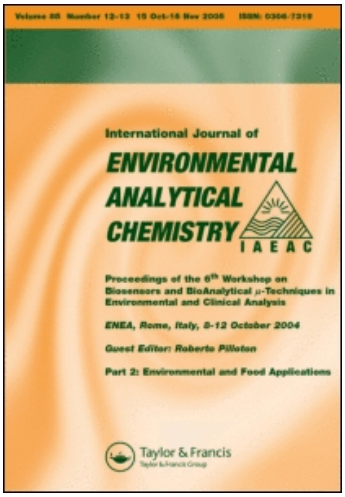


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# A Rapid and Precise System for Lead Determination in Whole Blood

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The paper describes a heated furnace atomic absorption method for determining lead in whole blood using an automated sample introduction system. It is direct and rapid. No sample pretreatment is required and up to 30 samples per hour can be analyzed. Precision and accuracy data are presented. The system has been applied to both macro (venipuncture) and micro (capillary) samples. The method uses physiologically-bound lead standards to compensate for matrix effects. It has been used in the screening of target populations and for confirming elevated blood lead levels in the California Department of Health Services Childhood Lead Program.

KEY WORDS: Blood lead, lead screening, lead poisoning, biological samples, atomic absorption.

## INTRODUCTION

A large number of analytical methods for the determination of lead in blood has appeared in the literature. Eller and Haartz[1] have compared the precision and accuracy of some widely-used methods. A few studies[2–4] have described non-flame or electrothermal atomic absorption techniques with automatic sample injection systems. These automated methods[2–4] as well as most non-automated methods[1,5–7] require sample pretreatment with acid and/or surfactant.

In support of the California Department of Health Services Childhood Lead Program, the State's Air and Industrial Hygiene Laboratory (AIHL) has validated and applied a direct and rapid method for lead analysis in whole blood. The method requires no sample preparation and has been used extensively for screening target populations and confirming elevated lead levels in macro as well as micro blood samples.

This report describes the technical details of the system. An electrothermal atomic absorption method with background correction and an automatic sample introduction system is employed.[8,9] It consists of a nebulizer that introduces the sample into a premix chamber, converting the sample into an aerosol. A sampling jet then deposits the aerosol onto a heated graphite cuvette under controlled conditions. Because the aerosol dries on contact with the cuvette, interferences associated with sample soaking into the graphite or those caused by crystal formation during evaporation are theoretically eliminated. The sample volume varies linearly with the deposit time chosen by the operator, thus achieving the desired sensitivity. No sample pretreatment is required other than the addition of distilled, deionized water to hemolyze and to reduce the viscosity of the blood.

## EXPERIMENTAL

### Apparatus and Reagents

All blood lead analyses were performed with an Instrumentation Laboratory (IL)† Model 257 atomic absorption spectrophotometer with background corrector and a controlled temperature furnace (IL-CTF atomizer, Model 555). An autosampler, FASTAC™ IL Model 254 was used to introduce samples automatically into the furnace.

A platform rocker was used for mixing blood samples prior to analysis. High precision automatic pipettors with disposable tips were used to dispense aliquots of whole blood into disposable analyzer cups used in the system's autosampler.

Lead standards included:

1. Certified atomic absorption standard, lead reference solution, 1,000 ppm, Fisher Scientific Company, U.S.A., and
2. Physiologically-bound lead standards obtained from lead acetate-fed cows.[10] The lead values were established by submitting the standards to reference laboratories, using several different methods, and averaging the results.

All reagents and solutions were prepared using distilled, deionized water.

### Procedures

The instrument was calibrated with physiologically-bound bovine lead standards to read the concentration directly in the linear range of the

†Mention of commercial products should not be construed as an endorsement.

analyte. An evaluation of the entire system including the autosampler was made, after slightly modifying the conditions recommended by the manufacturer.[11] Detailed operating parameters are given in Table I. With these instrument settings, the limit of detection was found to be 1 μg/dL, based on 12 measurements. The limit of detection is defined as the concentration which gives a reading equal to twice the standard deviation of a series of determinations at or near blank level.

