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Title	Assessment of LeadCare (R) II analysis for testing of a wide range of blood lead levels in comparison with ICP-MS analysis
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Citation	Chemosphere, 271, 129832 https://doi.org/10.1016/j.chemosphere.2021.129832
Issue Date	2021-05
Doc URL	https://hdl.handle.net/2115/89538
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Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Type	journal article
File Information	CHEM79109 Revised Manuscript_changes accepte.pdf

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Abstract

The LeadCare® testing system, which utilizes anodic stripping voltammetry (ASV) methodology, has been widely used worldwide for cost-effective blood lead level (BLL) screening. However, some concerns have recently been issued regarding inaccurate results obtained using LeadCare®. Hence, we aimed to evaluate the accuracy of BLL measured by LeadCare® II (BLL_{LC}) by comparison with ICP-MS (BLL_{IM}) by the Passing–Bablok regression, Deming regression, and Bland–Altman analyses by using 994 venous blood samples. BLL_{LC} ranged from 3.3 to 162.3 μg/dL, while BLL_{IM} ranged from 0.8 to 154.8 μg/dL. Although BLL_{LC} and BLL_{IM} exhibited a strong and positive correlation, BLL_{LC} values were generally greater than BLL_{IM} values, indicative of the overestimation of the LeadCare® analysis. A large positive bias of 19.15 ± 8.26 μg/dL and 29.25 ± 14.04 μg/dL for BLL_{LC} compared with BLL_{IM} were recorded in the BLL_{LC} range of 45.0 to 64.9 μg/dL and for ≥65.0 μg/dL, respectively. In contrast, a bias of ≤0.3 μg/dL was observed at a BLL_{LC} of less than 10.0 μg/dL. Blood copper, cadmium, and iron levels did not exhibit an effect on the bias of BLL_{LC}, indicative of the minimal potential interferences of the metals; these interferences are a cause for concern with the ASV method. In conclusion, LeadCare® analysis is thought to be a good tool for screening purposes at a lower BLL around the reference level of 5 μg/dL in the initial stage; however, conversion or retesting using a laboratory analyzer is recommended at a higher BLL for appropriate clinical evaluation and research.

Keywords:

LeadCare, ICP-MS, bias, Bland–Altman analysis, Passing–Bablok regression, Deming regression

1. Introduction

Environmental exposure to lead (Pb) still remains a public health concern. Pb poisoning occurs because of the current anthropogenic sources and historic air Pb emissions, including those of gasoline and from industries and mining operations (ATSDR, 2010). The Pb concentration of the bone reflects long-term exposure and body burden, while the blood Pb level (BLL) reflects more recent exposure. BLL is currently used widely as an indicator of Pb exposure (Barbosa et al., 2005). Although the Center for Disease Control and Prevention (CDC) stipulated an action level of 60 μg/dL for BLL in the 1960s, the action level for BLL has dramatically and gradually decreased. In 2012, the CDC has issued new guidelines for assessing children's BLL with its reference level of 5 μg/dL (CDC, 2012). On the basis of scientific evidence, chelation therapy is currently highly recommended at a BLL of greater than or equal to 45 μg/dL (CDC, 2002; Needleman, 2004).

Because of the demand for a rapid and inexpensive method for screening and monitoring BLL, some early instruments that utilize anodic stripping voltammetry (ASV) have been developed. However, potential interference by copper (Cu) was a major concern since it may affect the BLL result from the ASV method (Roda et al., 1988). In a recent review by Borrill et al. (2019), interferences of intermetallic compounds on solid electrodes are problematic because preconcentration occurs only on the electrode surface where interactions are likely to occur between different metals. Cadmium (Cd)–Pb (Zhao and Liu, 2018) and iron (Fe)–Pb (Chau and Lum-Shue-Chan, 1974) are additional examples of problematic intermetallic compounds at solid electrodes. Currently, only LeadCare® testing systems from Magellan Diagnostics Inc. (North Billerica, MA, USA) are commercially available and widely utilized in developed countries (Green et al., 2017; Sobin et al., 2011) and developing countries (Dooyema et al., 2011; Safi et al., 2019; Yabe et al., 2020). However, recently, the United States Food and Drug Administration

