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Graphite Furnace Atomic Absorption Spectrometry for Biomonitoring of Blood Lead Level in Quality Control Blood Materials

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Abstract

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Fasa University of Medical Sciences **Background & Objective:** Lead (Pb) is a major toxicant that has threatened human health for years. There is no safe level of exposure and deposition of a small amount of Pb in the human body has a negative impact on an individual's health. Despite recent reductions in its use, it has caused extensive environmental contamination as well. In the present study, the intralaboratory quality control assessment is reported as an initial step in lead determination by Graphite furnace atomic absorption spectrometry (GFAAS).

Materials & Methods: Varian SpectrAA-220 with partition tube and deuterium background correction was used for the analysis. The evaluation quality control blood material was Seronorm trace elements whole blood (levels I &II).

Results: The lowest method limit of detection was 0.74 μ g/dL, and based on the regression analysis, the lowest quantification concentration was 3.00 μ g/dL (CV = 4.6 %). In addition, obtained %CV for Seronorm (I) & Seronorm (II) was 7.3% & 5.4% respectively.

Conclusion: In recent years, several diverse technological advancements in blood lead level determination have occurred. At the same time, demands for blood lead (BPb) level determination by GFAAS has been increased by public health in order to identify children exposed to lead in the environment, and by occupational health to reduce excessive exposure in the lead industries. The obtained results indicated that for BPb analysis, the method described by GFAAS is still a reference routine analytical technique that requires less time and no matrix matched standard curve, allowing blood samples to be run against aqueous calibration standards which eliminates the need for standard additions.

Keywords: Lead Exposure- Graphite Furnace Atomic Absorption- Biomonitoring-Precision-Intralaboratory.

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Introduction

Heavy metals toxicity is one of the oldest serious health and environmental concerns because of a wide variety of their applications in nutrition, occupational health, environmental health, toxicology, agricultural, etc. (1). After their

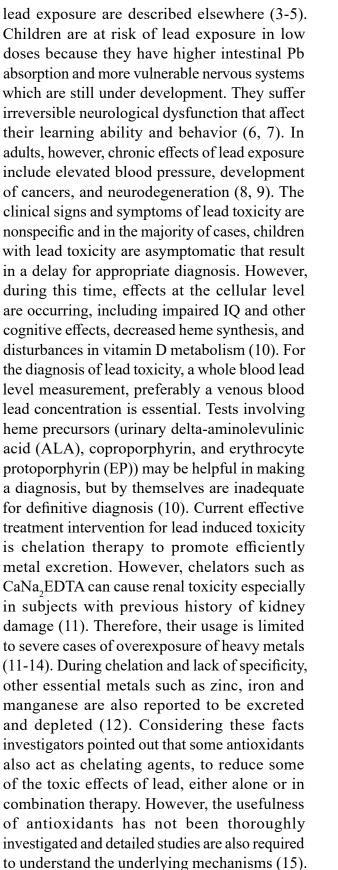
Corresponding Author: Aliasgharpour Mehri, Reference Health Laboratory, Ministry of Health & Medical Education, Tehran, Iran Email: aliasgharpour @health.gov.ir accumulation, they cause various adverse effects in the body system, characterized by heavy metal-mediated toxicity (2). Lead or Plumb (Pb) as a non-essential heavy metal with no biological function, is a major acute and chronic environmental and occupational toxin that has threatened human health. It has a wide range of industrial uses by humans and its important sources relating to human



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Absorption of Lead and Its Internal Distribution in the Body

Inorganic lead is absorbed through the respiratory and gastrointestinal (GI) tracts, but not through the skin, although organic lead compounds are absorbed (16). Adults absorb about 10% of dietary lead while young children absorb 40% to 50% of dietary lead and that about one-half the amount absorbed is retained. Once absorbed into the blood compartment, lead is distributed throughout the body with the major fraction in the bone (95% in adults and about 70% to 75% in young, growing children) (7) and soft tissue. The rate of turnover of lead in soft tissues is within days. In contrast, lead accumulates in bone with turnover of a half-life of about 30 years and it can be mobilized from bone under physiological and pathophysiological conditions of bone resorption. Furthermore, the rate of turnover of lead in bone is higher in children than in adults. Although lead toxicity affects all body systems (17, 18), the most susceptible ones to lead toxic effects are erythropoiesis, kidney function, and the central nervous system function (19). Approximately, 99% of the lead in blood is bound to RBCs. The remaining 1%, e.g, plasma lead, serves as an intermediate in transporting lead from the erythrocytes to other body compartments. The organs of excretion of lead are kidney and liver and the disappearance time largely depends upon the degree of overall excess exposure; the greater the body lead burden, the slower the rate of disappearance from the tissues, including blood (20). The underlying mechanisms for these systemic effects of lead have not been explained completely. However, several studies suggest that the most probable cause is the generation of free radical and/or depletion of the antioxidant defense system (21-24) that causes oxidative stress. Lead forms covalent bonds with the sulfhydryl groups of proteins, making the enzymes most susceptible targets for lead, including antioxidant enzymes. In addition, lead



