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# Hemolyzed, Lyophilized Bovine Blood for Quality Control of Lead Determination of Human Whole Blood

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The determination of Pb in human whole blood is perhaps the most common application of biological monitoring in occupational and environmental health (Grunder & Moffatt, Jr. 1982). The data from such determinations may be used for screening programs, diagnostic evaluation, assessment of workers exposed to Pb and its compounds, and in studies of environmental pollution (Boone et al. 1979). However, the lack of an adequate reference material has prevented a thorough evaluation of current analytical methods for Pb in blood and hence the evaluation of data generated by using such methods. As a result the concentration of Pb in human whole blood is not accurately known. This is evident from the wide scatter of interlaboratory data (Maher et al. 1979), and the wide ranges of values reported for the general population (Iyengar et al. 1978). In this paper we wish to report the preparation and analysis of two freeze-dried bovine whole blood reference specimens containing two different levels of Pb.

## MATERIALS AND METHODS

A Labconco Model 75040 Cascade Freeze Dryer 8 (Labconco Corporation, Kansas City, MO), equipped with a Labconco Model 75150 "Vac-Stop" Tray Dryer and a Model 8816 Director two-stage vacuum pump (Sargent-Welch Scientific Company, Skokie, IL), was used for freezing and freeze-drying the blood.

The blood sampling unit consisted of a cleaned and Pb-decontaminated plunger and barrel assembly of a Labindustries 10-mL Repipet dispenser with the barrel being set at the 5-mL mark and the liquid pick-up tube being replaced with a cleaned 40 cm x 0.5 cm o.d. x 0.3 cm i.d. Teflon tubing.

A Perkin-Elmer Model 603 atomic absorption spectrophotometer equipped with a Model HGA-500 graphite furnace, a deuterium arc background corrector and a Perkin-Elmer Intensitron Pb hollow cathode lamp operated at 6 mA and at a resonance wavelength of

283.6 nm (SBP=0.7 nm) was used for the determination of Pb. Nitrogen was the purge gas and its flow was interrupted during atomization.

High-purity water was obtained by passing tap water through a cellulose adsorbent, two mixed-bed ion-exchange columns and finally, distillation in a Corning AG-11 distillation unit. A 10% aqueous solution of diammonium hydrogenphosphate (Baker Analyzed Reagent, J.T. Baker Chemical Co., Phillipsburg, NJ) was prepared and purified as described by Subramanian & Méranger (1979). A 10% aqueous solution of Triton X-100 was prepared by adding 10 mL of the warm (40°C) reagent to 50 mL of water contained in a volumetric flask. The contents were vigorously mixed, and the final volume was adjusted to 100 mL with water.

A certified atomic absorption standard containing 1000 mg ± 1% Pb (II) per litre (So-L-21) was obtained from Fisher Scientific. Fresh working standards of lower concentrations of Pb were prepared by serial dilution of the stock solution in high purity water.

Two clinically healthy "Grade Holstein" heifers A and B, weighing 603 and 588 kg, respectively, were used as the source of blood. In order to provide a reference sample containing a baseline Pb level, cow A was not fed any lead salt other than that which would normally be derived from its regular diet of alfalfa and cereals. About 3 L of whole blood was withdrawn from its jugular vein and brought to the laboratory within ~30 min. The pooled blood was vigorously agitated for 1 h using an Eberbach shaker (Ann Arbor, Michigan).

The Nalgene bottle containing the blood, and the retort stand holding the sampling unit were placed on an oscillating hot plate with the oscillation turned on. The Teflon tubing was inserted into the blood. The oscillation was turned off and 5 mL aliquots of blood were immediately dispensed into 10-mL serum bottles. The bottles were partially stoppered and placed on a 28 x 28 x 2 cm stainless-steel tray. As soon as each tray was loaded it was placed in the Vac-Stop Tray Dryer. The samples were frozen to -35°C and freeze-dried for 24 h. The product temperature was then raised to 27°C and maintained at this temperature for 1 h to remove the last traces of moisture from the samples. At the end of this operation, the serum bottles were vacuum-stoppered automatically. The serum bottles were sealed, and the samples were stored in a refrigerator at 4°C.

The heifer B was given a single dose of 20 g of reagent-grade lead acetate trihydrate. The Pb content of the animal's blood was monitored at periodic intervals by drawing blood into triplicate Becton-Dickinson trace element-free vacutainers. When the Pb level was around 250 ng/mL, 3 L of blood was withdrawn and processed exactly as described above.

The Pb content of the composited liquid whole blood samples in quintuplicate was monitored using the $(NH_4)_2HPO_4$-TritonX-100-GFAA procedure of Subramanian & Méranger 1981). The Pb content of the freeze-dried blood materials was determined by using the above method and also a nitric acid-deproteinization-GFAA method (Stoeppler et al. 1978). The dilution factors used were 5 and 10 for the blood of heifer A and B, respectively, using the $(NH_4)_2HPO_4$-Triton X-100-GFAA method; and 4 and 8, respectively, using the $HNO_3$-deproteinization-GFAA method. The concentration of Pb was obtained by using the method of standard additions for both of these procedures.