(FDA) has issued a Class I recall, the most serious type, for the LeadCare® testing system (US FDA, 2017; 2018a). The US FDA has warned that LeadCare® testing systems may underestimate BLL for the processing of venous blood samples. In addition, in late 2018, the US FDA has concluded that there is a significant chance of obtaining incorrect results by using the LeadCare® system in cases where venous blood is collected in certain blood tubes containing a chemical called thiuram (US FDA, 2018b). The suspected tubes manufactured by Becton Dickinson & Company (NJ, USA) include BD Vacutainer® Lithium Heparin Green Top, which was used in our study before the FDA announcement. Thiuram in the rubber stopper of the tube can release reactive gases, carbon disulfide (CS_2), and carbonyl sulfide which can dissolve into the blood and tightly bind to Pb particles. Similarly, the CDC also has issued concerns regarding the inaccurate results obtained by using the LeadCare® instrument (CDC, 2018). Taken together, these indicate that careful consideration and further scientific investigation of the LeadCare® analyzer's validity are required.

To the best of our knowledge, only two studies comparing BLL measured using the LeadCare® analyzer (BLL_{LC}) and inductively coupled plasma–mass spectrometry (ICP–MS, BLL_{IM}) at around the reference value of 5 μg/dL have been reported (Johnson et al., 2019; Sobin et al., 2011). In those studies, which were conducted in the laboratory or a primary school in the USA, the LeadCare® analyzer was deemed to provide an acceptable screening result. For the field study, Neri et al. (2014) reported that the dilution method using human blood, which is verified to have a BLL below the lower detection limit of 3.3 μg/dL for the LeadCare® II analyzer, can give an adequate result for BLL greater than 65 μg/dL, corresponding to the upper detection limit of LeadCare® II. In addition, in the same study, the BLL_{LC} consistency overestimated BLL_{IM} by twofold or greater, and the concordance correlation coefficient was quite low (0.423) when blood was diluted with saline. However, a study investigating the suitability of the LeadCare®

system for field analysis in a wide range of BLL values has not been reported thus far. Moreover, there is a high demand for a portable instrument for rapid analysis, such as LeadCare® testing systems, especially in rural areas of developing countries with limited applicable resources.

As a core mining area, Kabwe Town in the Republic of Zambia has been in operation for almost a century. Despite the closure of the mine in 1994, alarming concentrations of Pb have been reported in the environment (Nakayama et al., 2011) and animals (Doya et al., 2020; Nakata et al., 2016; Toyomaki et al., 2020; Yabe et al., 2013). Recently, our study investigating human BLL_{LC} reported an increased BLL in Kabwe in the range of 1.65 to 162 μg/dL for 1190 participants, although the dilution was performed using hydrochloric acid (HCl) and not uncontaminated blood. The results below the lower detection limit of 3.3 μg/dL were adjusted to half their value of 1.65 μg/dL (Yabe et al., 2020). A similar elevated trend of BLL_{IM} in the range of 0.79 to 154.75 μg/dL was also reported for 504 representatives from Kabwe (Nakata et al., 2020).

From the above discussion, LeadCare® II analysis was compared with ICP–MS analysis in terms of validity in this study. To obtain a better picture, the maximum available number of samples collected in Kabwe was compared, whereas some blood samples were excluded from the analysis of two earlier studies (Nakata et al., 2020; Yabe et al., 2020) because of the research design and limited budget for further clinical assessment. As LeadCare® series demonstrates immense potential for field analysis due to its unique and convenient characteristics, the evaluation in this study is significant for the monitoring and control of global Pb pollution.

