Evaluation of a Second-Generation Portable Blood Lead Analyzer in an Occupational Setting

Noel V. Stanton, MS* and Tracy Fritsch, BS

Background *A new blood lead testing instrument has qualities that make the instrument attractive for on-site testing of occupational lead exposures. This study evaluated the accuracy of the instrument when used in a manufacturing setting, and examined the impact of blood storage and shipment on results.*

Methods *Venous blood specimens (n = 121) were obtained and immediately analyzed on-site using the new instrument. They were then shipped to a reference laboratory and analyzed using electro-thermal atomization atomic absorption spectrometry (ETAAS), and retested using the new instrument.*

Results *The cohort blood lead concentration averaged 40.1 μg/dl. Results obtained on the new analyzer with freshly collected blood averaged 38.7 μg/dl. The mean difference of 1.2 μg/dl on paired samples was not statistically significant. Following blood shipment and storage, results on the analyzer increased to an average of 42.4 μg/dl. The mean increase of 3.0 μg/dl on stored blood samples also failed to reach statistical significance. Under OSHA proficiency test acceptability requirements, 94% of the results had satisfactory agreement.*

Conclusions *The new analyzer might be a useful tool for on-site monitoring of occupational lead exposures. The manufacturer's instructions should be adhered to with respect to specimen age and storage requirements.* Am. J. Ind. Med. 2007

KEY WORDS: blood lead analysis; occupational lead exposure; instrument evaluation; on-site testing

INTRODUCTION

Adverse health effects associated with occupational lead exposure were first identified many years ago, and continue to be investigated and characterized today. A recent meta-analysis of studies examining the association between lead exposure and cardiovascular impacts concluded that there is a causal relationship between lead and hypertension, and that the data suggest a similar relationship with clinical outcomes, for example, coronary heart disease, stroke mortality, and peripheral artery disease [Navas-Acien et al., 2007]. A similar evaluation of 21 studies conducted after 1995 concluded that moderate evidence exists for an association between psychiatric symptoms and lead dose [Shih et al., 2007]. Overt effects on the central nervous system, renal, and hematopoietic systems from higher blood lead concentrations have been characterized previously.

Undue exposure to lead in occupational settings is tracked by the Adult Blood Lead Epidemiology and Surveillance (ABLES) program. The ABLES program has found undue lead exposures to be most frequent in the manufacture of storage batteries, painting and redecorating, and mining and smelting industries [CDC, 2006]. Based on evidence available at the time, the Occupational Safety and Health Administration (OSHA) developed lead exposure standards for general industry in 1978 [Federal Register,

Wisconsin State Laboratory of Hygiene, Toxicology Section, University of Wisconsin-Madison, Madison, Wisconsin

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*Correspondence to: Noel V. Stanton, Wisconsin State Laboratory of Hygiene, Toxicology Section, University of Wisconsin-Madison, PO Box 7996, Madison, WI 53707-7996.
E-mail: nvstox@mail.slh.wisc.edu

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1978] and for the construction industry in 1993 [Federal Register, 1993]. These standards require the monitoring of blood lead concentrations in workers with potential exposures, with several action thresholds beginning at blood lead concentrations exceeding 40 μg/dl (1.93 μmol/L).[1] The action thresholds have been challenged based on more recent data, and reviews have suggested that they be lowered to 10–20 μg/dl [Landrigan et al., 1990; Silbergeld et al., 1991; Kosnett et al., 2007].

Occupational monitoring frequently involves on-site blood collection, followed by shipment to a reference laboratory for testing. Laboratories testing worker blood are required to obtain approval by the OSHA. This approval is based exclusively on proficiency testing (PT) performance in a program recognized by OSHA. The OSHA PT requirements state that over a 1 year period testing laboratories must obtain results within ±6 μg/dl or 15% of the target value for ±95% of the individual PT specimens. Additionally, all laboratories performing blood lead testing on U.S. citizens, including workers, are subject to certification under the Clinical Laboratory Improvement Amendments (CLIA) of 1988. CLIA regulations also require successful PT participation, with tighter acceptability criteria of ±4 μg/dl or 10% for individual PT specimens and 80% sample acceptability for test events. In contrast to the OSHA approval process which relies exclusively on PT, CLIA also requires that many other general laboratory requirements be met.

