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 μmol/L (700 μg/L), although we routinely used a linear calibration up to 1.930 μmol/L (400 μg/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 μmol/L (10 μg/L). The within-day and between-day CVs, estimated by repeated measures (n = 21), were 11% and 7%, respectively, for a sample measuring 0.284 μmol/L (59 μg/L) and 2% (both within-day and between-day) for a sample measuring 1.739 μmol/L (360 μg/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.

We appreciate the invaluable help of Veronica Kestenberg, research assistant, for technical assistance and for helping to prepare this manuscript, as well as the technical assistance of Chester Bowen.

References

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
transportation would be longer than 30 min, in which case specimen pairs were placed immediately on ice and separated from cells within 4 h. Plasma samples were then kept frozen at −20 °C or lower until blinded analysis using a standard Abbott IMx analyzer and a single lot of Abbott Hcy reagents (kindly donated by Dr. Jessie Shih, Abbott Diagnostics, Abbott Park, IL) (10). Five pairs of specimens from healthy donors were omitted because of errors or delays in processing or collection, leaving 109 specimen pairs for analysis.

To compare the stability of Hcy in citrate vs EDTA-whole-blood specimens, five paired K3EDTA and citrate specimens were concurrently drawn from each of seven healthy volunteers. Plasma was separated from the cells within 30 min of phlebotomy and after 2, 4, 8, and 24 h at room temperature. Storage and analysis were as above. Informed consent from all volunteer donors and Institutional Review Board approval were obtained for this study. The paired Student t-test (two-tailed) was used to calculate statistical significance.

Among the 109 paired samples in the correlation study, Hcy concentrations were 4.8–17.8 µmol/L in EDTA samples and 4.0–16.7 µmol/L in citrate samples. One obvious outlier point was eliminated from further analysis. For the healthy donors, the mean and median Hcy concentrations and nonparametric reference interval (2.5th to 97.5th percentile) were 8.1, 7.9, and 5.0–12.3 µmol/L, respectively, in EDTA and 6.9, 6.9, and 4.3–10.3 in citrate (P <0.000001 for the difference between EDTA and citrate). The proportional bias can be seen in Fig. 1A, and in the following equation calculated by standard linear regression:

\[ \text{Hcy}_{\text{citrate}} = 0.85(\text{Hcy}_{\text{EDTA}}) + 0.13 \, \mu\text{mol/L} \]

\( r^2 = 0.839; 95\% \text{ confidence intervals (CIs) for slope and intercept, 0.78–0.92 and -0.48 to 0.73 \, \mu\text{mol/L}, respectively).} \)

Some difference between citrate and EDTA results was expected because citrate tubes contain a larger volume of anticoagulant than do EDTA tubes, which produces a slight dilutional effect on the citrate results. The K3EDTA tubes draw 10 mL of whole blood and contain 0.117 mL of EDTA solution (150 g/L), whereas the citrate tubes draw 4.5 mL of whole blood and contain 0.5 mL of sodium citrate (105 mmol/L). To correct for the difference in dilution of whole-blood volume, citrate results were multiplied by a correction factor, \( C_B \):

\[ C_B = \frac{(\text{blood volume, EDTA})(\text{blood + anticoagulant volume, EDTA})}{(\text{blood volume, citrate})(\text{blood + anticoagulant volume, EDTA})} \]

\[ (10)(5) \]

\[ (4.5)(10.117) = 1.098 \]

Multiplication of the citrate results by 1.098 improved the agreement between citrate and EDTA results. However, the difference between EDTA and corrected citrate results remained significant (\( P = 0.0000017 \)), and some proportional bias remained, as seen in Fig. 1B and in the following linear regression equation:

\[ \text{Hcy}_{\text{citrate}} = 0.93(\text{Hcy}_{\text{EDTA}}) + 0.14 \, \mu\text{mol/L} \]

\( r^2 = 0.839; 95\% \text{ CIs for slope and intercept, 0.85–1.01 and -0.52 to 0.80 \, \mu\text{mol/L}, respectively).} \)

