

Field Evaluation of a Portable Blood Lead Analyzer in Workers Living at a High Altitude: A Follow-up Investigation

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Background Field-portable instruments can offer expeditious analytical results to health professionals in field settings and in areas lacking laboratory infrastructure. This study further evaluated an electroanalytical field-portable instrument, which rapidly analyzes blood lead concentrations.

Methods A portable anodic stripping voltammetry (ASV) instrument was evaluated utilizing paired samples from 243 employees working at an elevation of approximately 3,800 meters in Peru. Each worker donated two venous blood samples, one of which was analyzed by the ASV device and the other by a reference analytical method, graphite furnace atomic absorption spectrometry (GFAAS).

Results According to the GFAAS results, the mean blood lead concentration measured was $46(\pm 16)$ $\mu\text{g}/\text{dl}$; this was significantly greater than the mean ASV measurement of $32(\pm 11)$ $\mu\text{g}/\text{dl}$ (paired *t*-test; $P < 0.0001$). The accuracy of the ASV estimation decreased as the measured blood lead concentration increased.

Conclusions The results from this investigation were significantly different from the previous study, which was conducted near sea level. The exact causes for the discrepancies between the portable ASV results from the two studies are unclear, but are thought to be related to differences in blood chemistry between the Midwestern United States and Peruvian Andes worker cohorts. Portable ASV blood lead measurements from populations living at high altitudes should be viewed with caution. *Am. J. Ind. Med.* 46:656–662, 2004. Published 2004 Wiley-Liss, Inc.[†]

KEY WORDS: anodic stripping voltammetry; blood lead analysis; high-altitude population; instrument evaluation; occupational lead exposure; smelter

INTRODUCTION

Field-portable instruments can offer expeditious analytical results to health professionals in field settings and in areas lacking laboratory infrastructure. In this project, we evaluated an electroanalytical field instrument, which is used to rapidly analyze venous blood lead levels in individuals. The instrument, which employs anodic stripping voltammetry (ASV) to measure lead in blood [Ashley, 1994; Wang, 1996], was used on samples from an Andean worker population in Peru. This instrument, developed by ESA, Inc. and AndCare, Inc. in conjunction with the Centers for Disease Control and Prevention (CDC), was designed to provide a

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prompt, cost-effective methodology to monitor lead exposures for pediatric screening.

In 1997, the LeadCare[®] instrument received 510(k) market clearance from the Food and Drug Administration (FDA), and was classified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988 as a moderately complex medical device [Federal Register, 1997]. This portable ASV instrument was designed to provide a prompt, cost-effective technique to monitor lead exposures, and has been used with success for pediatric screening [Shannon and Rifai, 1997]. However, the instrument's expeditious analysis also made it potentially valuable to occupational health professionals for on-site investigations of lead-exposed worker populations. The instrument utilizes pre-packaged reagents and does not require high-purity reagents, such as concentrated nitric acid or ultra-pure water. This is a distinct advantage for field investigations.

Accordingly, in an earlier study the authors evaluated this portable ASV instrument on blood samples from over two hundred employees in Midwestern United States [Taylor et al., 2001]. In that investigation, the portable ASV instrument overestimated the true venous blood lead concentration by less than 1 $\mu\text{g}/\text{dl}$ —a difference, which holds very little clinical significance [Taylor et al., 2002]. During the domestic evaluation, employee blood lead results ranged from 1.7 $\mu\text{g}/\text{dl}$ to 42 $\mu\text{g}/\text{dl}$. Since the LeadCare instrument has an operational range upward to 65 $\mu\text{g}/\text{dl}$, a goal of the current research study was to evaluate the instrument's performance across the entire working range, especially at blood lead levels greater than 42 $\mu\text{g}/\text{dl}$. Since it was difficult to recruit a large number of highly exposed workers from a U.S. company with blood lead concentrations between 40 $\mu\text{g}/\text{dl}$ and 65 $\mu\text{g}/\text{dl}$, international locations were pursued.