LeadCare® Blood Lead Instruments

A portable blood lead testing instrument, the LeadCare® analyzer (ESA Biosciences, Inc., Chelmsford, MA), was introduced in 1997, and has features that allow testing in non-specialized laboratory environments. Studies have demonstrated the suitability of this instrument for testing both occupationally exposed adults [Taylor et al., 2001] and children [Shannon and Rifai, 1997]. A second-generation instrument, LeadCare II® was granted 510(k) market clearance by the Food and Drug Administration (FDA) in September 2006. Like the earlier instrument, the LeadCare II measures lead by anodic stripping voltammetry. Blood is mixed with a reagent, and a portion dispensed onto single use disposable electrodes (sensors) where plating and subsequent stripping of lead takes place. Both instruments are portable, and can be operated with batteries or an AC adapter. Sensor-specific calibration information is stored on a calibration ''button'' that is placed against a port on the instrument, eliminating a requirement for user calibration. Materials needed for testing, including sampling capillaries, the calibration button, treatment reagent, transfer droppers (pipette), and sensors, are provided in a kit supplied by the manufacturer. Another similarity between the two instruments is that blood must be tested within 24 hr of collection and stored at ambient temperature to ensure proper measurement.

However, several important characteristics of the LeadCare II differ from the earlier analyzer. Volumetric capillary tubes are provided that eliminate the need for quantitative measurement of blood by users. Stepwise instructions and sensor calibration lot information are displayed on a small screen, and the testing process begins automatically following proper application of the blood-reagent mixture to the sensor. These characteristics led to the designation of waived status under CLIA, conferring a waiver of PT and many other general CLIA requirements attendant with all other blood lead test methods. This status allows blood lead testing to expand into a variety of new non-laboratory settings, including the on-site occupational monitoring of workers. A concern of this application is that ubiquitous lead in workplace environments adds an increased risk of contamination during sampling and analysis, with consequent falsely elevated results.

In this study, we evaluate the performance of the LeadCare II (the portable analyzer) in a storage battery manufacturing facility, and also examine the impact of shipment and storage of blood specimens on performance of the instrument.

MATERIALS AND METHODS

Study Configuration

The industrial partner participating in this study and performing the on-site analyzer testing was a North American storage battery manufacturing plant. The site was identified through a commercial laboratory performing clinical blood lead testing for the plant. The reference blood lead and secondary analyzer testing were performed at the Wisconsin State Laboratory of Hygiene (WSLH). The study protocol was approved by the University of Wisconsin Health Sciences Institutional Review Board (#M2006-1177) and provided to the Health Canada Research Ethics Board.

Following consent, venous blood specimens collected by plant medical staff in the course of their routine occupational monitoring program were immediately sampled and tested using the portable analyzer. The blood specimens were then forwarded via overnight air shipment to WSLH, where they were integrated into the clinical test workload and analyzed by electro-thermal atomization atomic absorption spectrometry (ETAAS, graphite furnace AAS). The blood specimens were then retested at WSLH using a second portable analyzer.

Donor Recruitment and Specimen Collection

Prior to the collection of blood for routine blood lead monitoring, workers were provided with an information

[1] To convert blood lead concentrations expressed as μg/dl to systems international (SI) units (μmol/L), multiply by 0.04826.

sheet and given the opportunity to participate. Informed consent was obtained immediately prior to blood collection. Following cleaning of the puncture site, plant medical staff employed standard phlebotomy procedures. Approximately 3 ml of blood was collected from the antecubital vein into evacuated tubes (Becton Dickenson, Franklin Lake, NJ) containing K_2EDTA preservative. The tubes used were ''brown top'' tubes certified by the manufacturer to contain a background lead concentration of <0.25 μg/dl when filled. The tubes were labeled with an alphanumeric identifier immediately following collection.

