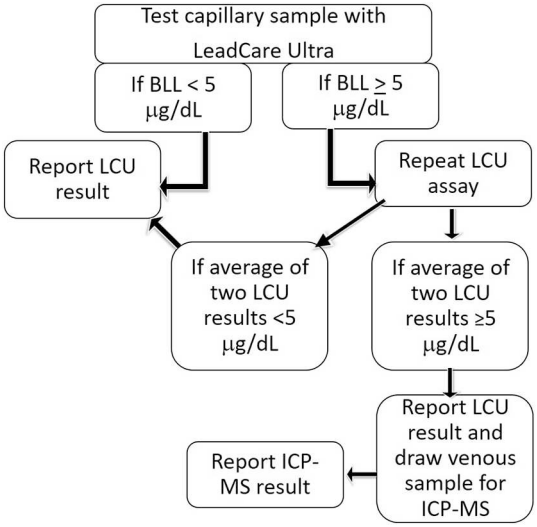

Fig. 1. Screening algorithm for elevated BLLs for our patient samples.

LCU assay (Fig. 1). If the BLL was ≥5 µg/dL, the LCU assay was repeated to ensure there was no contamination. The average LCU result was reported, and the physician was immediately notified that the patient should return for a venous blood draw. The venous sample was sent-out for confirmation testing by ICP-MS. The Minnesota Department of Health (MDH) was notified of all results (Minnesota Statutes, section 144.9502, [10]).

2.1. Precision

Precision was evaluated with a quality control (QC) sample at 8.2 µg/dL, a QC sample at 24.7 µg/dL, and a low BLL patient sample at 2.5 µg/dL. The QC material is supplied in the LCU kit. Within-day coefficients of variation (%CV) were calculated after repeating the QC material or low patient sample 10 times on the same day. Between day imprecision was found by analyzing QC material twice per day for 10 days. The patient sample was not analyzed for between day imprecision because the manufacturer instructions state that samples must be processed within 24 h. The desired imprecision was < 10%.

2.2. Method comparison for assay validation

A total of 75 capillary or venous whole blood samples were analyzed by both the LCU and by ICP-MS across the LCU measurement range of 1.9–65 µg/dL. For method comparison with ICP-MS, a sample was sent to Medtox (LabCorp, St. Paul, MN). The ICP-MS assay at MedTox had a limit of quantification of 1.0 µg/dL, analytical measurement range of 1.0–100.0 µg/dL, and inter-assay imprecision of < 7.0%. For results reported below the respective analytical measurement ranges (< 1.9 µg/dL for LCU and < 1 µg/dL for ICP-MS), half of the value was used for the comparison (i.e. 0.95 µg/dL for LCU and 0.5 µg/dL for ICP-MS). The results were compared with Deming Regression and Bland-Altman plots using an online website designed by Dr. Bahar [11]. Figures were plotted in Excel for clarity, but the Deming regression slope and intercept with confidence intervals, as well as the Pearson's correlation coefficient were computed using the online application [11].

2.3. Positivity rate

The results of patient capillary samples from May 2017 to April 2018 were reviewed in order to determine the positivity rate for the LCU screen in our patient population. If the capillary sample was found to be positive by the screening algorithm (Fig. 1), then the clinician was immediately notified that the patient should return for a venous blood collection to be confirmed by ICP-MS. We qualitatively compared the positive result of the LCU assay screen to the confirmatory venous result from ICP-MS to determine the true positive and false positive rate for our screen [12,13]. We also determined the percent of patients with elevated BLLs who did not have a confirmatory venous specimen. It should be noted that the time between capillary collections to venous blood draws could range from 0 days to 2 months, but most were collected within 14 days.

2.4. Method comparison for College of American Pathologists (CAP) proficiency testing surveys

We compared the mean measurement from the LCU to ICP-MS from CAP proficiency testing surveys: 2016 BL-C, 2017 BL-A, 2017 BL-B, 2017 BL-C, 2018 BL-A. The results were compared using Deming regression and Bland-Altman plot.

3. Results

3.1. Precision

The within day and between day imprecision was < 10%, which met the desired criteria (Table 1). The largest %CV was seen in the low BLL patient sample. Unfortunately, the low BLL patient sample could not be analyzed past 24 h to assess between-day CV, per manufacturer's instructions [9].