Lead in Peru

Because of potential health effects, substantial attention has been given to environmental and occupational sources of lead in the global community. Environmental lead sources such as refuse from metal mining and smelting, leaded gasoline, contaminated soil, and battery recycling are still commonplace in Peru. Unleaded gasoline has been introduced, although leaded gasoline is still used extensively, causing significant air and soil contamination [Romieu and Lacasana, 1996]. Peru also has the largest number of lead mines in South America, mining approximately 258 thousand tons per year [Lead Development Association International (LDAI), 1998].

Although the Peruvian Ministry of Health has implemented an annual maximum air concentration limit of 0.5 $\mu\text{g}/\text{m}^3$, there are consistent monthly violations of this limit in the capital city of Lima [Jacoby, 1998]. Due in part to airborne sources of lead contamination in the Lima metropolis, the average children's blood lead level in the Lima area was

estimated at approximately 10 $\mu\text{g}/\text{dl}$, although adults measured about a third of this value [Hernandez-Avila, 1999]. Apart from air, anthropogenic environmental sources in bulk materials (e.g., contaminated soils and refuse) can contribute to higher human blood lead levels. For instance, a recent CDC technical assistance project in Peru documented blood lead levels as high as 48 $\mu\text{g}/\text{dl}$ in children living in Lima's port city of El Callao [Rubin et al., 2000]. Although occupational exposures are regulated in Peru, employees with elevated blood lead concentrations (>40 $\mu\text{g}/\text{dl}$) are common.

As part of Peru's effort to privatize the industry, several international companies have purchased smelting facilities from the Peruvian government's central mining organization. Since the LeadCare instrument could potentially be a useful tool for these remote facilities, one smelting company in the Peruvian Andes invited CDC personnel to conduct an instrument evaluation at its facility (elevation \sim 3,800 meters).

MATERIALS AND METHODS

The LeadCare instrument was evaluated utilizing paired samples from 243 employee volunteers. Each employee gave two venous blood samples, one of which was analyzed by the LeadCare ASV instrument, and the other by a reference method, CDC Whole Blood Method 1080C (graphite furnace atomic absorption spectrometry, GFAAS).

To enable the evaluation of the higher concentration range of the LeadCare instrument, the employee population of approximately 4,000 workers was stratified so that a lottery could be conducted, whereby 50% of the workers chosen would have had their latest blood lead evaluation between 40 $\mu\text{g}/\text{dl}$ and 65 $\mu\text{g}/\text{dl}$, 40% between 20 $\mu\text{g}/\text{dl}$ and 39 $\mu\text{g}/\text{dl}$ and 10% below 20 $\mu\text{g}/\text{dl}$. Then, 243 employees were randomly selected from the three stratified groups. Small groups of the pre-selected employees (\sim 30–40 per session) attended a brief program describing the study; Spanish translation was provided during each meeting. At these gatherings, the informed consent (in Spanish) was reviewed and volunteers were enrolled in the study. After the informed consent was completed, each participant was interviewed by Spanish-speaking CDC personnel immediately prior to blood sampling. During each interview, participants were asked to provide categorical information, including age, gender, smoking history, and work practice information. Owing to a preliminary survey indicating that monetary compensation was culturally unacceptable, each participant also received a duffel bag as a nominal gift for his/her time and inconvenience for participating in the monitoring program.

The protocol was approved by the CDC, National Institute for Occupational Safety and Health (NIOSH), Human Subjects Review Board (HSRB), the CDC Institutional Review Board (IRB), and by the human studies review board of the Peruvian Ministry of Health.

