Anodic Stripping Voltammetry Compared with Graphite Furnace Atomic Absorption Spectrophotometry for Blood Lead Analysis, *Desmond I. Bannon*^{1*} and *J. Julian Chisolm, Jr.*² (¹ Kennedy Krieger Institute, Neurology, 707 North Broadway, Baltimore, MD 21205; ² Kennedy Krieger Institute, Trace Metal Laboratory, 3001 East Biddle St., Baltimore, MD 21213; * author for correspondence: fax 410-502-8093, e-mail dbannon@jhmi.edu)

According to a recent CDC report, blood lead concentrations in 1 000 000 US children are at values associated with irreversible damage to health (1). The effects of chronic lead poisoning on the developing nervous systems have been well documented (2), with children in inner city neighborhoods, where older housing stocks have deteriorating lead paint, most vulnerable (3). Accurate screening of children for lead exposure is, therefore, of paramount importance.

The current biomarker for assessment of lead exposure is venous blood lead, commonly measured by anodic stripping voltammetry (ASV) or graphite furnace atomic absorption spectrometry (GFAA). Although both of these techniques have been used in our laboratory for 15 years, with ASV being our instrument of choice for clinical blood lead analysis, there is surprisingly little published information on ASV as a clinical tool for blood lead analysis.

Here we present comparative data on ASV and GFAA analyses of blood lead in our clinic, with a novel reagent for calibration of ASV. For ASV, we used the ESA 3010B Trace Metals Analyzer (Environmental Science Associates) with a mercury-coated graphite electrode, a Ag/ AgCl reference electrode, and a platinum counter electrode. For GFAA (4), we used a Zeeman/5100 PC atomic absorption spectrophotometer with HGA-600 graphite furnace and AS-60 autosampler (Perkin-Elmer).

For analysis by ASV, instead of the manufacturer's reagent we used a novel reagent developed at our laboratory that is based entirely on chloride salts and HCl. HCl is suitable for electroplating of metals. Importantly, hemoglobin, the predominant protein found in erythrocytes, is soluble in hydrochloric acid but not in nitric or perchloric acids. In this reagent, Pb²⁺ ions are found within the pH range 1.3-1.4, above which there is slow conversion to PbOCl⁻, whereas at pH values less than this range, H₂ is driven off and recorded as a Pb signal, leading to false positives. We included nickel in the reagent to complex excess EDTA in the anticoagulant, as it has a higher affinity for EDTA than Pb²⁺ ions have. Additionally, EDTA was preferred to heparin because, in our experience, heparinized samples stored in the refrigerator tend to form gels and give inconsistent analysis (5).

This reagent is prepared by dissolving 52.6 g of KCl in 1500 mL of deionized water. To remove any trace quantities of lead, this solution was passed through a cation-exchange column. The following chemicals were added in the filtrate: 76 mg of HgCl₂ (Spex Industries), 320 mg of NiCl₂ (Johnson Matthey), 0.2 mL of 2-octanol (Sigma), and 0.4 mL of Triton X-100 (Sigma). The solution was thoroughly mixed and diluted to a total volume of 2 L with

deionized water. The pH was adjusted to 1.3–1.4 with 16.7 mL of 6 mol/L HCl [G. Frederick Smith (GFS Chemicals, Powell, OH)]. The manufacturers' tubes were used for sample analysis.

Venous blood samples were collected from children who were referred to the lead clinic at the Kennedy Krieger Institute. Collections were in accordance with the Institute's protocol on patients and were performed with stainless steel butterflies, polypropylene tubing, and 3-mL Vacutainers containing potassium EDTA as anticoagulant. Acceptable tubes were at least three-fourths filled to avoid interference because of excess EDTA. We combined 100 μ L of well-mixed blood with 2.900 mL of reagent and analyzed samples after a few minutes. Several samples can be prepared in advance. Analysis was carried out as recommended by the manufacturer.

Calibration of the ASV was carried out with calibrators made from bovine blood, which was filtered and homogenized by sonification. PbNO₃ was added to the approximate concentration required and thoroughly mixed. The values of these calibrators were then established by comparisons with human blood that had been assayed by thermal ionization mass spectrometry as described previously (4). The calibrators had assigned values of 0.284 μ mol/L (59 μ g/L), 1.736 μ mol/L (360 μ g/L), and 3.376 μ mol/L (700 μ g/L). The quality-control material had values of 0.385 μ mol/L (80 μ g/L) and 1.736 μ mol/L (360 μ g/L). These were analyzed after calibration and at least after every 10 samples. Our requirement for process control was a measured value within 0.097 μ mol/L (±20 μ g/L) of the certified value for each high and low qualitycontrol sample for both instruments.