## RESULTS AND DISCUSSION

Table 1 gives the values obtained for Pb in the two lyophilized control specimens by us and three other laboratories. In general there is good agreement in the values obtained for the two control samples. Thus, the mean value for heifer A ranges from 44 to 52 ng/mL with an overall average of 48 ng/mL. The % coefficient of variation was 4 to 11. Heifer A was not fed any Pb salt other than that which would normally be derived from its diet. Therefore, the value of 48 ng/mL corresponds to baseline Pb level of heifer A. The mean value of Pb in the blood of heifer B ranges from 223 to 246 ng/mL with an overall average of 238 ng/mL. Again the agreement is good. The % CV ranges from 1 to 7 indicating good precision. The good correlation in the values obtained using GFAA and ASV (anodic stripping voltammetry) by two different analysts in two different laboratories lends considerable confidence to the reference value given above for this control.

The Pb values of the composited liquid whole blood from which aliquots were dispensed into the serum bottles for freeze-drying were 46 ± 5ng/mL and 251 ± 26 ng/mL for heifers A and B, respectively as determined by the $(NH_4)_2HPO_4$-Triton X-100-GFAA method (Subramanian & Méranger 1981). These values are in good agreement with those obtained for the corresponding freeze-dried material. Thus, the Pb value was not affected by the freezing and freeze-drying process. In other words, the lyophilization of liquid whole blood did not result in any volatilization loss of Pb and freeze-drying is a good approach in the production of reference whole blood samples for Pb determination.

The bovine blood matrix is similar to that of human whole blood in its general physical and chemical characteristics. In particular nearly all of the Pb is bound to the erythrocytes in both human and cattle blood (Penton & Bissell 1978). Furthermore, the freeze-dried material containing the high amount of Pb (238 ng/mL) was prepared by feeding the cow with a lead salt so that the Pb in the blood will be derived from natural metabolic process and will be present in its natural chemical form. Up to 3 L of blood can

Table 1. Concentration of lead in two lyophilized bovine whole blood control materials

| Lab no. | Method | Concentration of lead, ng/mL Heifer A | Heifer B |
|---|---|---|---|
| 1 | Triton X-100-GFAA[a] (Fernandez 1975) | 52 ± 2[b](5)[c] | 244 ± 12 (5) |
| 2 | $(NHO_4)_2HPO_4$-Triton X-100-GFAA[d] (Subramanian & Meranger 1981) | 45 ± 5 (24) | 223 ± 15 |
| 2 | $HNO_3$-GFAA[e] (Stoeppler et al. 1978) | 50 ± 4 (24) | 242 ± 11 |
| 3 | $(NH_4)_2HPO_4$-Triton X-100-GFAA | 44 ± 3 (10) | not analyzed |
| 4 | ASV[f] (ESA 1983) | ≤50 (5) | 246 ± 2 (5) |
| | Overall mean | 48 ± 4 | 238 ± 11 |

a 8-fold and 16-fold dilution with 0.1% Triton X-100 for heifer A and heifer B, respectively, followed by 10 µL injections into Perkin-Elmer Model HGA-500 furnace.
b The results are expressed as the average ± standard deviation.
c The numberwithin the bracket indicates the number of samples analyzed.
d 5-fold and 10-fold diluted blood for heifers A and B, respectively.
e 4-fold and 8-fold dilutions for heifers A and B, respectively.
f ASV ≡ Anodic stripping voltammetry. The methodis not sensitive for values of Pb ≤ 50 ng/mL.

be removed from a 600 kg heifer without any harm (Swenson 1970), and this ensures production of the reference material in sufficient quantity for repeated analyses. Also, a continuous supply of the bovine blood material can be ensured by bleeding several Pb-fed cows, compositing their blood and then freeze-drying the latter.

The freeze-dried bovine blood material maintains its integrity during its useful shelf life. Over a period of ten months 5 randomly selected samples from each Pb level were analyzed at approximately 3-month intervals. The consistent results in the Pb values (Table 2) shows that the lyophilized samples are stable for at least for ten months. However, once the freeze-dried samples are reconstituted with water, the Pb value in the liquid sample remains stable only for two weeks provided it is stored frozen (Subramanian et al. 1983).

Table 2. Stability of lead in two lyophilized bovine whole blood control materials

| Measurement interval[a] | concentration of lead, ng/mL[b] | |
|---|---|---|
| | heifer A | heifer B |
| October, 1983 | 45 ± 5 | 223 ± 15 |
| January, 1984 | 47 ± 4 | 229 ± 12 |
| April, 1984 | 44 ± 3 | 227 ± 11 |
| August, 1984 | 48 ± 5 | 229 ± 8 |

[a] During each interval 5 randomly selected freeze-dried samples of heifers A and B were analyzed. [b]The analytical method used was that of Stoeppler et al. (1978). The values given represent the average ± standard deviation.

The homogeneity of the composited liquid whole blood was ensured by hemolysis with ammonium heparin and by intensive agitation. The good precision for the Pb values in the liquid pool shows its excellent homogeneity. The homogeneity of the freeze-dried blood was tested by determining Pb in an intercomparison survey involving 21 laboratories under the auspices of the International Union of Pure and Applied Chemistry. The mean values obtained for the two control materials were 53.74 ± 11.49 ng/mL and 242 ± 33 ng/mL, respectively, showing excellent homogeneity.

As more data become available, the values given here may be updated and more accurate numbers assigned. It is hoped that the production of lyophilized bovine whole blood quality control materials such as these will aid meaningful measurements of Pb in human whole blood samples.

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