Venous Blood Sampling

Analysis of venous blood samples is currently considered to be the primary method for monitoring occupational lead levels, due to potential contamination problems with capillary samples taken from workers [Esswein et al., 1994; NIOSH, 1997]. Blood sample collection was conducted in a hospital about 2 miles from the metals smelter facility. "Universal precautions" [CDC, 1988] were followed during each blood draw. Prior to the venous blood draw, the employee's arm was cleaned with a lead-free soap and sterile gauze to remove any lead contamination. An alcohol swab was used to sterilize the sampling area. Blood sampling consisted of taking two samples of venous blood, drawn into 5-ml Vacutainer[®] tubes (K₃EDTA, BD# 36-9651) from the antecubital area (inside portion of the arm, opposite the elbow). Each blood tube was labeled with a bar-coded label for identification. One Vacutainer tube was refrigerated at 4°C for subsequent GFAAS analysis in the CDC, National Center for Environmental Health (NCEH) laboratory in Atlanta, GA. The other paired Vacutainer sample was analyzed on-site for blood lead using the portable ASV LeadCare instrument. Figure 1 illustrates the venous blood

sampling and applicable analysis scheme for each blood sample.

GFAAS Blood Lead Analysis

One of the venous samples was refrigerated and later hand carried to the CDC reference laboratory. GFAAS analysis of lead was conducted in the CDC/NCEH Division of Laboratory Sciences, Elemental Analysis Laboratory in Atlanta, GA. Blood lead samples were stored and transported at ~4°C until analyzed. Venous blood lead was determined according to the CDC Whole Blood Method 1080C by GFAAS (Perkin-Elmer Model 4100-ZL with Zeeman background correction) [Miller et al., 1987].

The reported GFAAS lead concentration result was the average of two measurements drawn from the same venous blood sample. The blood lead instrument employed an aqueous calibration using calibration samples, which were prepared by diluting a performance evaluation material (National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 955B, Lead in Bovine Blood; NIST, Gaithersburg, MD). Analytical quality control (QC) was monitored by utilizing four concentrations of