neutralizes glutathione that serves as a major thiol

antioxidant. Furthermore, it interferes with some

essential metals needed for antioxidant enzyme

activities, and/or increases susceptibility of cells

to oxidative attack by altering the membrane

integrity and fatty acid composition. These interferences result in an imbalance between

production and neutralization of reactive oxygen

species (ROS) due to lead action (25, 26). Free

radicals have been implicated into DNA damage,

oxidation of thiol group(s) of proteins, and lipid

peroxidation (23) which are associated with the

From the early 1940s through the mid-1960s,

the method of choice for blood lead BPb

analysis was colorimetry/spectrophotometry

using dithizone diphenylthiocarbazone (27, 28).

Since then several new analytical advancements

in BPb concentration determination have

developed. These methods are very diverse and

include Flame atomic absorption spectrometry

(FAAS)(29, 30); Delves-cup FAAS (31, 32);

Anodic stripping voltammetry (ASV)(33, 34);

Graphite furnace atomic absorption spectrometry

(GFAAS)(35,36); Inductively coupled plasma

mass spectrometry (ICP-MS)(37); Thermal

ionization mass spectrometry (TIMS; ID-MS)

(38) and Point-of-care /near-patient testing,

or a rapid diagnostic/ rapid screening test (39,

40). Point of-care methods are very simple

to use and do not require trained laboratory

personnel. These methods differ significantly in

their analytical capacities (limits of detection,

accuracy and precision), costs (purchase

and maintenance, laboratory infrastructure

required, reagents and supplies) and technical

requirements (sample preparation, calibration

and skilled personnel). Of the various analytical

techniques available for measuring BPb, GFAAS

continues to dominate methods routinely used

by clinical laboratories for this purpose. Most

GFAAS systems use an electrically heated

graphite tube and programmed to control the

temperatures for drying, ashing, and atomizing the sample injected onto the carbon rod (41, 42).

onset of various diseases.

Methodology





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In the present study, intralaboratory quality control assessment as an initial step in BPb determination by GFAAS-Varian SpectraAA-220 system; with partition tube and deuterium background correction is reported (10).

Materials and Methods

Calibration of the System

GFAAS: A Varian SpectrAA-220 system with deuterium background correction was set for the analysis. Spectrometer was equipped with Varian GTA-110 partition graphite tube atomizer and a programmable sample dispenser. Inert gas was high purity argon. Peak height was used as a measurement mode. The instrument was calibrated using aqueous working pb standards (10, 20, 30, 40 & 60 μ g/L) made from Pb stock standard solution (1g/L). Solution volume injected was constant at 10 µL and two replicate readings were taken for standard solutions. At the time of analysis, quality control blood material (levels I & II) were manually diluted (1:10) with working ammonium dihydrogen phosphate modifier solution (NH₄H₂PO₄, Triton X-100 and HNO₂ modifier) and dispensed to the graphite tube. Then, the sample was heated to a temperature above 1700°C to atomize lead by absorbing light emitted from the Pb hollow cathode lamp at 283.3 nm. Other necessary chemicals and solutions used in the analysis were of a grade suitable for trace metal analysis and were purchased from Merck Co. The quality control blood material used for the evaluation procedures was Seronorm trace element & heavy metals whole blood (levels I & II) (Sero AS Asker-Norway). Deionized water (D.I-H₂O) was used throughout the reagent preparation and all working glassware was pre-acid-washed (10).

Precision Studies

Between-runs-precision studies were performed by reconstituting and pooling five Seronorm whole blood vials separately; quality control material levels (I & II) and analyzing aliquots of them (n=3) in 10 runs.