2. Methods

2.1. Venous blood sample collection

The study was approved by the University of Zambia Research Ethics Committee (UNZAREC; ref. no. 012-04-16) and the Ministry of Health through the Zambia National Health Research Ethics Committee and the Kabwe District Medical Office. After two-stage random selection to capture the representative data of this area, blood sampling was performed in Kabwe in July and August 2017. Geographical information including the distance between the mine and each sampling point are shown in Supplementary Figure S1. The details of sample selection were described in a recent paper (Hiwatari et al., 2019). For blood collection in clinics, all collection items were placed in plastic Ziploc® storage bags until use to avoid contamination as described in a previous study (Nakata et al., 2020). After the cleaning and wiping of the collection site on the arm with alcohol swabs to eliminate environmental contaminants, blood was collected from the cubital vein. The collected samples were then immediately subjected to LeadCare II (Magellan Diagnostics, North Billerica, MA, USA) analysis as described below. Additionally, 200 μL of blood were separated into 1.5 mL plastic tubes immediately after blood collection for metal extraction and ICP-MS analysis. The separated blood samples and the remainder of the blood samples were stored at −20°C until transportation. After the Material Transfer Agreement (MTA) was granted by the Zambian Ministry of Health through the National Health Research Ethics Committee (approval no. E03618), the samples were packed in cooler boxes and transported to the Laboratory of Toxicology, Faculty of Veterinary Medicine, Hokkaido University, Japan, for laboratory analysis by ICP–MS.

2.2. LeadCare II analysis

Analysis was performed according to the manufacturer's instructions and as recently described (Yabe et al., 2020). In brief, 50 µL of venous blood from a heparinized tube (BD Vacutainer® Lithium Heparin Green Top (Becton, Dickinson and Company, Franklin Lakes, NJ, USA)) was transferred into a vial containing the LeadCare II treatment reagent (250 µL of 0.1% HCl) for hemolysis and for the release of Pb into the solution. The mixed solution was then applied onto the electrochemical sensor for single analysis. For quality assurance, the instrument was calibrated using a calibration probe assigned to a specific reagent kit box (48 tests) by the manufacturer. In addition, analyses of standard control reagents supplied by the manufacturer were performed as per the manufacturer's instruction to confirm the accuracy. Those samples with a BLL_{LC} above the detection limit of 65 µg/dL were diluted for reanalysis. Next, 50 µL of blood was added into 100 µL of 0.1% HCl for three times dilution. Subsequently, 50 µL of the mixed solution was transferred into a vial in the same way as that performed for undiluted blood.

2.3. Blood digestion and metal extraction

Blood digestion and metal extraction were performed as described recently (Nakata et al., 2020). First, 200 µL of whole blood was digested with 5 mL of twofold diluted ultrapure nitric acid (Cica reagent, specific gravity of 1.38, 60%; Kanto Chemical Corp., Tokyo, Japan) and 1 mL of ultrapure hydrogen peroxide (Cica reagent, 30%; Kanto Chemical Corp.) using a microwave digestion system (Speed Wave MWS-2; Berghof, Eningen, Germany). The extracted solutions were then transferred into 15 mL plastic tubes and diluted to a final volume of 10 mL with double-distilled and deionized water (Milli-Q; Millipore, Bedford, MA). Supplementary Table S1 summarizes the detailed heating program of the microwave digestion system.