Venous Sampling and Analysis

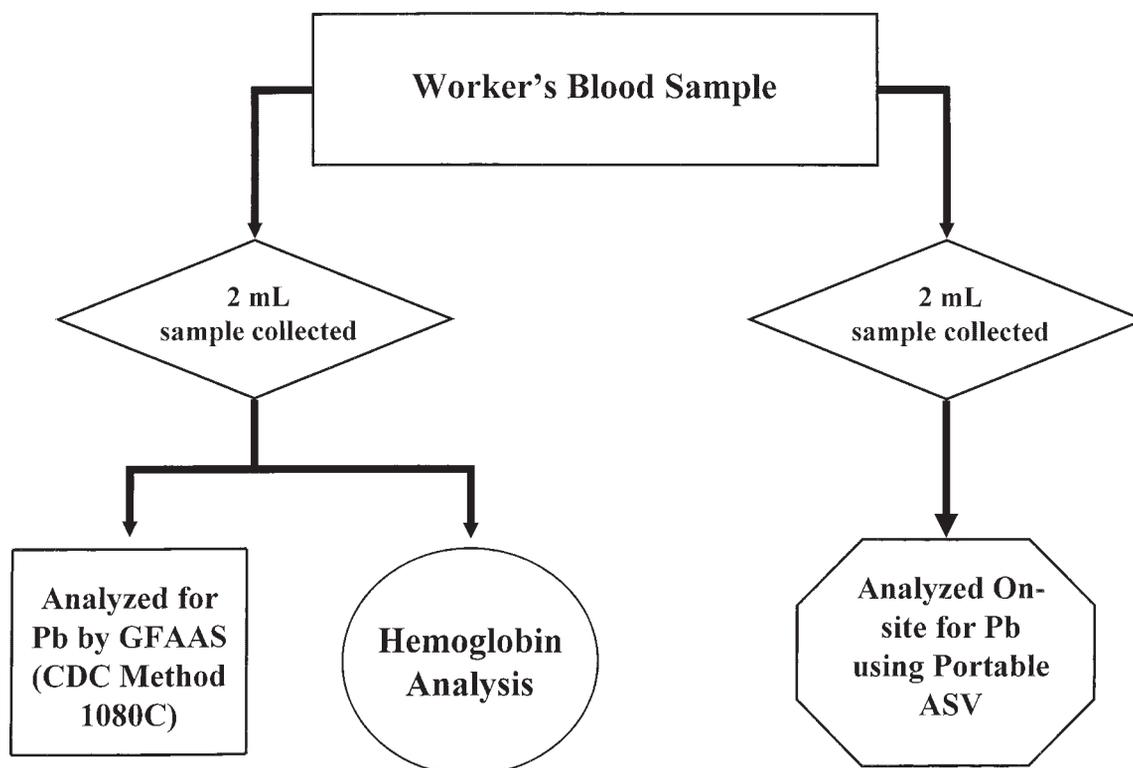


FIGURE 1. Schematic of blood lead sampling and analysis protocol.

bench QC materials and two levels of blood lead QC materials.

Blood Lead Measurement by ASV

The other paired blood sample collected was analyzed on-site by ASV using the LeadCare instrument [ESA, Inc., 1997]. This analysis was completed using appropriate sample preparation and measurement procedures, and has been described in detail elsewhere [Taylor et al., 2001]. The analytical range of the instrument is 1.4–65 $\mu\text{g}/\text{dl}$. If the sample contains more than 65 $\mu\text{g}/\text{dl}$ of lead, the instrument displays the message “HI” in the digital display. The LeadCare instrument had an electronic calibration pre-determined for the lot of electrodes used. Analytical QC was monitored by utilizing three concentrations of bench QC materials. Two of these materials were standard QC materials available from the vendor, and one was specially prepared for this study by ESA, Inc. It consisted of a higher blood lead concentration for checking the high range of the instrument.

Hemoglobin Analysis

The blood samples were analyzed for hemoglobin using a HemoCue[®] instrument (HemoCue, Inc., Mission Viejo, CA). To conduct the hemoglobin analysis, a small bead of blood was pipetted from the second blood specimen (i.e., that analyzed by GFAAS) onto a small piece of paraffin film. A 10- μl sample of blood was drawn into a disposable hemoglobin microcuvette by capillary action. The microcuvette was then inserted into the HemoCue instrument and the hemoglobin concentration (g/dl) measured. As part of the QC process, a control cuvette with an optical interference filter was used to verify the instrument’s basic electronic/optical status. Liquid QC materials (HemoCue) at three concentration levels were also used to ensure that both the instrument and cuvette performance were within pre-defined limits.

Statistical Analysis

The sample size was chosen to be able to detect a statistically significant difference in means of at least 12.5% between paired results for the LeadCare[®] instrument and the GFAAS laboratory analysis. The significance levels chosen for type I and type II errors in testing the null hypothesis of no difference between paired results were $\alpha = 0.05$ and $\beta = 0.90$, respectively. These significance levels are typical for bioanalytical measurements where it is desired to have less than 5% error, while choosing a two-sided confidence interval in which at least 90% of the analytical results fall [Bland and Altman, 1986]. The percent difference of 12.5% is approximately the allowable range for blood lead proficiency testing programs (± 4 $\mu\text{g}/\text{dl}$ or 10%, whichever is

greater). Using $\alpha = 0.05$ and $\beta = 0.90$, a minimum required population size of $n = 200$ was calculated.

A stratified analysis was conducted on the categorical data obtained from the questionnaire to verify confounders such as age, gender, and tobacco usage. Demographic characteristics of the study population were summarized using frequency distributions.

