Evaluation of the Delves Micro System for Blood Lead Analysis

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Correlation between results of a conventional macro-scale (5 ml of blood) atomic absorption procedure for lead determination and results of the Delves micro-scale (10 μl blood) atomic absorption method was high (0.96). Analyses of lead in capillary and intravenous blood samples also correlated well (0.93). The coefficient of variation of the Delves micro-scale method was 8% for a concentration of 46 μg of lead per milliliter. We conclude that the Delves micro-scale method—which is simpler, requires smaller samples than previous methods, and can be readily applied to capillary ("finger-stick") blood—is very suitable for a pediatric practice and for the screening of large numbers of patients.

Additional Keyphrases: conventional atomic absorption technique compared • results for capillary and intravenous blood lead compared • mass-screening procedure • lead poisoning • environmental hazards

Mass screening programs in old neighborhoods (1-3) indicate that many children in these areas have blood lead concentrations of more than 40 μg/100 ml. This means that undue absorption of lead by children in these areas is still a continuing problem in the practice of pediatrics.

Conventional methods for lead analysis have included the classical dithizone colorimetric technique (4) as well as many variations in the treatment of blood in order to prepare it for atomic absorption spectrophotometry. These latter methods, which have involved either wet or dryashing or extraction or a combination of both (5-7), are time consuming and usually require a minimum of 5 ml of blood. Because children in affected areas sometimes need to have blood drawn frequently, and since intravenous sampling can be a traumatic experience for children, we decided to evaluate a micro-scale method, the Delves atomic absorption procedure (8), which requires only 10 μl of capillary ("finger-stick") blood. The sampling-cup system used in this procedure makes it possible to determine low concentrations of easily atomized elements, such as lead, by atomic absorption spectrophotometry. The Delves technique has been described in detail in a manufacturer's publication (9).

Materials and Methods

Equipment

A Model 303 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn. 06852) was used under the following conditions.

Lamp: lead hollow cathode, 8 mA; wavelength, 283.3 nm; slit, 1.0 mm; band-pass, 0.7 nm. We used a 3-slot-head burner, used to produce a nonluminous air-acetylene flame, with an acetylene flow setting of 3.5 and an air flow setting of 10. A Perkin-Elmer 56 chart recorder was used with a chart speed of 120 mm minute⁻¹ and a range of 10 mV; it was controlled by a foot pedal. A Perkin-Elmer recorder readout was used with the following settings: Full-scale expansion (×1); noise suppression, ×1. A deuterium background corrector was used.

Hot plate: This was a “Thermolyne,” set at 140°C, thermostatically controlled (Sybron, Dubuque, Iowa 52001).

Sample trays: Perkin-Elmer, 20-cup holders.

Cups: Perkin-Elmer, Delves system nickel micro cups.

Eppendorf pipets: 10 μl and 20 μl (Brinkmann Instruments, Inc., Westbury, N. Y. 11590).

Sample collecting tubes for micro leads: 13 × 75 mm tubes soaked in dilute nitric acid (10 volumes of concd acid plus 90 ml of water) for 16 h, and washed with de-ionized water. Sodium heparin (50 μl, 1000 USP units/ml) was added to each tube, and the solution evaporated to dryness at 130°C for 10 min.

Collecting tubes for macro leads: Lead-free brown stopped tubes (Cat. No. L3200XF313; Becton-Dickinson and Co., Rutherford, N. J. 07070).

Reagents

All reagents, including water, must be assayed to ensure that they contain no lead.

Hydrogen peroxide: 30% by weight.

Stock lead standard: Pb(NO₃)₂ (1000 μg/ml of di-
lute nitric acid; Cat. No. 7635; Harleco, Philadelphia, Pa. 19143).

Stock working standard, 10.00 mg/liter: The stock standard was diluted 100-fold with de-ionized water on the day of analysis.

Working standards, 20, 40, 60, 80, and 100 μg/100 ml, are prepared from the stock working standard.

Procedure

Blood was drawn intravenously from 76 patients into disposable plastic syringes, and then transferred to lead-free heparinized tubes. These samples were analyzed by the technique of Berman (6). This procedure involves precipitation of protein with trichloroacetic acid, chelation of the lead with ammonium pyrrolidone dithiocarbamate, and extraction of the chelate into methylisobutylketone. The same intravenous samples were then analyzed by the Delves method (8). A comparison of the two techniques was made.