2.4. Blood Pb, Cu, Cd, and Fe analysis using ICP–MS

153 Levels of BLL_{IM} as well as blood Cu (BCuL), Cd (BCdL), and Fe (BFL) were
154 determined by ICP–MS (7700 series, Agilent Technologies, Tokyo, Japan) as reported
155 by Nakata et al. (2020). Supplementary Table S2 summarizes the detailed operating
156 conditions. Analytical quality control was performed using the certified reference
157 material of Seronorm™ Trace Elements Whole Blood L-2 (Sero, Billingstad, Norway).
158 Replicate analysis of these reference materials revealed good accuracy (relative standard
159 deviation (RSD) of less than 3%) and recoveries (95–105%). The instrument detection
160 limit was 0.001 µg/L for all the targeted metals. The limits of detection (LOD) of the
161 extracted sample analysis were 0.048, 0.024, 0.008, and 11.65 ug/L for Pb, Cu, Cd, and
162 Fe, respectively.
163
164 **2.5. Statistical analysis**
165 JMP Pro version 14 (SAS Institute, NC, USA) was employed for all statistical
166 analyses, except the Passing–Bablok regression analysis, which was carried out using
167 Analyze-it Method Validation Edition version 5.65.3 (Analyze-it Software, Ltd., Leeds,
168 UK). Following lack of normality in the BLL data distribution based on the Shapiro-Wilk
169 test, the data were log-transformed. The log-transformed data fitted a normal distribution
170 for statistical analysis as was confirmed by the Shapiro-Wilk test. However, the results
171 for the actual and log-transformed numbers were recorded in this study for easy
172 comparison with other studies. Those samples with a BLL_{LC} below the detection limit of
173 3.3 µg/dL were excluded from the analysis and comparison. The Passing–Bablok
174 regression analysis and Deming regression analysis were performed to assess the
175 correlation between BLL_{LC} and BLL_{IM} in real and log-transformed data, respectively. In
176 addition, Bland–Altman tests were conducted to evaluate comparability across BLL
177 measurement methods. The Pearson correlation coefficient was utilized to assess the
178 relationship of log-transformed BCuL with log-transformed BLL_{LC}, BLL_{IM}, and the bias

179 (log-transformed BLL_{LC} − log-transformed BLL_{IM}); log-transformed BCdL with log-
180 transformed BLL_{LC}, BLL_{IM}, and the bias; as well as log-transformed BFL with log-
181 transformed BLL_{LC}, BLL_{IM}, and the bias. Statistical analyses were performed at a
182 significance level of 0.05 ($p < 0.05$).
183

3. Results

3.1. Overall trend of BLL_{LC} and BLL_{IM}

In total, 1208 venous blood samples were analyzed using the LeadCare II analyzer and ICP–MS. Supplementary Table S3 provides the characteristics of the studied population by area. Among 1208 blood samples, 214 samples (17.7%) exhibited a BLL_{LC} below the detection limit of 3.3 μg/dL; hence, these samples were excluded from the study, and the remaining 994 samples were used for data analysis and comparison. Table 1 summarizes the statistical distribution of the obtained BLL_{LC} and BLL_{IM}, and Figure 1 shows the corresponding analysis using the box-and-whisker plot. The BLL_{LC} ranged from its lower detection limit of 3.3 to 162.3 μg/dL with a mean ± standard deviation (SD) of 25.9 ± 21.8 μg/dL. In contrast, the range and mean ± SD of BLL_{IM} were 0.8 to 154.8 μg/dL and 18.1 ± 14.8 μg/dL, respectively. The BLL_{LC} values were greater than the BLL_{IM} values for all descriptive statistical values, including the mean, 95% confidence interval (CI), SD, and minimum and maximum values, as well as percentiles.

3.2. Comparability between BLL_{LC} and BLL_{IM}

Strong and positive correlations between BLL_{LC} and BLL_{IM}, as well as between $\log BLL_{LC}$ and $\log BLL_{IM}$, with correlation coefficients (r^2) of 0.904 and 0.903, respectively, were observed (Figure 2, Supplementary Figure S2, Supplementary Figure S3). A regression-line slope of <1 indicated that BLL_{LC} is generally greater than BLL_{IM}. The Bland–Altman analysis was performed to assess the bias of BLL_{LC} as compared with BLL_{IM}, as well as that of $\log BLL_{LC}$ against $\log BLL_{IM}$ (Figure 3). Overall, the mean bias of BLL_{LC} was 7.76 μg/dL, with a CI of 7.103 to 8.412 μg/dL. The lower and upper limits of agreement were −12.87 and 28.39 μg/dL, respectively. The increasing tendency of bias at a higher Pb level was observed rather than constant bias. Compared with the log-transformed data, $\log BLL_{LC}$ exhibited a positive mean bias of 0.129 with a CI of 0.119 to