To test for a statistically significant difference between data sets, a pair-wise *t*-test was conducted to compare the participant venous GFAAS and LeadCare results. Normally, blood lead results from laboratory analysis are reported to patients in integer figures. But to avoid biasing the statistical analysis by rounding, blood lead results were left to one decimal place during the statistical analysis. Descriptive statistics were determined for the GFAAS, ASV, and hemoglobin results. Regression analysis was used to investigate a potential relationship between hemoglobin concentrations and (reference) blood lead levels, as measured by GFAAS.

RESULTS

Study Demographics

Of the 243 eligible participants who were enrolled in the study, four of the participants failed to give blood, or their blood samples were unacceptable for GFAAS analysis. Results for an additional two volunteer blood samples could not be reported by portable ASV owing to blood lead results greater than 65 $\mu\text{g Pb}/\text{dl}$ (the upper reporting limit of the instrument).

The total study population had a mean age of 44 years ($SD = 9$), was almost entirely male (99.6%), and 7% used tobacco products. The mean period of employment was 20 years ($SD = 10$), and 98% reported current use of a respirator. After the study was complete, each participant received a letter giving that individual’s blood lead results. Since mail delivery was inconsistent, these letters were hand-delivered in sealed envelopes by a Peruvian Ministry of Health physician. If desired, the employee could open the letter immediately and ask the physician questions.

Blood Lead Measurement Results

The mean GFAAS blood lead concentration of the study population was 46 $\mu\text{g}/\text{dl}$ ($SD = 15.5$), while the mean measured ASV blood lead concentration was 32 $\mu\text{g}/\text{dl}$ ($SD = 10.7$). The mean difference between the blood lead levels obtained from the portable ASV instrument and GFAAS was -14.2 $\mu\text{g}/\text{dl}$ ($SD = 7.6$; $P = 0.0001$), indicating that, on average, the portable ASV instrument underestimated the true blood lead value by more than 14 $\mu\text{g}/\text{dl}$. As shown by Figure 2, the difference between the two analytical techniques worsened as the true blood lead concentration increased.

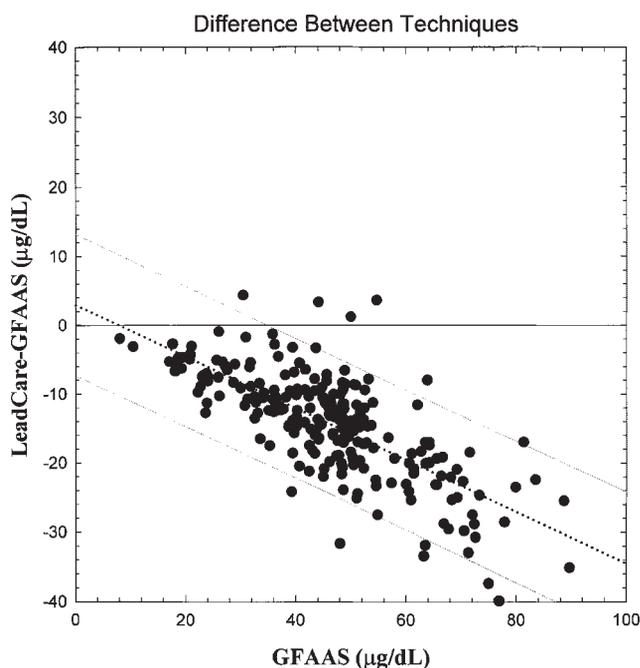


FIGURE 2. Plot of difference between portable ASV results and reference laboratory results versus reference laboratory atomic spectrometric results. The dotted and dashed lines through the data points represent the best fit line and prediction interval, respectively.