We first compared the performance of ASV and GFAA in the Wisconsin State Laboratory of Hygiene Proficiency Testing Program (CDC). From the fitted regression lines (summarized in Table 1), both linear models fit the data and there was no systematic error associated with a plot of the residuals (data not shown). There was no bias associated with either method, although the proportional error, represented by the slope, was slightly greater for GFAA than for ASV. In both cases, the model accounted for >96% of the variance (regression coefficient, R^2). As expected, these two methods performed well in the measurement of blood lead from single-blind proficiency programs over a representative range of blood lead concentrations. Results of a previous study (4) comparing an earlier model, the 3010A, with GFAA were as follows: ASV, y = 0.984x + 0.476 ($R^2 = 0.982$); GFAA, y = 0.977x $+ 0.292 (R^2 = 0.996).$

ASV and GFAA are compared in Fig. 1 for the analysis of human blood samples. The model shows that 98% of

Table 1. Linear regression statistics for the performance of
ASV and GFAA in the Wisconsin State Laboratory/
CDC Blood Lead Proficiency Program for 53 samples.

	Slope	Intercept	R ²
ASV	0.999 ± 0.0119	-0.010 ± 0.0168	0.995
GFAA	0.966 ± 0.0096	-0.002 ± 0.0136	0.996



Fig. 1. Linear regression lines and statistics of the relationship between ASV and GFAA for blood lead analysis of 53 clinical samples.

the variability in ASV is explained by the GFAA measurement. We saw no systematic error associated with a plot of the residuals (data not shown). The linear model accounted for the data, and the underlying assumptions (linearity, stable variance) of the model have not been violated.

The upper limit of linearity was 3.377 μ mol/L (700 μ g/L), although we routinely used a linear calibration up to 1.930 μ mol/L (400 μ g/L), which encompassed almost all of our clinical samples. The limit of detection, measured as 3 SD of seven replicates of the low calibrator, was 0.048 μ mol/L (10 μ g/L). The within-day and betweenday CVs, estimated by repeated measures (n = 21), were 11% and 7%, respectively, for a sample measuring 0.284 μ mol/L (59 μ g/L) and 2% (both within-day and betweenday) for a sample measuring 1.739 μ mol/L (360 μ g/L).

These data demonstrate that the newer ASV technology (3010B) is comparable to GFAA for blood lead analysis. The ASV Model 3010B blood lead analyzer is well suited in size, cost, and operation for a clinic. The ASV 3010B showed a marked improvement in stability, ease of operation and precision over the earlier model (4) and, according to our results, performs as well as or better than the 5100 GFAA.

This paper's coauthor, J. Julian Chisolm, Jr., MD, professor emeritus of pediatrics at Johns Hopkins School of Medicine and director emeritus of the Lead Poisoning Prevention Program at Kennedy Krieger Institute, died on June 20, 2001. He will be fondly remembered as a colleague, mentor, and friend. This work was funded by the Lead Poisoning Prevention Program at the Kennedy Krieger Institute.

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Compatibility of the Abbott IMx Homocysteine Assay with Citrate-Anticoagulated Plasma and Stability of Homocysteine in Citrated Whole Blood, Darryl E. Palmer-Toy,* Zbigniew M. Szczepiorkowski, Vivian Shih, and Elizabeth M. Van Cott[†] (Massachusetts General Hospital, Department of Pathology, Division of Laboratory Medicine, Boston, MA 02114; * Current address: Johns Hopkins School of Medicine, Department of Pathology, 600 N. Wolfe St./Meyer B-125, Baltimore, MD 21287; † address correspondence to this author at: Coagulation Laboratory, Massachusetts General Hospital, Department of Pathology, Division of Laboratory Medicine, Gray-Jackson 235, 55 Fruit St., Boston, MA 02114; fax 617-726-7758, e-mail evancott@partners.org)

Hyperhomocysteinemia is widely regarded as a risk factor for arterial thrombosis (1, 2), and it is also implicated as a risk factor for venous thrombosis (3-6). Therefore, homocysteine (Hcy) often is included in hypercoagulability evaluations (7). The Abbott IMx Hcy fluorescence polarization immunoassay instructions recommend EDTA- or lithium heparin-anticoagulated plasma or serum, whereas other coagulation tests are generally performed on citrate-anticoagulated specimens. To simplify specimen collection and avoid unnecessary phlebotomy, we investigated the compatibility of the Abbott IMx Hcy assay with citrate-anticoagulated plasma. Previous reports have suggested that acidic citrate stabilizes the Hcy concentration in whole-blood specimens for at least 6 h at room temperature (8, 9). Therefore, we also studied the stability of Hcy in whole-blood specimens collected in sodium citrate.

To evaluate the correlation between citrate and EDTA Hcy values, 114 sets of paired specimens were concurrently obtained from 96 nonfasting individuals (87 healthy volunteer platelet donors and 9 patients undergoing hypercoagulability evaluation) in Becton Dickinson Vacutainer lavender-top [tripotassium EDTA (K₃EDTA)] and blue-top (3.2% sodium citrate) tubes. For each patient, the citrate tube was drawn immediately before the EDTA tube, in accordance with the NCCLS guidelines. The conditions and timing for specimen collection, processing, storage, and assay performance were identical for the two anticoagulants. The specimen pairs were separated from the cells within 30 min of phlebotomy unless specimen