clarified by recognizing that ZPP indicates the iron being delivered in the bone marrow for heme (hemoglobin) synthesis (1). In other words, ZPP/H must be recognized as different from other indicators of iron status, such as stores and transport. When one considers exactly what the ratio is measuring, i.e., low marrow iron, the test becomes very specific. Thus, every abnormal ZPP/H finding is of medical importance, whether it be a result of nutritional iron deficiency, infection, blood loss, anemia of chronic disease, or accelerated erythropoiesis, to name a few underlying causes of relative iron deficiency anemia (2).

Given our results in this evaluation, we believe that whole blood specimens are suitable for ZPP/H determination and that washing erythrocytes is required only on a limited basis for obtaining clinically valuable results by hematofluorometry. Furthermore, use of a detergent to ensure complete hemolysis is a valid modification of the procedure to obtain consistent results in the determination of the ZPP/H ratio. The hemolyzing agent (Triton X-100) can be combined with the ProtoFluor reagent if desired. Obviously, these hemolysis findings raise questions about the principle of front-surface fluorescence/absorbance as described for hematofluorometry (3, 8). Perhaps the principle is more closely related to the right-angle fluorometry method used to study erythrocyte fluorescence (11).

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References

Evaluation of Vacutainer Plus Low Lead Test Tubes for Blood Lead and Erythrocyte Protoporphyrin Testing, Debra Eserson-Jensen,1* Valerie Bush,2 and Patrick J. Parsons3 (1 Division of General Pediatrics, North Shore University Hospital, 865 Northern Boulevard, Great Neck, NY 11021; 2 Becton Dickinson Vacutainer Tube Systems, 1 Becton Drive, Franklin Lakes, NJ 07417; 3 Wadsworth Center, New York State Department of Health, P.O. Box 509, Albany, NY 12210-0509; * address correspondence to this author at: Division of General Pediatrics, Suite 108, 410 Lakeville Road, New Hyde Park, NY 11042; fax 516-465-5399, e-mail DIPBJMJ@aol.com)

Lead poisoning is an important preventable environmental health problem. Blood lead concentrations as low as 100 μg/L (0.48 μmol/L) in whole blood have been shown to affect children’s neuropsychologic or cognitive performance adversely (1–3). In view of this, in 1991 the Centers for Disease Control and Prevention (CDC) lowered the concentration of lead in blood considered safe from 250 to 100 μg/L (from 1.21 to 0.48 μmol/L) (4). This lowered threshold has been reaffirmed by the CDC in their 1997 document on screening children for lead exposure (5). Before 1991, erythrocyte protoporphyrin (EP) was the standard test for screening children for lead poisoning; however, it was subsequently found to be an insensitive predictor of blood lead concentrations above the lower threshold (6). However, serial EP measurements, paired with matching blood lead measurements, are still useful in managing children with lead poisoning. In the absence of additional exposure of the child to lead, the EP concentration will continue to decrease at a faster rate than is reflected by the blood lead concentration as lead reequilibrates among blood, soft tissue, and bone stores.

Another recommendation in the 1991 CDC statement was to screen all children 6 months to 6 years of age for lead poisoning, using a direct blood lead measurement. Venous blood is preferred for blood lead measurement because capillary measurements may be falsely increased by skin contamination with lead. The issue of lead contamination of capillary blood specimens obtained by fingerstick was reported earlier (7). For diagnostic purposes, EDTA-containing evacuated tubes are traditionally used in collecting blood for lead and/or EP determinations. Each manufactured lot of tubes should be certified as free of analytically significant lead contamination by the blood lead testing laboratory before use. Such a practice is proposed in a recent NCCLS document on blood lead testing (8). A significant lead contamination is defined as one that would increase the blood lead concentration by >5 μg/L. Alternatively, the testing laboratory may recommend the use of trace element tubes.
(stoppers of tubes used in the US are royal blue) containing Na₂EDTA or heparin.