3.2. Method comparison for assay validation

The accuracy of the LCU was compared to ICP-MS. The Deming regression showed a slope of 0.91 (95% CI: 0.87–0.94) and intercept of 1.3 µg/dL (95% CI: 0.9–1.7). The Pearson's correlation coefficient was 0.986. (Fig. 2)

The mean bias and 2 standard deviations (2SD) from the mean are listed in the plots (Fig. 3) and Table 2. Although the overall bias is 0.7 µg/dL, the bias changes from positive, zero, to negative when BLLs were below, near, or above the cut-off, respectively. Table 2 summarizes the data from the Bland-Altman plots (Fig. 3) of the different BLL measurement ranges. There is a large positive bias of 1.7 µg/dL (220%) for the LCU assay in the BLL measurement range < 5 µg/dL. ICP-MS reported 11 samples as below the assay measurement range of 1 µg/dL, and therefore BLL's of 0.5 µg/dL were used in the comparison. LCU did not report any samples less than the assay measurement range of 1.9 µg/dL during the method comparison. If the 11 results below the assay measurement range of ICP-MS were not used in the analysis, then the average mean bias would be 1.6 ± 0.6 µg/dL in the BLL range of < 5 µg/dL. The BLL range near the cut-off, 5 to 10 µg/dL, showed no mean bias between the LCU and ICP-MS assays. The BLL range > 10 µg/dL, had a slight negative mean bias of −0.8 µg/dL.

3.3. Summary of patient data collected from May 2017 to April 2018

Between the beginning of May of 2017 to the end of April of 2018 (one year of testing), 5979 patient samples were screened using the

Table 1
Within day and between day imprecision of two QC samples and a low BLL patient sample.

Sample	Concentration µg/dL	Within day %CV	Between day %CV
Low QC	8.2	4.7%	6.3%
High QC	24.7	5.1%	5.6%
Patient	2.5	7.9%	*N/A

*per the manufacturer's instructions, the patient sample was not analyzed past 24 h.

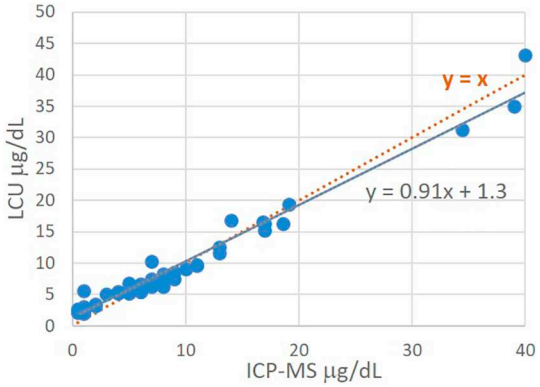

Fig. 2. Deming Regression of ICP-MS versus LCU for all BLL comparison measurements.

algorithm (Fig. 1). The percent positive rate of the LCU screen was 1% (59 samples). Of the 59 screen positive samples, 19 (32%) were confirmed positive by ICP-MS, 30 (51%) were confirmed negative by ICP-MS, and 10 (17%) did not have a confirmed result by ICP-MS. Additionally, we examined how many patient samples would screen positive by the LCU assay at a theoretical reference value of 3.5 µg/dL. A total of 243 (4.1%) had BLLs above 3.5 µg/dL by the LCU assay.

3.4. CAP proficiency testing data

CAP proficiency testing data for peer groups of LCU and ICP-MS were compared. The Deming regression had a slope of 1.0 (95% CI: 1.01–1.05) and intercept of 0.5 µg/dL (95% CI: 0.18–0.85). The Pearson's correlation coefficient was 0.999. The average mean bias seen in the Bland-Altman plot was 0.9 ± 0.7 µg/dL. For CAP samples BL-05 and BL-10 in 2017, some LCU users reported a result below the assay measurement range (< 1.9 µg/dL). However, a majority of LCU users reported a BLL concentration above 1.9 µg/dL; therefore, the higher LCU peer group mean BLL was used in the comparison between LCU and ICP-MS for those two samples (Fig. 4).

Table 2
Mean bias, standard deviation (SD) of the bias, and the absolute percent bias in the different measurement ranges.

BLL Range	Number of Samples	Mean Bias µg/dL	SD of Bias µg/dL	% Bias
1.9–65 µg/dL	75	0.7	1.5	
< 5 µg/dL	38	1.7	0.6	220%
5 to 10 µg/dL	23	0.0	1.0	13%
> 10 µg/dL	14	−0.8	2.0	11%

4. Discussion

Overall, the LCU was compared to ICP-MS to analyze BLLs in children and found to be acceptable as an initial screen for a BLL of 5 µg/dL due to the low bias in the BLL range of 5–10 µg/dL. Our data showed that the LCU does have a large positive bias in the BLL range of < 5 µg/dL (Table 2, bias of 1.7 µg/dL). A previous study from 2011 found that an earlier version of the LeadCare system had a negative bias of 0.457 µg/dL in the range from 1.4–10 µg/dL [8]. A negative bias would not be acceptable for lead measurements, since the LeadCare result would be falsely lower than an ICP-MS result, and thus more prone to false negative results near the cut-off. The positive bias implies the LCU result is, on average, higher than the ICP-MS result, and thus more likely to be a false positive (than false negative) result. Since a false