TABLE I

Detailed operating parameters for lead determination in whole blood

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Spectrophotometer: | Wavelength | | | 283.3 nm | | | |
| | Hollow cathode lamp | | | 5 ma | | | |
| | $D_2$ arc (background corrector) | | | 15 ma | | | |
| | High voltage meter | | | 530 | | | |
| | Energy meter | | | within green zone | | | |
| | Integrate[b] | | | 10 sec | | | |
| Furnace: | $N_2$ purge gas[b] | | | 34 psi | | | |
| | gas flow[b] | | | 10 SCFH[a] | | | |
| | temperature | | | 25°C | | | |
| | operation mode[b] | | | auto | | | |
| | temperature program[b] | | | | | | |
| | Stage | 1 | 2 | 3 | 4 | 5 | 6 |
| | Temp | — | 150 | 500 | 500 | 2000 | — |
| | Time | 0 | 1 | 2 | 2 | 0 | 2 |
| | integrate start | | | stage 5 | | | |
| | sample size[b] | | | 80 μL | | | |
| Autosampler: | nebulizer flow (deposition rate) | | | 3 mL/min | | | |
| | delay | | | 10 sec | | | |
| | deposit time | | | 20 sec | | | |
| | repeat | | | 2 | | | |
| | door | | | set at 3 (150°C) | | | |

[a]Standard cubic feet per hour.
[b]Denote modified parameters.

Before adopting this method for routine analysis, a thorough comparison with other atomic absorption methods[5,12] was made, using blood samples of known lead content. These samples were obtained through the U.S. Center for Disease Control's (CDC) Blood Lead Proficiency Testing Programs. Having established the equivalency of these methods, the method described below was used for the subsequent

analysis of all blood samples submitted to the California Childhood Lead Program.

The blood standards, controls, and samples were stored at 4°C. Prior to analysis they were well mixed on a platform rocker until room temperature was reached. For macro samples obtained by venipuncture, 4 mL of distilled, deionized water and 80 $\mu$L of the well-mixed blood were pipetted into a disposable analyzer cup, capped, and shaken thoroughly by hand. For capillary or micro samples, containing less than 150 $\mu$L, the entire blood sample was dispensed into a cup. Then a 40 $\mu$L aliquot was transferred to another cup, to which was added 4.04 mL of water. The cups were then positioned in the autosampler for aspiration into the furnace cuvette and each cup analyzed in duplicate. A blood control and a blank sample of water were analyzed after every five samples. (The analytical results for capillary samples were multiplied by two to account for dilution.)

Shown in Figure 1A and 1B are absorbance profiles of human blood samples containing 2 and 51 $\mu$g Pb per dL blood respectively, analyzed by this method, using an identical atomic absorption system equipped with a cathode ray tube and a video printer. These samples cover a range of concentrations which includes greater than 90% of the samples analyzed in AIHL and were analyzed by A. Rattonetti, Instrumentation Laboratory, Inc. The dotted curves represent the total uncorrected absorption due to both the background and the lead present. The solid curves represent the absorption of lead.



## RESULTS AND DISCUSSION

### Comparison of bovine and aqueous standards

When blood samples of known lead content were analyzed using aqueous lead standards to calibrate the system, the measured lead concentrations were less than 40% of the true values. When concentrations of these samples were calculated using physiologically-bound bovine lead standards for calibration, the measured values were approximately 100% of the true values (Table II). The absorbance values measured for bovine blood lead standards were lower than the corresponding aqueous lead standards of the same lead concentrations due to differences in matrix composition. Only when exhaustive sample preparation, e.g. liquid–liquid extraction procedures, are employed can aqueous standards be used for analysis of lead in blood.[14] Since it is necessary to compensate for matrix effects, physiologically-bound lead standards were used subsequently for the routine analysis of lead in whole blood.