210 0.140. The lower and upper limit of agreement were −0.207 and 0.465, respectively.
211 Table 2 summarizes the mean and SD of the bias in the different BLL_{LC} range groups
212 calculated by the Bland–Altman analysis. Compared with that observed for BLL_{IM}, large
213 positive biases of 19.15 ± 8.26 μg/dL and 29.25 ± 14.04 μg/dL for BLL_{LC} were recorded
214 in the range of 45.0–64.9 μg/dL and at ≥65.0 μg/dL, respectively. On the other hand, the
215 bias was within 0.3 μg/dL in the ranges of 3.3–4.9 μg/dL and 5.0–9.9 μg/dL. Generally,
216 the higher was the BLL_{LC}, the higher was the bias.

3.3. Distribution of BCuL, BCdL, and BFL, as well as the associations with BLL_{LC} and BLL_{IM}

Table 1 summarizes the BCuL, BCdL, and BFL distributions. The recorded BCuL ranged from 0.27 to 3.06 mg/L, with a mean of 1.15 ± 0.24 mg/L. The comparison of the log-transformed values for BLL_{LC} and BLL_{IM} revealed significant positive associations ($p < 0.0001$ and $p < 0.0001$, respectively) for BCuL despite the low r^2 values of 0.13 and 0.17, respectively (Table 3). On the other hand, a statistical relationship was not observed between log BCuL and the BLL measurement bias ($p = 0.06$). BCdL and BFL were within the range of 0.02 to 2.27 µg/L and 0.07 to 0.91 mg/mL, with mean values of 0.25 ± 0.24 µg/L and 0.47 ± 0.08 mg/mL, respectively. Compared with the BLL measurement bias, BCdL did not exhibit a statistically significant association ($p = 0.43$). The significant relationship between BLL measurement bias and BFL ($p < 0.01$) was observed albeit the low r^2 value of -0.09.

4. Discussion

A portable LeadCare® testing system has been widely considered as a good tool for screening the Pb exposure level because of its convenient characteristics. However, the US FDA and CDC have expressed concerns related to the possible inaccurate results of BLL_{LC} (CDC 2018; US FDA, 2017; 2018a; 2018b). Even before these reports, Bossarte et al. (2007) raised concerns that LeadCare may provide a falsely low BLL result. On the basis of this concern, several earlier studies were carried out to assess the suitability of LeadCare® analysis compared with a laboratory metal analyzer such as ICP–MS in a specific range of BLL. In this study, the comparison between the 994 pairs of BLL_{LC} and BLL_{IM} was done with a wide range of BLL and a larger sample size as compared with previous investigations to further verify the validity of LeadCare® II analysis. For this purpose, three statistical comparative methods were applied although the Passing-Bablok regression does not account for random variation between the two analytical methods.

Our results revealed a good correlation between BLL_{LC} and BLL_{IM} when compared in the overall BLL_{LC} range of 3.3 to 162.3 µg/dL. In contrast, the overestimation of LeadCare® measurement was indicated by regression analyses and Bland–Altman analysis, with an overall positive bias of 7.76 µg/dL. However, the comparison of different BLL_{LC} ranges revealed a small bias in the BLL_{LC} ranges of 3.3–4.9 µg/dL and 5.0–9.9 µg/dL, indicating the validity of BLL_{LC} in a lower BLL range. This trend is similar to that previously reported for the bias of mainly around 5 µg/dL for children's BLL (Johnson et al., 2019; Sobin et al., 2011). In addition, a small positive bias of 0.45 µg/dL was reported for a Pb-exposed employee's BLL of less than 10 µg/dL upon comparison of the LeadCare® instrument with graphite furnace atomic absorption spectrometry (GFAAS) (Taylor et al., 2001). For fresh blood samples of the Scandinavian brown bear (*Ursus arctos*), the BLL_{IM} of which ranged from 3.3 to 17.3 µg/dL, a bias of 0.225 in log-transformed data the real number of which is described by the unit µg/L

257 (equivalent to 0.17 µg/dL for a real number) was recently recorded (Boesen et al., 2019).
258 As a BLL of 5 µg/dL is considered as a reference value (CDC, 2012), the LeadCare®
259 analyzer can be considered as a good tool for primary screening. In the BLL_{LC} range of
260 10.0–19.9 µg/dL, a higher positive bias of 2.84 µg/dL was detected, while a slight
261 negative bias (−0.8 µg/dL) was previously reported for the samples for which BLL mostly
262 ranged from 10 to 20 µg/dL (Johnson et al., 2019).