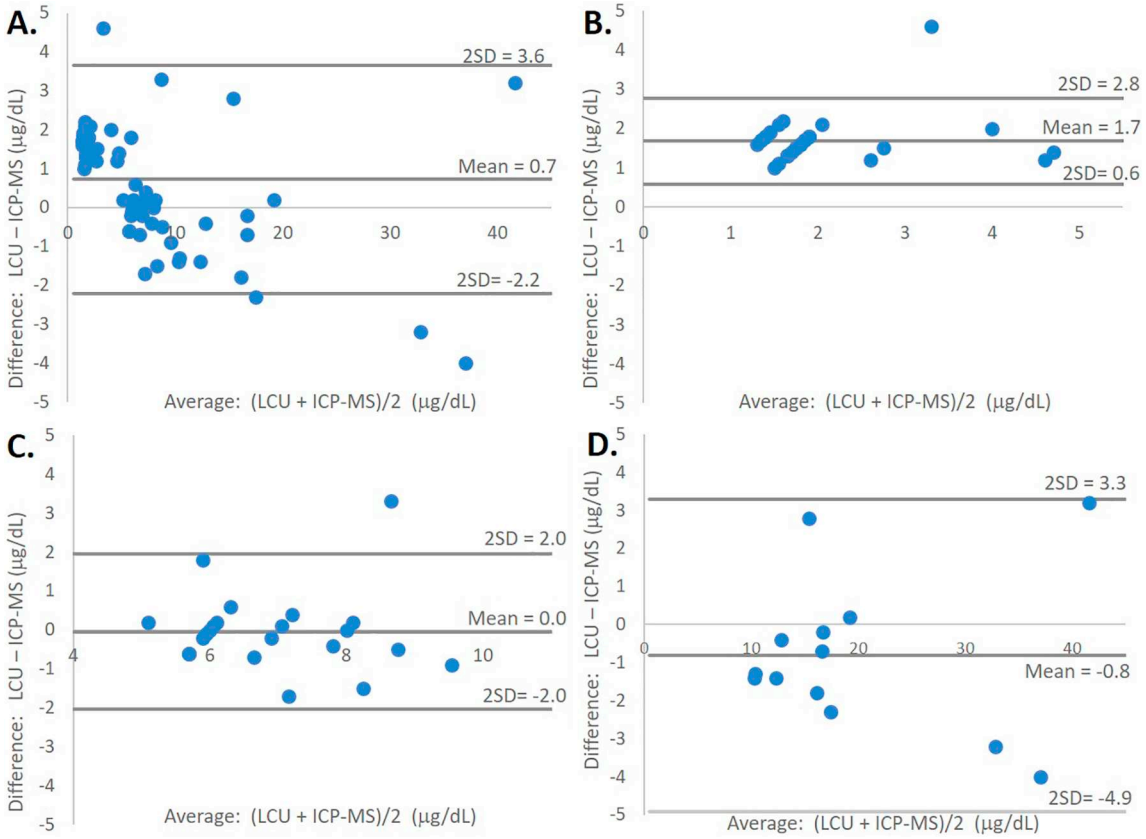

Fig. 3. Bland-Altman plots comparing LCU to ICP-MS for A.) analytical measurement range of BLL 1.9–65 µg/dL; B.) measurement range of BLL < 5 µg/dL; C.) measurement range of BLL from 5 to 10 µg/dL; and D.) measurement range of BLL > 10 µg/dL.