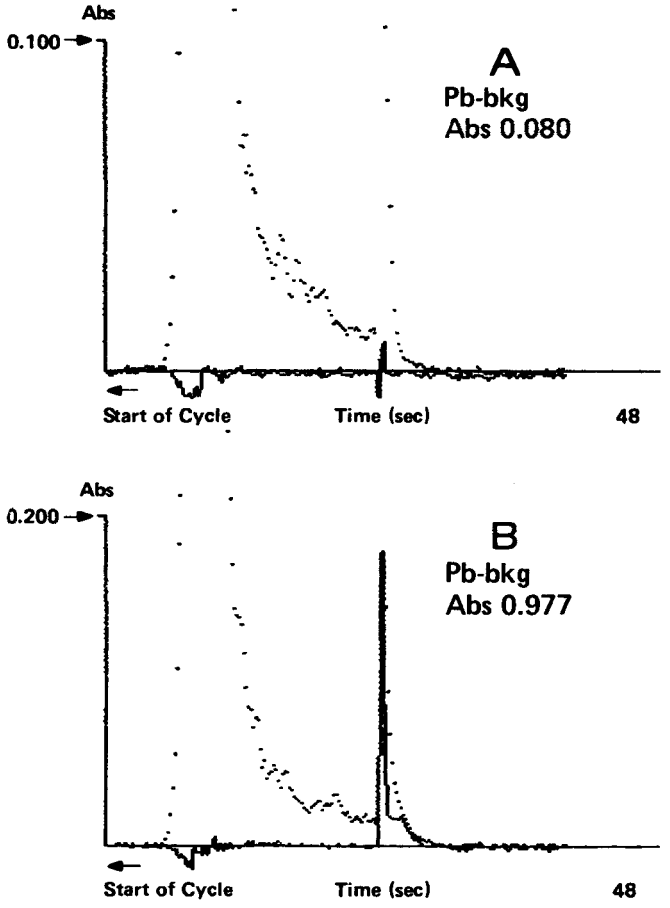


FIGURE 1 Absorbance profiles of human blood samples. A. Low lead (2 μg/dL), 10 × scale expansion B. High lead (51 μg/dL), 5 × scale expansion.

## Precision

The precision of the method varied from 5 to 12% depending on the blood lead concentration range. Within-run precision of 5 to 6% and between-run precision of 4 to 7% were obtained for blood lead concentration of ≧45 μg/dL (Table III). Comparable precision data have been reported for a reference method, namely, the MIBK extraction procedure.[14] As with other methods,[4,5] precision obtained at lower concentrations (≦14 μg/dL) was poorer, (10 to 12%) although well within the variability acceptable to CDC and other blood lead proficiency programs.

TABLE II

Comparison of lead recoveries using aqueous and bovine blood lead standards for calibration

| Known sample concentration μg/dL | Aqueous calibration concentration found, μg/dL | % recovery | Bovine calibration concentration found, μg/dL | % recovery |
|---|---|---|---|---|
| Aqueous 72 | 72 | 100 | — | — |
| 45 | 46 | 102 | — | — |
| 14 | 15 | 107 | — | — |
| Bovine 73 | 28[a] | 38 | 74[d] | 101 |
| 45 | 11[b] | 24 | 47[e] | 104 |
| 14 | 4[c] | 28 | 16[f] | 114 |

[abc]mean of 9 determinations from a single sample.
[d]mean of 2 determinations from a single sample.
[ef]mean of 4 determinations from a single sample.



TABLE III

Precision of lead measurements by the AIHL proposed method

| Control value, | μg/dL | Range, μg/dL | Mean | Std. Dev. | % Coef. of Var. |
|---|---|---|---|---|---|
| | | | $n=10$ | | |
| Within-run | 72 | 61.0–75.6 | 70.3 | 4.25 | 6.0 |
| | 45 | 44.6–53.9 | 48.6 | 2.59 | 5.3 |
| | 14 | 11.2–16.3 | 13.3 | 1.44 | 10.7 |
| | | | $n=15$ | | |
| Between-run | 73 | 67.3–80.0 | 75.3 | 2.84 | 3.8 |
| | 45 | 40.3–50.4 | 46.3 | 3.21 | 6.9 |
| | 14 | 10.1–18.0 | 14.1 | 1.75 | 12.4 |

## Accuracy

Accuracy of the method was determined through a series of on-going interlaboratory comparisons. AIHL's results have been subjected to continuous evaluation through our participation in two separate CDC blood lead proficiency programs. Results obtained by us in the Childhood Lead Screening and Toxicology Proficiency Testing Programs are plotted in Figures 2 and 3. An estimate of the accuracy of our method is indicated by the slopes, 1.03 and 0.978, respectively. These were obtained by regression analyses comparing our results with the CDC target values. Bovine blood samples[10] of various concentrations were prepared and sent to several reference laboratories. As shown in Table IV, analytical data