Blood was then obtained from 65 patients, taking one sample by the intravenous route described above, and one sample by capillary puncture. Capillary specimens were obtained from the end of a finger, with great care to avoid contamination. The whole finger, including under the nail, was scrubbed with "pHisohex" and then wiped off with an alcohol wipe. The finger was quickly punctured and the first drop of blood was discarded; the next drop was collected into the prepared lead-free tubes. The tube was then sealed with "Parafilm." The two types of samples were compared in a blind study of the Delves technique.

Samples for the Delves technique were prepared by transferring 10 μl (Eppendorf pipet) of well-mixed, clot-free blood into the small nickel cups, which had been preconditioned by placing each cup into the flame until the recorder pen returned to the baseline. The sample was evaporated to dryness at 140°C for 1.5 min. Hydrogen peroxide (20 μl of 30% H₂O₂, by weight) was then added to partially oxidize the sample. The cup was then reheated for an additional 2 min. The sample cup was then placed in the loop of the Delves cup assembly, and introduced into the flame of the atomic absorption spectrophotometer; the peak height was recorded on the strip-chart recorder, and the height of individual peaks determined from the chart graduations.

Standards for the Delves cup system were prepared by addition of 10 μl (in triplicate) of each of the working standards to the sample cups. After drying the samples at 140°C for 1 min, 10 μl of a blood known to have a low lead concentration was added to each cup. The concentration of lead in this blood was determined by the macro procedure and by comparison of the results obtained by another laboratory. After drying the samples at 140°C for 1.5 min, hydrogen peroxide (20 μl of 30%) was added and the samples again dried at 140°C for 2 min.

Results

Observations on the Analytical System

A typical recorder tracing of analyses of standards, a control blood, and a sample from a patient by the Delves micromethod is shown in Figure 1. The first peak of each determination is the smoke peak, which is caused by unashed organic matter. The deuterium background corrector greatly increased the resolution of the sample peaks, and diminished, but could not completely eliminate the smoke peak; the efficiency with which this smoke peak was decreased varied from day to day.

Figure 2 shows a typical standard curve in which triplicate analyses of each standard were made by the Delves micromethod. Absorption and the amount of lead were linearly related.

Figure 3 shows the comparison between analyses of 76 intravenous blood samples by the macro- and the
Delves micro-systems. The coefficient of correlation was 0.96, and the regression coefficient 0.92; the standard error of the latter was 0.03, and standard error of the estimate was 4.4 μg/100 ml.

Figure 4 compared the results for analyses done for intravenous blood and for the corresponding capillary blood specimens, drawn from each of 64 patients. These analyses were done as a blind study. In agreement with an unpublished observation of Delves (10), we noted a slight trend for the capillary analyses to be higher than those of the intravenous blood, although the coefficient of correlation was high (0.93), as was the regression coefficient (0.97); the standard error of the regression coefficient was 0.05, that of the estimate 2.2 μg/100 ml.

**Precision**

The precision of the Delves micromethod was tested by performing 50 replicate determinations on a single sample of whole blood. The coefficient of variation was 8% at the 46 μg/100 ml concentration, with a standard deviation of 3.7 μg/100 ml.

Twenty-five daily analyses on a sample of blood, 16 μg of lead per 100 ml, showed a standard deviation of 1.4; the results for another blood sample, which contained 76 μg of lead per 100 ml, was analyzed daily for 39 days, and had a standard deviation of 6.8 μg/100 ml.

**Accuracy**

To determine our laboratory's accuracy with the Delves micro technique, we obtained a number of blood samples of known lead content from Dr. S. C. Chen, Medical College of Ohio at Toledo, as part of a quality-control program sponsored by the USPHS. Dr. Chen uses three different methods: that of Farrelly and Pybus (7); that of Berman (6); and that of an independent laboratory, in which low temperature ashing is followed by a conventional colorimetric dithizone technique (11). For 24 analyses done over a six-month period, the coefficient of correlation between our method and Dr. Chen's values was 0.98.

Recovery experiments were also carried out by adding known amounts of lead nitrate to normal blood. As shown by the data of Table 1, the average recovery of the added lead was 97%.

**Discussion**

The Delves micro cup system appears to offer several distinct advantages over many of the previous methods available for the analysis of blood lead. It is not only precise and accurate, but it offers speed, and lead may be measured in capillary-blood specimens. Because only 10 μl of blood is required for an analysis, duplicate analyses are feasible, questionable analyses can be repeated without drawing additional blood. It has enabled our hospital, which is situated in the middle of an "inner-city" poor neighborhood, to begin routine screening for increased lead absorption.

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*Mean ± SD.*
Addendum: After this manuscript was submitted for publication, a paper by Olsen and Jatlow appeared (12), which described improvements in the Delves method as applied to intravenous blood samples and to urine.

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References