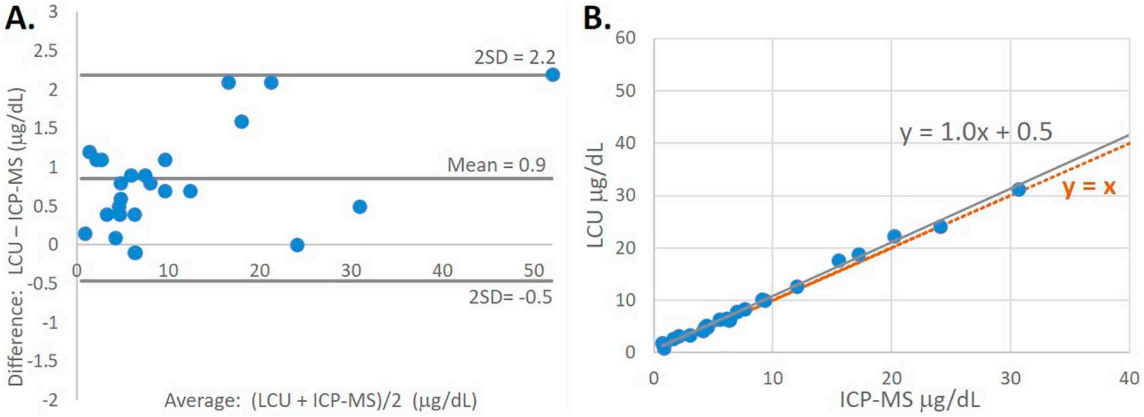

Fig. 4. Comparison of the peer group (LCU and ICP-MS) mean measurement from CAP proficiency testing surveys: 2016 BL-C, 2017 BL-A, 2017 BL-B, 2017 BL-C, and 2018 BL-A. A.) The Bland-Altman plot with mean bias and B.) Deming regression shows the comparison of peer group means.

positive result would be confirmed by a venous sample measured by ICP-MS, it is preferable for a lead screening test to have a positive rather than negative bias, to reduce the risk of missing truly elevated BLLs. However, due to the large positive bias, the LCU should not be relied upon to accurately quantify BLLs below the reference value of 5 µg/dL.

Additionally, laboratories should investigate the positivity rate and accuracy of their method for BLLs < 5 µg/dL because ACCLPP recommended that the CDC update the reference value every 4 years. Data from the 2011 to 2014 NHANES study suggests that the reference value should be lowered to 3.48 µg/dL based on the 97.5th percentile (95% CI 2.65–4.29 µg/dL) [2]. If the reference value for BLL is lowered to 3.48 µg/dL, then the LCU would still be a cost-effective screen because an estimated 4.1% of samples would need confirmation by ICP-MS (versus 1% for BLL reference value of 5 µg/dL). However, further methodology performance studies to examine the accuracy of the LCU at a BLL of 3.5 µg/dL would be needed. Given the high positive bias (Table 1, bias of 1.7 µg/dL) in the BLL range < 5 µg/dL, our screen would likely produce more false positive results and be less effective.

Unfortunately, a Letter to the Editor in *Clinical Chemistry* from 2007 [14] and a recent FDA recall in May of 2017 [15] raised the possibility that the LeadCare kits may produce falsely low BLL results. The Letter to the Editor specifically stated that a 2005 recall was due to defective reagent lots of disposable sensors which caused falsely low BLLs [14]. In 2017, the FDA sent a safety communication to warn clinicians of the possibility of falsely low BLL in venous samples, but capillary samples were not affected by the recall [15]. Recently, the FDA has described that a compound, thiuram, present in the rubber stopper of certain collection tubes is believed to cause the falsely low BLL in venous samples [16]. Therefore, it is important to be aware of interfering compounds and defective sensor lots with the anodic stripping voltammetry method; these issues did not affect GFAAS and ICP-MS.

We did not find evidence to suggest that the LCU produced falsely low results during our validation process and with our patient samples. Instead, the data supported a positive bias when comparing the LCU to ICP-MS results. This is consistent with a positive bias for LCU seen in CAP proficiency testing data, with the caveat that we cannot rule out a potential impact of non-commutability for CAP samples (Fig. 4). Both venous and capillary samples were used during our method comparison study for the validation, and we did not observe a negative bias at low BLLs in our data (Table 1). Additionally, we offered to re-test all patients with LCU results reported from a venous specimen prior to May 2017. After reviewing all of the data, we did not detect a negative bias after comparing the BLL from the venous specimen prior to May 2017 versus the capillary specimen collected after May 2017 for those patients who opted to be re-tested (data not shown).

Our patient population had a percent positivity rate of approximately 1%. Of the 1% patients with a BLL > 5 µg/dL, 32% were confirmed positive, 51% had a confirmed negative result, and 17% did not have a confirmed result. Therefore, the LCU does have a high false positive rate. This is likely due to contamination issues associated with capillary samples [12], and the positive bias of the LCU assay compared to ICP-MS. Mostly high risk children are screened for elevated BLLs at our institution [17]. The Minnesota Department of Health (MDH) lists the positivity rate for our metropolitan area to be 0.9% in 2015 [18]. Although our positivity rate for the screen is roughly similar at 1%, our true positive rate of ~0.32% is lower than that found by MDH, suggesting that we should review our screening criteria to ensure that we are testing all high risk children [17]. It is important to note that MDH receives all results, both LCU and ICP-MS. Therefore, if a patient with a positive screen elects to not return for confirmatory testing, then MDH will reach out to local public health services to contact the family and provide them with educational materials about the harmful effects of lead, with the hope that the patient will return for confirmation testing. If confirmation testing with a venous specimen shows BLL ≥ 5 µg/dL, then MDH will begin an official environmental investigation [10].