On-Site Portable Analyzer Testing

On-site analyzer testing was performed by the plant quality assurance (QA) officer and nursing staff. The QA officer received training from the manufacturer prior to commencement of the study. Testing was performed according to manufacturer's instructions. The venous blood specimens were mixed by hand for a minimum of 60 s, then sampled using the manufacturer-supplied capillary tubes. Contents of the filled capillary tubes were dispensed into labeled treatment reagent vials, and thoroughly mixed by hand. A portion of the blood/reagent mixture was then transferred to a sensor using the provided transfer dropper, and analyzed for blood lead concentration. Single analyses were performed, as would be typical with patient specimens. Two levels of blood-based controls provided by the manufacturer were analyzed, one before and one after each set of patient samples. Results were required to fall within the manufacturer-specified acceptability limits for results to be accepted. The blood specimens and blood/reagent mixtures were maintained at room temperature throughout the analytical process. The remaining blood was then packaged without refrigerant and shipped to WSLH for additional testing.

Reference ETAAS Testing

Upon receipt at WSLH, the blood specimens were wiped with D-Wipe® Towels (Esca Tech, Inc., Milwaukee, WI) to remove lead that might be present on the outer surface of the evacuated tubes. The specimens were then assigned laboratory identification numbers, and stored at $4 \pm 2^{\circ}$C. until analysis, which was performed the same or following day. The storage time averaged 17 hr. Prior to analysis, the specimens were brought to room temperature and mixed on a mechanical rocker for a minimum of 15 min. The specimens were then sampled using an automated diluter (Hamilton Company, Reno, NV) and analyzed on a Perkin Elmer AAnalyst 600 atomic absorption spectrophotometer (Perkin Elmer, Inc., Norwalk, CT) utilizing longitudinal Zeeman-effect background correction. The analytical procedure was adapted from the method described by Parsons and Slavin [1993].

All specimens were analyzed in duplicate, and the values averaged. Duplicate results were required to agree within 2.0 μg/dl for specimens with blood lead concentrations $\leq$20 μg/dl, or 10% for specimens exceeding that threshold. Blood-based analytical controls were tested following calibration and after every 15th injection, so that all specimens were bracketed by acceptable controls. Control results were required to fall within $\pm$2.0 μg/dl of the assigned value for specimens with blood lead concentrations $\leq$20 μg/dl, or 10% for specimens exceeding that threshold. Accuracy was further assured through successful participation in four external PT programs administered by the College of American Pathologists, New York State Department of Health, Pennsylvania Department of Health, and the WSLH. Specimens with results exceeding the calibration range of the method were retested using a reduced injection volume and subsequent calculation, and confirmed by testing a blood-based reference material with similar elevated concentration [NCCLS, 2001].

Repeat Portable Analyzer Testing at WSLH

Following sampling for the ETAAS analysis, the specimens were placed back on the rocker for same-day analysis on the portable analyzer. The specimens were mixed for a minimum of 15 min, and tested according to the manufacturer's instructions, using the same test procedure described for the on-site analysis. A single analysis was performed for each specimen. For this testing, both levels of the manufacturer-supplied blood based controls were run before and after testing of the patient specimens. All QC results were required to fall within the acceptable range.

Statistical Analysis

Results for the three test groups were evaluated using univariate regression analysis. Results of the on-site portable analyzer testing were also examined by the distribution of differences relative to OSHA proficiency test sample accuracy requirements. Bland and Altman [1986] limits of agreement were determined for paired data.

RESULTS

Group Measurement Results

ETAAS results were obtained for 121 samples tested over a 60-day period. The samples were not all from different individuals; some were sampled more than one time over the course of the study. The mean ETAAS blood lead concentration was 40.1 μg/dl; results ranged from 0.4 to 59.0 μg/dl.

Nearly all results (97%) fell in the range 16.1–59.0 μg/dl. The average difference of the duplicate ETAAS test results was 0.1 ± 1.3 μg/dl. Portable analyzer results were obtained on 120 on-site (fresh blood) samples. The mean concentration was 38.7 μg/dl. Portable analyzer results were obtained on 118 samples tested later at WSLH (shipped blood), yielding a mean concentration of 42.4 μg/dl. An average of 2 days (range 1–3) elapsed between the on-site and WSLH portable analyzer testing. Three samples could not be tested at WSLH due to a lack of sensors. In addition, three samples had results that fell below the 3.3 μg/dl limit of quantification for the portable analyzer. These samples were excluded from evaluation.