263 On the other hand, a strong positive bias were detected at a higher BLL_{LC} range
264 of >20 µg/dL. A previous study investigating the suitability of the LeadCare® analyzer
265 for raptor venous blood reported an adverse bias of −1.12 µg/dL for blood samples for
266 which BLL_{IM} ranged from 10 to 80 µg/dL (González et al., 2019). Pineau et al. (2002)
267 performed recovery tests using pooled blood (3.68 ± 0.21 µg/dL) and additive blood, the
268 BLL of which is 22 µg/dL. The LeadCare® analyzer returned a mean value of 27.1 ± 1.8
269 µg/dL, in contrast to that of 24.9 ± 0.35 µg/dL by GFAAS and indicative of 2.2 µg/dL
270 overestimation of LeadCare analysis as compared with GFAAS. Compared with earlier
271 observations, a higher bias was observed herein for the BLL_{LC} range of 20.0 to 44.9 µg/dL
272 and between 45.0 and 64.9 µg/dL. As chelation therapy is recommended at a BLL of
273 ≥45 µg/dL (CDC, 2002; Needleman, 2004), the observed large bias at around the
274 threshold for treatment would be crucial. Although the bias did not exhibit a statistically
275 significant relationship with BCuL, BCdL, and BFL, one of the possible reasons for this
276 large bias is the interference of intermetallic compounds, which are concerns reported
277 previously (Borrill et al., 2019; Chau and Lum-Shue-Chan, 1974; Zhao and Liu, 2018).
278 In addition to Cu, Cd, and Fe, which were verified in this study, the elevated concentration
279 of some metals (such as cobalt, nickel, and chromium) and a metalloid (arsenic) in the
280 environment (Nakayama et al., 2011) and animals (Doya et al., 2020; Nakata et al., 2016;
281 Toyomaki et al., 2020; Yabe et al., 2013) has been previously reported for the examined
282 area of Kabwe. Borrill et al. (2019) reported that Pb speciation is another factor that

should be carefully considered. To the best of our knowledge, data have not been reported about Pb speciation in humans or any other animals in Kabwe. Cerussite ($PbCO_3$) and anglesite ($PbSO_4$) are the two main crystalline states of Pb in leached residues from the Kabwe mine (Silwamba et al., 2020a; 2020b). Further investigation should be performed to answer this inexplicable large bias.

At a BLL of greater than 65 µg/dL, at which blood samples need to be diluted for LeadCare® measurement because of the exceeding upper detection limit, the mean bias was extremely high (29.25 µg/dL). A limitation of this study was possibly the dilution medium used for readings that were above the detection limit. In this study, use of the LeadCare® II treatment reagent of 0.1% HCl for dilution was done instead of the recommended dilution protocol using "unpolluted blood" (Neri et al., 2014) because of the practical challenge in the investigated area considerably contaminated with Pb.

BCuL, BCdL, and BFL exhibited significant positive relationships with BLL_{LC} and BLL_{IM}, whereas correlation coefficients were small. This result is in agreement with the elevated Cu, Cd, and Fe levels in soil (Nakayama et al., 2011) and animals (Doya et al., 2020; Nakata et al., 2016; Toyomaki et al., 2020; Yabe et al., 2013) around the mine area, along with that observed for Pb. However, the recorded range of BCuL was comparable to the formerly reported normal values of 1.4 ± 0.32 and 1.5 ± 0.38 mg/L for men and women, respectively, with ages between 46 to 60 years (Kazi et al., 2008). This result could be explained by the function of Cu in homeostasis in the body; it is an essential metal for the human body (Araya et al., 2006). On the other hand, the comparison with previously reported normal values of BFL for men (0.71 ± 0.05 mg/mL) and women (0.70 ± 0.06 mg/mL) suggested that the observed BFL in this study is relatively lower (Kazi et al., 2008). In contrast to the correlation with BLL_{LC} and BLL_{IM}, the associations of BCuL, BCdL, and BFL with the bias of BLL_{LC} against BLL_{IM} were not significant. On the other hand, intermetallic interference has been suggested as one of