Linearity Studies

The linearity study was determined by assaying neat & diluted pooled Seronorm II (n=8). Dilution was performed by a factor of 2 (1/2, 1/4, 1/8) & 1/20. Data were analyzed with linear regression using SPSS software.

Blank/Quantitation Limits

As a conventional model, Mean \pm 3SD for detection limits was used. A blank (modifier) solution was run 15x and its concentration was determined against calibration curve. Mean \pm SD was calculated and 3SD was chosen as instrument detection limit. For the method detection limit, Seronorm Ι (by diluted factor 2) was until its

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concentration reached closer to the instrument detection limit and then was run 10x. Mean \pm SD was calculated and its 3SD was set as method detection limit (43).

<u>Results</u>

Precision study results for Seronorm levels I & II whole blood lead quality control materials are listed in Table 1 and obtained %CVs for Seronorm (I) & Seronorm (II) were 7.3% & 5.4% respectively. Linearity studies and quantitation determinations results are summarized in Table 2. Based on the obtained data, the lowest detectable concentration was 3.0 µg/dL which gave %CV of 4.6% and the "lowest method limit of detection" was 0.74 µg/dL.

 Table 1. Between-runs precision assay evaluation for seronorm level I & II whole blood quality control materials

Seronorm Whole Blood Level	Measured value Pb μg/dL * mean ± sd	Obtained Range Pb µg/dL	%CV **	Brushour Range Pb μg/dL	Target value Pb μg/dL
Ι	3.3±0.24	2.96- 3.60	7.3	3.1 -3.9	3.4
Π	36.95±2.0	33.60 -39.8	5.4	35.3- 44.3	40.1

* Between Runs Precision (10 runs & n=3) & ** Coefficient of Variation

Table 2. Linear regression analysis & quantitation determinations

Neat & Diluted *	Measured value μg/dL * mean ± sd	Slope	%CV
Seronorm Whole Blood Level II-Neat	34.6±0.69	0.96	2
1/2	17.96±0.24	0.96	1.4



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1/4	9.30±0.20	0.97	2.3		
1/8	6.0±0.23	0.78	3.8		
1/20	3.19±0.15	0.76	4.6		
LLD (Instrument) **	0.15±0.097				
LLD (Method) ** *	0.74±0.18				
Independent Variable= Conc * - n=8					

** - n=15; 0.097x3=0.29 ** * - n=10; 0.18 x 3=0.55

Discussion

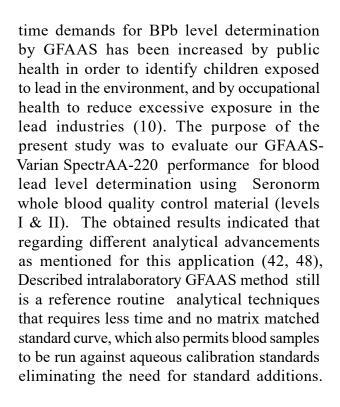
The development of portable technologies for the measurement of lead concentration in blood has been strongly supported and encouraged by the CDC and has also been expected to measure a BPb concentration of 5 μ g/dL (0.24 μ mol/L) with lower cost per test than current laboratorybased methods (10). It is expected that these technologies will permit screening for BPb in locations such as lead clinics. They may also permit immediate retesting of children who are determined to have elevated BPb results. However, these devices tend to overestimate blood lead level compared to GFAAS (44). With ICP-MS it is feasible to determine lead in blood at trace amounts below 1 μ g/dL (0.05 µmol/L) concentration and usually it is expected of methods that will meet modern research requirements. The greater sensitivity of the GFAAS (about 50 times) to the FAAS has made it possible to determine lead concentration in blood with no preconcentration, and requiring

samples of only μ L volumes (42). Furthermore, additional improvements in GFAAS technology for example; deuterium and zeeman background effect to correct the vaporized matrix components which are being atomized at the same time as the analyte and use of platform on which to deposit the sample has made it current for routine basis of a consensus method for BPb determination (44-46). Furthermore, for the purpose of analyzing blood Pb level, there are no spectral interferences from blood requiring zeeman background correction. High speed deuterium instruments have been successfully used for determining lead in blood (47).

Conclusion

In recent years, several technological advancements in blood lead level determination have occurred. These advancements are very diverse and include updated and enhanced older technologies as well as new analytical laboratory based technologies. At the same





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Conflict of Interest

None.

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