Comparison of Portable Analyzer and ETAAS Results

Regressions of the two sets of portable analyzer results against the ETAAS results are displayed in Figure 1. Correlation was very good for both sets, $r^2 = 0.928$ and 0.952 respectively for the on-site and WSLH analyzer results. Slope and intercept parameters are included in Figure 1; the slope did not differ significantly from one and the intercept did not differ significantly from zero for the fresh blood results. The mean difference between the two methods for paired data was 1.2 μg/dl; the two standard deviation Bland and Altman limits of agreement were −7.8 to 5.35 μg/dl. This was not observed for the WSLH results on shipped blood, where the slope of 1.09 was significantly above one ($P < 0.001$). The y-intercept of −1.7 (std. error = 0.97) was not statistically distinguishable from zero. The mean difference for paired data was 2.0 μg/dl; the two standard deviation Bland and Altman limits of agreement were −4.1 to 8.0 μg/dl.

One pair of ETAAS/on-site blood results exhibited a difference of >3 standard deviations, and was excluded as a statistical outlier (ETAAS result = 45.3 μg/dl, portable analyzer = 30.4 μg/dl). The reason for the discrepancy could not be determined.

Evaluation Using OSHA Approval Criteria

The on-site analyzer results were also evaluated in terms of their absolute difference from the ETAAS results, and used to predict performance when the OSHA PT acceptability criteria were applied. This evaluation is displayed in Figure 2. The dashed lines in Figure 2 denote the OSHA PT sample acceptability criteria of the target value ±6 μg/dl or 15% (larger). Under these criteria, 94% (111/118 samples) would be viewed as acceptable, not quite meeting the OSHA threshold of 95% acceptability. Though waived from PT requirements under CLIA, results were also examined using the CLIA acceptability criteria. Eighty-one percent (95/118) of the samples met the CLIA PT sample acceptability limits of ±4 μg/dl or 10%, just above the CLIA test event threshold of 80% for satisfactory performance.

Comparison of Portable Analyzer Results, Fresh Versus Shipped Blood

Regression analysis was also performed on the paired on-site and WSLH portable analyzer results. Figure 3 displays the linear regression of these data. Good correlation was observed, $r^2 = 0.887$. The slope and intercept parameters did not differ significantly from one and zero respectively, though the y-intercept figure of 2.72 μg/dl fell just outside the

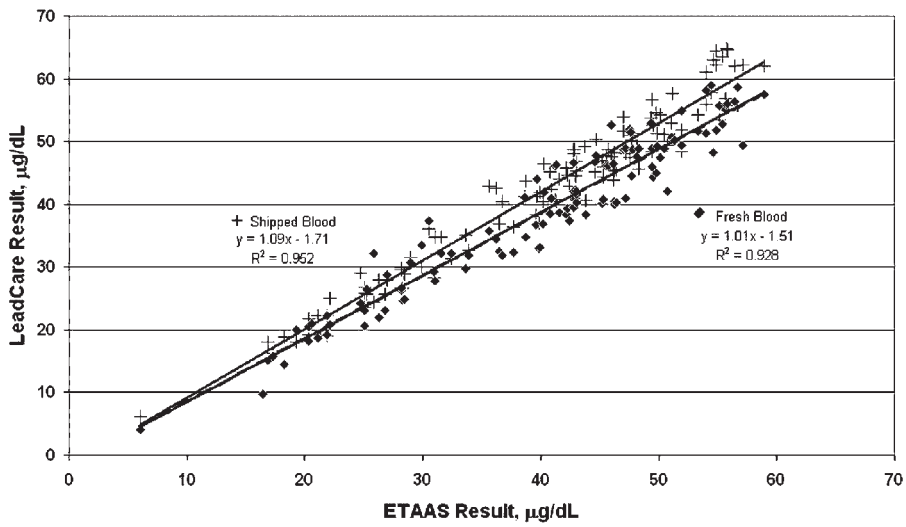

FIGURE 1. Regression plot of reference electro-thermal atomization atomic absorption (ETAAS) versus LeadCare II portable analyzer results for fresh blood specimens analyzed at the work site, and the same blood following shipment and storage to the reference laboratory.