The correction factor, \( C_B \), corrects for the dilution of whole blood by the anticoagulant in the tube. However, if the citrate and EDTA anticoagulant solutions remain extracellular, then the correction factor should only reflect the centrifuged specimen plasma volume and not the whole blood volume:

\[ C_P = \frac{(\text{plasma volume, EDTA})(\text{plasma + anticoagulant volume, EDTA})}{(\text{plasma volume, citrate})(\text{plasma + anticoagulant volume, EDTA})} \]

Assuming a plasma volume of 57% (hematocrit, 43%), the revised correction factor, \( C_P \), is 1.171. When we used this correction factor, the differences between citrate and EDTA results were no longer significant (\( P = 0.4 \)), and the proportional bias was essentially eliminated \([Hcy_{\text{citrate}} = 0.99(\text{Hcy}_{\text{EDTA}}) + 0.15 \, \mu\text{mol/L}; \, r^2 = 0.839; 95\% \text{ CIs for slope and intercept, 0.91–1.08 and -0.56 to 0.86, respectively; not shown} \])

Hematocrit values for the specimens were not available to determine individual specimen plasma volumes, but gender and hemoglobin concentrations were known. Therefore, two additional types of plasma correction factors were calculated: gender- and hemoglobin-based. The gender-based correction factors, assuming average hematocrits of 40% for females and 46% for males, were 1.163 and 1.18, respectively (Fig. 1C). The hemoglobin-based correction factor, calculated assuming that hematocrit is three times the hemoglobin (g/dL), was 1.177 on average, with a range of 1.157–1.218 (Fig. 1D). Both types of correction essentially eliminate the proportional bias: \( \text{Hcy}_{\text{citrate}} = 1.00(\text{Hcy}_{\text{EDTA}}) + 0.10 \, \mu\text{mol/L} \) (\( r^2 = 0.839 \)) for gender; \( \text{Hcy}_{\text{citrate}} = 0.99(\text{Hcy}_{\text{EDTA}}) + 0.20 \, \mu\text{mol/L} \) (\( r^2 = 0.839 \)) for hemoglobin (95% CIs for slope and intercept, 0.92–1.08 and -0.62 to 0.81 \( \mu\text{mol/L} \) for gender-based and 0.90–1.07 and -0.52 to 0.92 \( \mu\text{mol/L} \) for hemoglobin-based corrections, respectively). There was no significant difference between these corrected citrate and EDTA results (\( P = 0.3 \) and 0.2, respectively). The Hcy reference interval (nonparametric range from 2.5th to 97.5th percentile) in citrate when the \( C_P \) (hematocrit, 43%) or the hemoglobin- or gender-based correction factor was used was 5.0–12.0 \( \mu\text{mol/L} \). We recommend the gender-based correction factor for citrate Hcy measurements because patient gender is generally provided with all clinical specimens, the residual bias is negligible and perhaps slightly less than when the single plasma volume correction factor \( C_P \) is used, and its use is simpler than the hemoglobin-based approach. We suspect that such a correction factor would be appropriate for correlating most analyses performed in both citrate and EDTA, but
each assay should be evaluated for specific interactions of the anticoagulant with analytes or reagents (11).

Several authors have compared chemistry assay performance in sodium citrate and EDTA, and generally a negative proportional bias has been noted in which citrate results underestimate EDTA results, similar to the unadjusted results in the present study. Among the explanations for this effect are whole blood dilution (12, 13), osmotic effects (14), and specific interactions with the analytes (15). In the present study, the proportional bias was best eliminated by correcting for differences in plasma dilution.

In the stability study, no significant difference was evident in the stability of Hcy in blood over 24 h in the two anticoagulants (Table 1). Initial Hcy concentrations were 6.1–10.8 μmol/L in EDTA and 5.5–10.1 μmol/L in citrate (uncorrected). In both anticoagulants, an upward trend in Hcy concentration was evident starting at 4 h. Acidic citrate prevented this upward trend in previous studies (8, 9). In contrast to the standard sodium citrate tubes used in the present study, acidic citrate tubes contain 0.5 mL of 0.5 mol/L citrate acidified to a pH of 4.3.