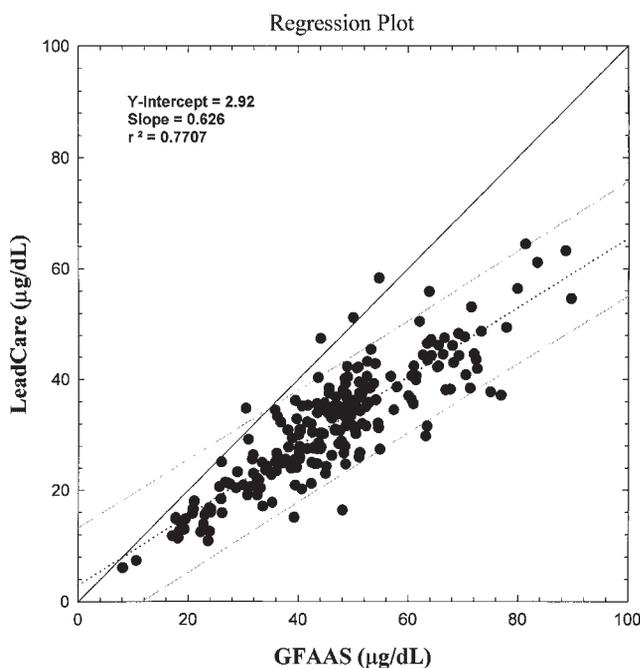


FIGURE 3. Regression plot of portable ASV blood lead results versus reference fixed-site laboratory atomic spectrometric results. The dotted line through the data points represents the best fit regression line; the prediction interval is represented by the dashed lines above and below the best fit regression line. For comparison, the solid diagonal line indicates unity slope and zero intercept (i.e., ideal correspondence).

A simple linear regression detected a statistically significant association between the portable ASV instrument and the laboratory results. The ASV versus GFAAS comparison is shown in Figure 3: slope = 0.63 ± 0.022 ; intercept = $2.92 \pm 1.07 \mu\text{g Pb/dl}$.

QC results were analyzed to estimate the ASV instrument's precision. Table I presents the measured mean values and precision estimates (relative standard deviations) for the three QC materials.

Hemoglobin Concentrations

The mean hemoglobin concentration of the study population was 17.3 g/dl (SD = 1.3). Regression analysis indicated there was no significant relationship between hemoglobin concentration and GFAAS blood lead results ($r^2 = 0.00905$; $P = 0.143$). The workers' mean hemoglobin concentrations were $\sim 1\text{--}2$ g/dl lower than those reported for other Andean populations living at high altitudes [Beall et al., 1990, 1998].

DISCUSSION

The discrepancies between measured GFAAS and the ASV blood lead concentrations for this very high-elevation Peruvian worker population are quite dramatic (Figs. 2 and 3). However, disparities of this magnitude were not seen in the portable ASV instrument evaluation that was carried out on a U.S. worker cohort [Taylor et al., 2001]. Even for blood concentrations below 42 $\mu\text{g/dl}$, the portable ASV instrument significantly underestimated the true blood lead concentration (Figs. 2 and 3). In the previous evaluation, which was conducted on a US worker population near sea level, the ASV instrument overestimated the true blood lead concentration by less than 1 $\mu\text{g/dl}$. However, in the current study, blood lead concentrations were underestimated by a mean difference of approximately 14 $\mu\text{g/dl}$, and in one case by 39 $\mu\text{g/dl}$ (see Fig. 2). These differences in blood lead concentrations are clinically significant.

In contrast, QC procedures and results corresponded closely in the two evaluations. Both precision and bias for the QC materials were approximately equivalent in the two studies (Table I). This suggests that the differences in performance between the blood lead methods were likely not a function of instrument error. Likewise, operators were

TABLE I. Blood Lead Quality Control Samples Measured On-Site by Portable ASV

QC sample	N	Mean \pm SD ($\mu\text{g Pb/dl}$)	Precision (RSD, %)
Level1 (low)	12	6.62 ± 1.4	21
Level2 (med)	14	22.5 ± 2.2	9.8
Level3 (high)	16	45.1 ± 3.9	8.6

common in both U.S. and Peruvian studies, and had completed comprehensive training procedures prior to both studies.