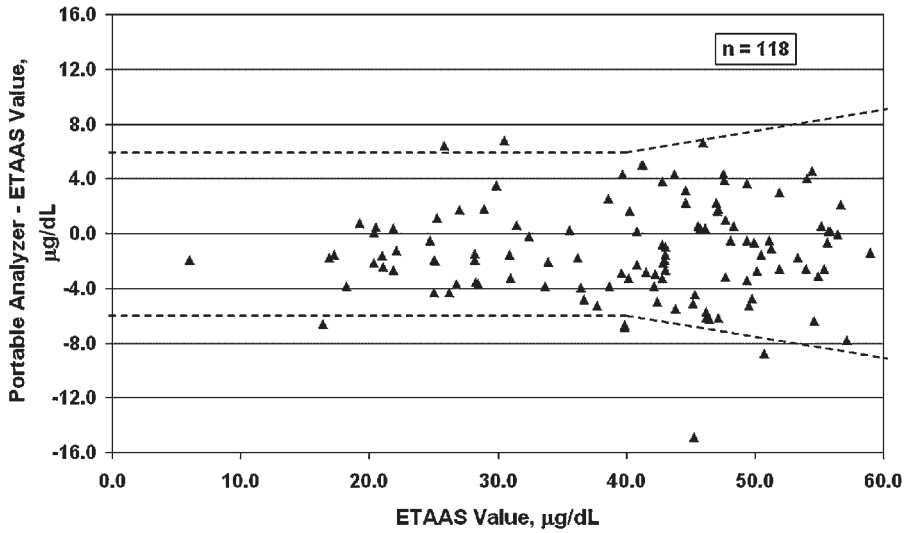

FIGURE 2. Difference plot of LeadCare II portable analyzer results versus reference electro-thermal atomization atomic absorption (ETAAS) results. The dashed lines represent proficiency test sample acceptability criteria under OSHA standards.

threshold of statistical significance ($P = 0.052$). The mean difference for paired portable analyzer data was 3.0 µg/dl; the two standard deviation Bland and Altman limits of agreement were −5.4 to 11.4 µg/dl.

DISCUSSION

On-Site Analyzer Performance

This study examined the performance of the LeadCare II portable analyzer on a population with substantially elevated blood lead concentrations averaging >40 µg/dl. Based on regression analysis, on-site results obtained on the portable analyzer did not differ significantly from the reference ETAAS results in this study. On evaluated data pairs, the portable analyzer results underestimated the ETAAS results by an average 1.2 µg/dl. In the context of current occupational monitoring thresholds, this bias would have little impact. Because a large majority of specimen concentrations fell above 20 µg/dl, the impact of lowering OSHA action levels cannot be effectively addressed by this study.

With respect to OSHA PT performance standards, performance of the portable analyzer in this study also appears acceptable, with 94% acceptability under the OSHA