The aim of this study was to evaluate a recently introduced 3-mL draw volume, plastic Vacutainer Tube (VACUTAINER PLUS⁴ Low Lead; Becton Dickinson Vacutainer Tube Systems) containing powdered K₂EDTA, designed specifically for pediatric lead testing. Each manufactured lot of tubes is pretested and contains ≤2.5 μg/L of lead per tube before market.

Patients were recruited into the study from the Division of General Pediatrics, North Shore University Hospital, Manhasset, New York. Two tubes of venous blood were drawn from each participant for lead testing. One tube was a standard lavender-stoppered K₂EDTA Vacutainer Tube (control), which had already been independently certified by the testing laboratory for use in blood lead testing; the other tube was the VACUTAINER PLUS Low Lead tube (evaluation). The study included samples with lead concentrations within (Pb <100 μg/L, EP <350 μg/L) and above the health-related reference limits. Informed consent was obtained from all parents/guardians of participants before blood collection. The study protocol and the informed consent forms were reviewed and jointly approved by the Human Subjects Institutional Review Boards of North Shore University Hospital and the New York State Department of Health.

The control tubes were provided precertified by the Lead Poisoning/Trace Elements Laboratory, Wadsworth Center, New York State Department of Health, Albany, NY. The laboratory randomly prechecks each manufacturer’s lot of tubes, as well as the needles used to collect blood for lead testing, by an well-established protocol (8). The laboratory serves as a referee laboratory in several national proficiency testing programs for blood lead and free EP, and participates in several international interlaboratory programs for lead lead. The VACUTAINER PLUS Low Lead tubes (evaluation) were provided by Becton Dickinson.

Blood specimens were protected from exposure to light by wrapping the tubes with aluminum foil to prevent photodegradation of EP. Control and evaluation specimens were shipped at ambient temperature to the Wadsworth Center for analysis within 5 business days of collection. The time between receipt and analysis in the laboratory varied from 1 to 3 days, during which time the specimens were stored refrigerated at 4 °C. Blood lead was determined by graphite furnace atomic absorption spectrometry with Zeeman background correction on a Perkin-Elmer LS 50B fluorometer (10).

We obtained 62 paired samples. All specimens were satisfactory for lead and EP determinations. Whole blood lead ranged from <10 to 265 μg/L. Differences in blood lead between the control and evaluation tubes ranged from <10 to 32 μg/L. EP values ranged from 150 to 1490 μg/L, with differences between the control and evaluation tubes ranging from −60 to 85 μg/L.

Difference plots (Fig. 1) were used to analyze the data (11). The graphs were generated by plotting the difference between each experimental and control tube value vs the mean of the respective control and evaluation values. As shown, the evaluation tube values fell within ±2 SD of the control tube ranges. The SD limits represent the allowable variation as calculated from the control tube. The values suggest a slight upward trend in abnormal lead (>100 μg/L) and EP (>350 μg/L). The slopes of the upward trends, for lead and EP, were significantly different from zero at P <0.001.

The mean (± 2 SD) of the control tube for blood lead was 67 ± 9.7 μg/L compared with 69 ± 9.7 μg/L for the evaluation tube. The average difference (evaluation minus control) was 7 ± 1.8 μg/L for lead. For EP, the mean (±

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⁴ Use of product or trade names is for informational purposes only and does not imply an endorsement by the New York State Department of Health.

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Fig. 1. Difference plots for whole blood lead (A) and EP (B). The means between evaluation and control tubes are plotted on the x axis; the differences between the two tubes are plotted on the y axis. The ± 2 SD limits based on allowable differences within the controls are shown as solid lines.
2 SD) was 360 ± 23 μg/L for the control tube and 370 ± 23 μg/L for the evaluation tube, with an average difference between tube types of 35 ± 4.3 μg/L. The test results for blood lead and EP on samples collected in the evaluation tubes were clearly equivalent to those collected in the independently certified, lead-free control tubes. The availability of pediatric pretested lead-free collection tubes will contribute to accurate and reliable diagnosis of lead poisoning, a preventable environmental disease.