In summary, Hcy concentrations measured by the Abbott method in EDTA- and citrate-anticoagulated plasma agree well after a plasma-volume correction factor is taken into consideration. Specifically, citrate Hcy results

![Figure 1](image-url)

Fig. 1. Bland–Altman plots showing Hcy concentrations in EDTA- and citrate-anticoagulated blood samples. All plots show the difference (y axis) vs the mean (x axis). (A), no correction for citrated samples (n = 108). (B), whole-blood volume correction for citrated samples (n = 108). (C), gender-based plasma volume correction for citrate (n = 108). (D), hemoglobin-based plasma volume correction for citrate (n = 105). Three patients were omitted from D because neither hemoglobin measurements nor hematocrits were available.
Table 1. Stability of Hcy in EDTA- and citrate-anticoagulated whole blood.

<table>
<thead>
<tr>
<th>Hours post collection</th>
<th>EDTA a</th>
<th>Citrate b</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>8</td>
<td>1.5 ± 0.2</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>24</td>
<td>1.9 ± 0.2</td>
<td>1.8 ± 0.4</td>
</tr>
</tbody>
</table>

a For each time point, n = 7 healthy volunteers.
b Relative to EDTA result from immediate analysis.
c Relative to citrate result from immediate analysis.

agree well with EDTA results if the citrate value is multiplied by 1.163 for females and 1.18 for males. No difference in specimen stability over time was evident in citrate tubes compared with EDTA tubes.

We wish to thank the platelet donors and the staff of the Massachusetts General Hospital Blood Transfusion Service and Amino Acid Laboratory for their invaluable assistance in this study. We thank Dr. Jessie Shih (no relation to author) of Abbott Diagnostics for kindly donating the Abbott reagents used in this study.

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Serum C-Reactive Protein in Canadian Inuit and Its Association with Genetic Variation on Chromosome 1q21, Robert A. Hegele, Matthew R. Ban, and T. Kue Young (1 John P. Robarts Research Institute, London, Ontario, N6A 5K8 Canada; 2 Department of Community Health Sciences, University of Manitoba, Winnipeg, Manitoba, R3E 0W3 Canada; * address correspondence to this author at: Blackburn Cardiovascular Genetics Laboratory, John P. Robarts Research Institute, 406-100 Perth Dr., London, Ontario, N6A 5K8 Canada; fax 519-663-3789, e-mail robert.hegele@rri.on.ca)

Mortality from cardiovascular disease among Inuit living in the far north of Canada is ~40% lower than in the rest of the country (1). This might be attributable to the protection resulting from environmental factors such as dietary ω-3 fatty acids in Arctic fish (1). Genetic factors may also be important. For example, the thermolabile variant of methylenetetrahydrofolate reductase is much less prevalent among Inuit than among subjects of European origin (2). However, there are also paradoxical genetic findings in these people. For example, some common genetic variants associated with a higher risk of cardiovascular disease, such as the APOE E4 and AGT T235 alleles, are more prevalent among Inuit than among subjects of European origin (3). These apparent inconsistencies may be related to the fact that there are numerous determinants of susceptibility to cardiovascular disease and that these determinants may differ among ethnic groups (4). As newer determinants of cardiovascular disease risk are identified, these can be evaluated in the Inuit.

C-Reactive protein (CRP), an acute-phase reactant originally detected through its interaction with pneumococcal C polysaccharide (5), has been proposed to be a risk factor for cardiovascular disease (6). When detected with a high-sensitivity assay (6), increased serum CRP could be related to increased vascular disease risk either directly through its association with inflammation (7) or indirectly through its association with obesity and insulin resistance (8). The serum CRP concentration in the Inuit has not been reported. Furthermore, the role of possible genetic determinants of serum CRP concentration has not been explored in depth. Our recent discovery of a silent single-nucleotide polymorphism (SNP) in the CRP gene, namely 1059G→C within exon 2 (9), and of other chromosome 1q21 SNPs near CRP (10) has allowed analysis of the association between the CRP locus and serum CRP concentration in Inuit.

The Northwest Territories are located above the 60th parallel of latitude and comprise one-third of the landmass of Canada. In 1986, the population of Northwest Territories was 52,000. Of these, 35% were Inuit (or Eskimos), 15% were Dene (or Athapaskan Indians), and 50% were predominantly migrants of European origin from other parts of Canada. The present study involved residents of eight communities from the Nunavut region, mainly from the western shore of Hudson Bay (2, 3, 11).

Randomly selected individuals (n = 516; age range,