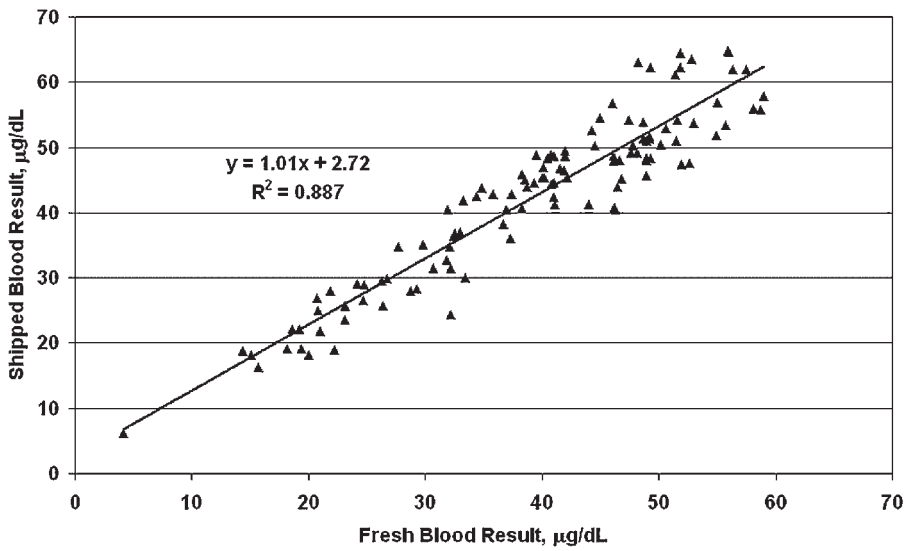

FIGURE 3. Regression plot of LeadCare II portable analyzer results for fresh blood specimens versus the same blood following shipment and storage.

criteria vs. a requirement of 95%. This level of performance surpasses that reported for the earlier LeadCare instrument by Taylor et al. [2001] in the Journal, where a 95% acceptability proportion was attained only at a much wider variability of ±11 μg/dl. The use of dual fixed and proportional PT acceptability limits allows higher proportional error for measured blood lead concentrations below 40 μg/dl, and in part for this reason blood lead PT performance has been observed to decline as blood lead concentration increases [Stanton, 1993; WSLH, 2007]. Only 3% of the test samples in this study had blood lead concentrations below 16 μg/dl, and as noted earlier, the mean blood lead concentration exceeded 40 μg/dl. Consequently, the observed level of performance would likely be higher in populations with lower average blood lead concentrations. Though not examined in detail due to the CLIA-waived status of the instrument, the CLIA PT event acceptability criteria of >80% of results falling within +4 μg/dl or 10% was also met in this study, with 81% acceptability observed following the application of these criteria to the data.

The ubiquity of lead in the industrial environment creates concerns of sample contamination during the sampling and testing process. Falsely elevated portable analyzer results caused by environmental contamination did not prove to be an issue in this study. Only three (2.5%) of the samples had on-site test results ≥6 μg/dl higher than the corresponding ETAAS results, and maximum observed positive bias was 6.8 μg/dl. Given the overall data distribution, this is more likely attributed to inter-method analytical variation rather than to sample contamination. One sample exhibited a gross negative bias in the on-site analyzer result compared to the ETAAS and follow-up analyzer test: ETAAS = 45.3 μg/dl, on-site analyzer = 30.4 μg/dl, follow-up analyzer = 45.9 μg/dl. It should be noted that this portable analyzer result would constitute a false negative result for follow-up actions under current the OSHA lead standard.

An additional potential benefit of the on-site testing allowed by this analyzer is that elevated results can be immediately confirmed, and any necessary actions can be undertaken immediately. Based on the results of this study, the LeadCare II analyzer will be a useful tool for monitoring worker exposures under the OSHA lead standards.

Effect of Blood Shipment and Storage on Analyzer Results

The manufacturer states that for accurate analyzer results, blood should be tested <24 hr post-collection, and not subjected to refrigerated or frozen storage temperatures. Mean portable analyzer results increased from 38.7 to 42.4 μg/dl following the shipment and storage of blood at WSLH, with a mean difference of 3.0 μg/dl for paired data. Despite the difference, the regression analysis slope and intercept displayed in Figure 3 did not differ significantly from one and zero respectively, though the y-intercept of 2.72 nearly reached significance ($P = 0.052$).

The portable analyzer results also increased relative to the reference ETAAS results, with mean values 42.4 and 40.1 μg/dl, respectively, and a mean difference of 2.0 μg/dl. The data in this study are therefore insufficient to conclude whether the observed increases are significant. Pending additional study, the manufacturer's instructions for blood age and storage requirements should be adhered to.

Study Limitations

This study cohort exhibited substantial exposure to lead, and very few blood lead results below 16 μg/dl were obtained. The paucity of results at lower concentration levels may have impacted the regression statistics obtained, particularly the y-intercept values. Additional study of a cohort with lower average blood lead concentrations would be useful. This study took place at a single industrial site that employed trained testing staff. This worksite environment and training level may not be typical of conditions encountered in the field. Additional study in other workplace environments is suggested.

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