This study was carried out with the cooperation of Becton Dickinson Vacutainer Tube Systems, Franklin, NJ, who provided the evaluation tubes and covered the costs of laboratory tests.

References

Measurement of Plasma Total Homocysteine by HPLC with Coulometric Detection, Steven C. Martin,* Ioannis Tsakas-Ampezis, William A. Bartlett, and Alan F. Jones (Department of Clinical Chemistry, Heartlands Hospital, Birmingham, West Midlands, B9 5SS, United Kingdom; *author for correspondence: fax 44 121 766 8693, e-mail s.c.martin@bham.ac.uk)

Homocysteine (HcySH) is an intermediate in the metabolism of methionine. High plasma HcySH concentrations are an independent risk factor for stroke, peripheral vascular disease, deep venous thrombosis, coronary disease, and neural tube defects (1). Because HcySH circulates mostly as disulfides complexed with albumin, measurement of plasma total HcySH involves reduction of these disulfide bonds and detection of the generated HcySH. Once reduced, HcySH can be separated chromatographically using ion-paired reversed-phase HPLC and is amenable to oxidative mode coulometric electrochemical detection, which avoids time-consuming pre- or postcolumn derivatization. The use of electrochemical detection with an appropriate internal standard, penicillamine, has led to substantial improvements over our previously reported method (2).

The assay required 400 μL of EDTA plasma, which was reduced with 100 μL of 50 mmol/L dithiothreitol [DTT; modified from Ref. (3)] containing the internal standard (200 μmol/L penicillamine) at 37 °C for 15 min. After incubation, protein precipitation was achieved by the addition of 100 μL of 150 g/L sulfosalicylic acid. We injected 20 μL of the supernatant onto a 250 × 4.6 mm octadecyl silica column (Spherisorb ODS-2; particle size, 5 μm; Jones Chromatography Ltd.), with a 10 × 4.6 mm guard column packed with the same material. The mobile phase (flow rate, 1 mL/min) consisted of 10 mmol/L diammonium hydrogen phosphate adjusted to pH 2.58 (2.63 mmol/L H+ ion) with orthophosphoric acid, 137.5 mL/L methanol, and 12 mmol/L octane sulfonate (OSA). The mobile phase was filtered through a 0.45 μm cellulose membrane before use and was not recycled during chromatography.

We detected HcySH with an ESA Coulochem 5100A electrochemical detector (ESA Analytical Ltd), using an ESA 5010 coulometric cell and with electrode 1 (E1) set at a screening potential of +0.4 V and electrode 2 (E2) set at +1.0 V. An ESA 5020 guard cell was set to +1.1 V and installed between the pump and the autosampler (ISS200; Perkin-Elmer). The guard cell cleans the mobile phase electrochemically before addition of sample and reduces the background current at detector E2. The signal (peak height) resulting from oxidation of HcySH was measured at E2 and processed using a computer integrator (Gilson Unipoint Software).

HcySH calibrators (0.625–40 μmol/L) were prepared by dissolving l-homocystine (Sigma Chemical Co.) in 2 mmol/L disodium hydrogen phosphate. l-Homocystine has been reported to be purer than commercially available d,l-homocystine (4). The internal quality control was citrated plasma (time-expired, fresh-frozen plasma; West Midlands Blood Transfusion Service) with HcySH added at 8.5 and 26 μmol/L.

Each component of the mobile phase was adjusted independently to achieve optimal separation of HcySH from other electrochemically active components. When an initial mobile phase of 10 mmol/L diammonium hydrogen phosphate buffer, pH 2.75, containing 6 mmol/L OSA and 100 mL/L methanol was used, sulfosalicylic acid was not retained by the column. Reduced DTT eluted early (at ~5.7 min), and oxidized DTT eluted at ~9.6 min. When the phosphate concentration of the mobile phase was increased, the retention times for all peaks were decreased without alteration of their elution sequence. Previous methods used sodium dihydrogen phosphate as the