309 the potential factors that may affect the BLL result from the ASV method (Borrill et al.,
310 2019; Chau and Lum-Shue-Chan, 1974; Roda et al., 1988; Zhao and Liu, 2018).
311 Interferences such as Cu, Cd, or Fe were not observed in this study using the modern
312 LeadCare® testing system.

313 A recent FDA recall suggested the potential falsely underestimated BLL in
314 venous blood when using the LeadCare® analyzer (FDA, 2017). In addition, the FDA
315 again reported that a chemical compound of thiuram in the rubber stopper of certain blood
316 collection tubes can result in a negative bias for the result (FDA, 2018b). Unfortunately,
317 as these suspected tubes are common, they were used in this study before the FDA
318 announcement. With respect to specimen collection, it was not practically possible to
319 collect blood samples twice from one person, one from a capillary by using the pricking
320 method for LeadCare® analysis only, and the other from the vein using a blood collection
321 needle for ICP–MS analysis and subsequent laboratory analysis, because of ethical
322 reasons and limited field capacity. Because this study was not originally designed to
323 assess the potential falsely low Pb levels due to thiuram as pointed out by the FDA, a
324 clear answer was unfortunately not obtained although the observed bias was found to be
325 generally positive.

326 However, it should be emphasized that the results obtained herein suggest that
327 the LeadCare® testing system is appropriate and acceptable for screening with a BLL
328 cut-off of 5 μg/dL, as recently concluded (Johnson et al., 2019). At higher range, greater
329 than 10 μg/dL, converting from BLL_{LC} to BLL_{IM} or simple retesting using a laboratory
330 metal analyzer such as ICP–MS for data confirmation would be recommended as
331 proposed before (Boesen et al., 2019; Sobin et al., 2011), although a positive bias should
332 be preferred than a negative bias in terms of risk management. Our results are also in
333 agreement with the suggestion of Sobin et al. (2011) that LeadCare® measurement is not
334 recommended for investigating the threshold for health effects or any other associated

335 factors. In contrast, the statistically strong positive association between BLL_{LC} and BLL_{IM}
336 indicates that the LeadCare® system is applicable to the assessment of concentration-
337 dependent effect of Pb on the associated factors. In addition, LeadCare® analysis is
338 beneficial to determining the pollution status in the initial stage in the field.
339
340 **5. Limitations**
341 There are some limitations in the current study. First, we used the LeadCare® II
342 treatment reagent of 0.1% HCl for dilution of blood samples whose BLL_{LC} at initial
343 measurement were above the detection limit of 65 µg/dL. This is because it was
344 realistically difficult to follow the recommended dilution protocols using "unpolluted
345 blood" (Neri et al., 2014) due to the capacity issue. Second, venous blood samples were
346 used for LeadCare® measurement instead of capillary blood because of the ethical
347 reasons and limited capacity in the field. The venous blood samples may have caused an
348 underestimation of BLL measured by the LeadCare® instrument as the US FDA
349 suggested (US FDA, 2017). Finally, the blood collection tubes with thiuram-contained
350 rubber stoppers were used for venous blood collection. The US FDA reported that there
351 is a possibility of obtaining inaccurate results of BLL because thiuram can release
352 chemical compounds which can interfere with Pb particles (US FDA, 2018b).
353
354 **6. Conclusions**
355 A possibility of underestimation or overestimation of the BLL following
356 LeadCare® analysis exists. The observed bias differed considerably between the low and
357 high concentration ranges. Our results suggest that LeadCare® analysis is a good
358 screening method for Pb exposure at a lower BLL at around the current reference level of
359 5 µg/dL. However, conversion or retesting using laboratory analyzers, such as ICP–MS,
360 is recommended at a higher BLL of greater than 10 µg/dL for the adequate clinical