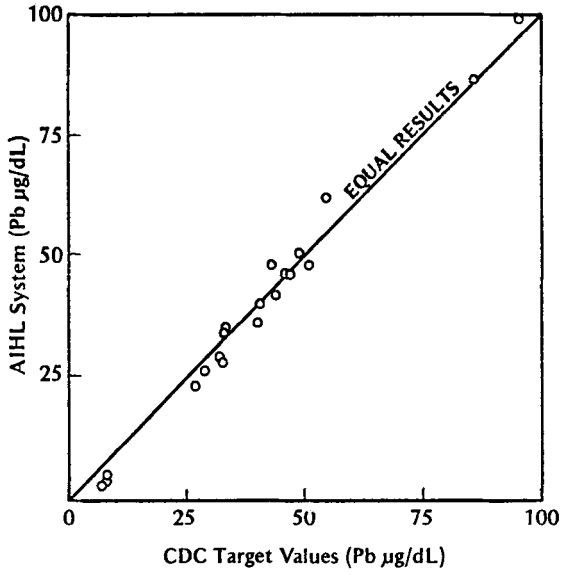


FIGURE 2 Results using AIHL system vs. results obtained by CDC Childhood Lead Proficiency Program in 1980.

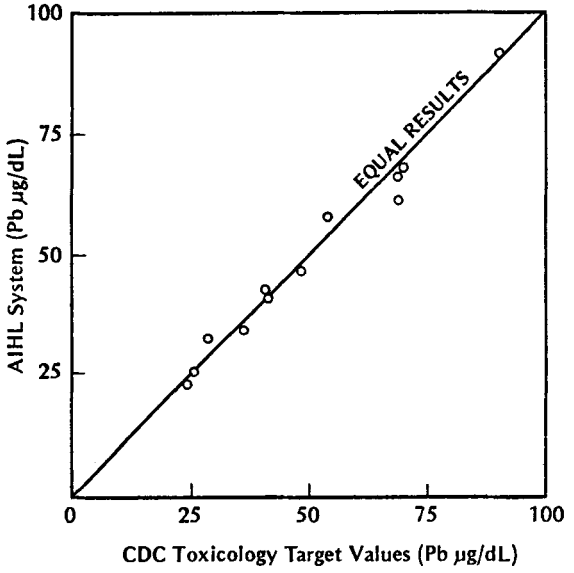


FIGURE 3 Results using AIHL system vs. results obtained by CDC Toxicology Proficiency Program in 1980.

TABLE IV

Interlaboratory comparison of AIHL bovine blood lead values with other reference laboratories in the Air and Industrial Hygiene Laboratory Lead Proficiency Testing Network

| Reference sample number | MIBK extraction | Delves cup | ASV[a] | HGA[b] | AIHL |
|---|---|---|---|---|---|
| | | Lead found, μg/dL[c] | | | |
| WG | 82, 80 | 86 | 83 | 79, 85 | 76 |
| WD | 69 | 70, 67 | 70 | 71 | 72 |
| GGRR | 14, 22 | 17, 18 | 13 | 13, 16 | 14 |
| WY | 44 | 47 | 46 | 49 | 48 |
| WO | 69 | 70, 67 | 70 | 71 | 72 |

[a]Anodic Stripping Voltametry.
[b]Heated Graphite Analyzer.
[c]Each value represents one laboratory.



obtained by the current AIHL method agreed closely with those reported by the other methods.

A limited comparison between the present method and a definitive reference method for lead determination, by mass spectroscopy-isotope dilution, was also made (Table V). Like other heated graphite-atomic absorption spectroscopy methods examined by CDC,[15] the AIHL system exhibited a 5% positive bias at the lower concentrations and a 6 to 9% negative bias at the higher concentrations. These biases are still well within acceptable ranges. In the CDC blood lead proficiency testing programs, results within $\pm 15\%$ or $\pm 6\,\mu g/dL$, whichever is greater, of the means obtained by the reference laboratories are considered acceptable.

TABLE V

Comparison of AIHL lead concentrations with NBS–MSID[a] data

| Sample number | Lead concentration, μg/dL | |
|---|---|---|
| | AIHL | NBS–MSID |
| DE1–A01 | 56.6 | 61.9 |
| DE1–A02 | 29.4 | 28.1 |
| DE1–A03 | 73.4 | 78.3 |

[a]Mass spectroscopy isotope dilution data from CDC Proficiency Testing Blood Lead Toxicology Survey-1, 1981.

## CONCLUSIONS

An instrumental system for the routine testing of whole blood for lead has been described. In use for over two years, the system is rapid, convenient and reliable and provides results well within acceptable ranges of precision and accuracy. Samples are not prone to contamination because minimal handling and no sample preparation other than dilution is required. Physiologically-bound lead standards were used in order to compensate for matrix effects. In conjunction with strict quality assurance and control procedures, the system has proven most useful in the California Childhood Lead Screening Program.

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