Portable ASV performance evaluated in this work differs considerably from a related study wherein the LeadCare instrument was used to measure blood lead in capillary samples from an Ecuadorian Andean population [Counter et al., 1998]. In the present work, venous samples were analyzed by both portable ASV and the reference laboratory method. On the contrary, capillary finger-stick samples were analyzed by ASV in the Ecuador study, but venous samples were analyzed by reference atomic spectrometric methods [Counter et al., 1998]. Contamination of finger-stick blood samples could result in a positive bias for ASV results, and this is suggested by slopes exceeding unity in the Ecuador study.

The underestimation of the ASV instrument may be attributed to the significant difference in blood chemistry in individuals living at an average altitude of $\sim 3,800$ meters ($\sim 12,500$ feet). For example, the blood samples from this Andean worker population contained an average hemoglobin concentration of 17.3 g/dl, which is significantly higher than the mean of 15.3 g/dl for sea-level U.S. males [Gunter et al., 1996]. However, the lack of an observed association between measured hemoglobin concentrations and blood lead levels in this study population suggests no direct effect from hemoglobin itself.

Hematocrit levels are related to hemoglobin concentrations [Imai et al., 1995], and higher percent hematocrit results in higher blood viscosity [Moore et al., 2000]. After transport and refrigeration, it was found that the blood lead samples required lengthy preparation steps for re-suspension of the samples; such procedures are not required for lower viscosity blood samples from individuals living at low altitudes. Indeed, pH and blood constituents such as glutathione and albumin can alter blood chemistry with respect to low-altitude populations [Winslow et al., 1989; Imai et al., 1995; Moore et al., 2000; D. Jones, Emory University, personal communication]. These factors may have an additive or synergistic effect on the ASV analysis, either through interference or by alteration of baseline instrumental response [Roda et al., 1988].

Previous work by the instrument manufacturer indicated that the ASV measurement is influenced by alterations in the level of glutathione concentrations; owing to this, the instrument manufacturer revealed that the ASV instrument had been adjusted for glutathione concentrations in the typical U.S. population [E. Zink, ESA, Inc., personal communication]. However, blood constituents such as glutathione may be very different in populations living in other regions of the world, notably very high altitudes. Glutathione is a potential interferant to blood lead electroanalysis, owing to the presence of sulfhydryl groups, which may bind to the electrode surface and block active sites.

Absolute glutathione peroxidase activities are significantly higher in Andean populations versus sea-level populations [Imai et al., 1995], and high glutathione levels may be a contributor to the negative bias that we observed in portable ASV blood lead measurements. However, glutathione could not be measured accurately in these samples, owing to the instability of this species in blood samples that are not stored at liquid nitrogen temperature [Richie et al., 1996].

Despite problems observed in this evaluation, a portable blood lead measurement instrument still holds promise in offsite applications at lower elevations. QC monitoring results during this investigation and data from ESA, Inc. (up to $\sim 2,400$ m [E. Zink, personal communication]) has shown that the instrument works correctly with multiple QC materials (both bovine and human), indicating that the problems with patient results may be due to some unique blood issues related to extreme high elevations. Our previous evaluation demonstrated that the portable ASV instrument could prove useful in monitoring blood lead concentrations in a transient workforce [Taylor et al., 2001]. International locations, especially where a high quality laboratory or a rapid specimen transport infrastructure does not exist, seem to provide a fruitful application for on-site monitoring devices such as the portable ASV instrument. Until the discrepancies of the results seen in this instrument evaluation are corrected, portable ASV measurements of blood lead samples from populations living at extremely high altitudes should be viewed with caution.

The current study was also conducted with the objective of evaluating the portable ASV instrument over its complete operating range. To date, until the issues arising from this work are resolved, we cannot state whether the instrument should be used in occupationally exposed populations with blood lead concentrations above 42 $\mu\text{g}/\text{dl}$. Practitioners are encouraged to evaluate the recent investigations of this portable ASV instrument and to determine whether this technology is appropriate for their particular application.

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