Our data shows that it is important to validate the accuracy of the LeadCare system for the desired BLL reference value and to assess the bias above and below the reference value. Overall, we found that the LCU is an effective screen for a BLL cut-off of 5 µg/dL. The assay does have a large, positive bias in the BLL range of < 5 µg/dL; however, from a risk management perspective, this is preferable to a negative bias because a false positive result triggers a venous blood collection for confirmatory testing. Users of the LeadCare system should perform a similar analysis at their desired BLL reference value. Lastly, if the CDC does lower the reference value in the future to 3.48 µg/dL based on recent NHANES data, the LCU will need improved accuracy at BLL < 5 µg/dL to avoid too many false positive results.

References

[1] Center for Disease Control, Low level lead exposure harms children: a renewed call for primary prevention. [cited 02/25/2018.] Available from: https://www.cdc.gov/nceh/lead/acclpp/final_document_030712.pdf
[2] K.L. Caldwell, et al., Measurement challenges at low blood Lead levels, Pediatrics 140 (2) (2017).
[3] Clinical and Laboratory Standards Institute (CLSI), Measurement Procedures for the Determination of Lead Concentrations in Blood and Urine, Approved Guideline-Second Edition, (2013) (CLSI document C40-A2).
[4] A. Pineau, et al., Determination of lead in whole blood: comparison of the LeadCare blood lead testing system with Zeeman longitudinal electrothermal atomic absorption spectrometry, J. Trace Elem. Med. Biol. 16 (2) (2002) 113–117.
[5] R. Gajek, F. Barley, J. She, Determination of essential and toxic metals in blood by ICP-MS with calibration in synthetic matrix, Anal. Methods 5 (2013) 2193–2202.
[6] S.M. Roda, et al., Anodic stripping voltammetry procedure modified for improved accuracy of blood lead analysis, Clin. Chem. 34 (3) (1988) 563–567.
[7] D.I. Bannon, J.J. Chisolm Jr., Anodic stripping voltammetry compared with graphite furnace atomic absorption spectrophotometry for blood lead analysis, Clin. Chem. 47 (9) (2001) 1703–1704.

[8] C. Sobin, et al., A Bland-Altman comparison of the LeadCare(R) system and inductively coupled plasma mass spectrometry for detecting low-level lead in child whole blood samples, J. Med. Toxicol. 7 (1) (2011) 24–32.
[9] Magellian Diagnostics, I, LeadCare Ultra Blood Lead Test Kit. [cited 4/22/2018], Available from www.lcultra.com.
[10] Minnesota Legislature, *144.9502, 144.9503, 144.9504 Minnesota Statutes*. Statewide Lead Surveillance System, Primary Prevention, Secondary Prevention [cited 1/2/2019], Available from https://www.revisor.mn.gov/statutes/cite/.
[11] B. Bahar, et al., An interactive website for analytical method comparison and bias estimation, Clin. Biochem. 50 (18) (2017) 1025–1029.
[12] N.H. Johnson, et al., The adequacy of capillary specimens for determining whole blood lead, Ann. Clin. Lab. Sci. 27 (3) (1997) 179–184.
[13] T.L. Schlenker, et al., Screening for pediatric lead poisoning. Comparability of simultaneously drawn capillary and venous blood samples, JAMA 271 (17) (1994) 1346–1348.
[14] R.M. Bossarte, M.J. Brown, R.L. Jones, Blood lead misclassification due to defective LeadCare blood lead testing equipment, Clin. Chem. 53 (5) (2007) 994–995.
[15] US Food & Drug, LeadCare testing systems (with blood obtained from a vein) by Magellan Diagnostics, FDA Safety Communication [cited 4/22/201], May 2017 Available from https://www.fda.gov/MedicalDevices/Safety/AlertsandNotices/ucm558733.htm.
[16] FDA, Safety Issue on Magellan Diagnostics LeadCare Testing Systems. [cited 4/22/2018], Available from https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/LabTest/ucm566188.htm.
[17] Center for Disease Control, *Sources of Lead*. [cited 1/2/2019], Available from https://www.cdc.gov/nceh/lead/tips/sources.htm.
[18] Minnesota Department of Health, Annual Blood Lead Testing: Facts & Figures, cited [4/22/2018]; Available from , 2015. https://data.web.health.state.mn.us/lead_annual_level.