evaluation of exposure status, albeit the good correlations between BLL_{LC} and BLL_{IM} even at ≥10 μg/dL. Moreover, the use of the LeadCare® instrument to determine the threshold for any factor should be carefully considered because of the analytical bias with ICP–MS.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan awarded to M. Ishizuka (No. 16H01779, 18K19847, and 18KK0287), Y. Ikenaka (No. 17K20038, 18H04132), S.M.M. Nakayama (No. 17KK0009, 20K20633), and H. Nakata (No. 19K20472) and the foundation of JSPS Bilateral Open Partnership Joint Research Projects (JPJSBP120209902). We also acknowledge financial support from The Japan Prize Foundation and The Akiyama Life Science Foundation. This research was also supported by JST/JICA SATREPS (Science and Technology Research Partnership for Sustainable Development; No. JPMJSA1501), JST aXis (Accelerating Social Implementation for SDGs Achievement No. JPMJAS2001) and Program for supporting introduction of the new sharing system (JPMXS0420100619). We are highly indebted to the communities that participated in the study. We are also grateful to the health centers in Kabwe, the Kabwe District Health Office, Kabwe Municipal Council and the Ministry of Health, Zambia, for facilitating our work. We also want to thank all the laboratory technicians and nurses at the health centers for their technical support. Our appreciation is extended to Hisao Odagiri, Michiko Nyanga and Susumu Makino for their coordination, Sayako Yamamoto for her assistance as well as Takahiro Ichise and Nagisa Hirano for their technical support.

Competing Interests Statement

The authors declare no competing interests.

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Figure legends

Figure 1. Box-and-whisker plot of BLL_{LC} and BLL_{IM}. Percentile lines represent 0, 0.5, 2.5, 10, 25, 50, 75, 90, 97.5, and 99.5% percentiles from the bottom to top. Parts of BLL_{LC} (Yabe et al., 2020) and BLL_{IM} (Nakata et al., 2020) data were reported in earlier papers and cited.

Figure 2. Passing–Bablok regression analysis between BLL_{LC} and BLL_{IM} (A), as well as Deming regression analysis between log BLL_{LC} and log BLL_{IM} (B). Parts of BLL_{LC} (Yabe et al., 2020) and BLL_{IM} (Nakata et al., 2020) data were reported in earlier papers and cited.

Figure 3. Bland–Altman plot of differences between BLL_{LC} and BLL_{IM} (left) and between log BLL_{LC} and log BLL_{IM} (right). Mean difference is indicated by a red line, with a 95% confidence interval indicated by the red dotted line. Black lines show upper and lower limits of agreement of Bland–Altman analysis (±1.96 × standard deviation). Blue = sample whose BLL_{LC} ranges from 3.3 to 4.9, light green = sample whose BLL_{LC} ranges from 5.0 to 9.9, orange = sample whose BLL_{LC} ranges from 10.0 to 19.9, pink = sample whose BLL_{LC} ranges from 20.0 to 44.9, red = sample whose BLL_{LC} ranges from 45.0 to 64.9, and black = sample whose BLL_{LC} is ≥65.0.

Table list

Table 1. Statistical distributions of BLL_{LC}, BLL_{IM}, BCuL, BCdL, and BFL.

Table 2. Bias of BLL_{LC} relative to BLL_{IM} calculated using the Bland–Altman analysis, as well as the regression equation with a correlation coefficient calculated using the Passing–Bablok regression analysis.

608 Table 3. Pearson's coefficients of correlation (r^2) between BCuL with BLL_{LC}, BLL_{IM},
609 and the bias; BCdL with BLL_{LC}, BLL_{IM}, and the bias; and BFL with BLL_{LC}, BLL_{IM}, and
610 the bias.

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