Effects of Hemoglobin C and S Traits on Glycohemoglobin Measurements by Eleven Methods, *William L. Roberts,[1*] Sekineh Safar-Pour,[2] Barun K. De,[3] Curt L. Rohlfing,[4] Cas W. Weykamp,[5] and Randie R. Little[4]* ([1] Department of Pathology, ARUP Institute for Clinical & Experimental Pathology, University of Utah, Salt Lake City, UT; [2] ARUP Laboratories, Salt Lake City, UT; [3] Department of Pathology, University of Arizona, Tucson, AZ; [4] Departments of Pathology & Anatomical Sciences and Child Health, University of Missouri-Columbia School of Medicine, Columbia, MO; [5] Queen Beatrix Hospital, Winterswijk, The Netherlands; * address correspondence to this author at: ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108; fax 801-584-5207, e-mail william.roberts@aruplab.com)

Patients with diabetes mellitus routinely have glycohemoglobin (GHb) testing performed to monitor glycemic control and assess risk for developing complications of their disease *(1)*. The accuracy of several GHb methods can be adversely affected by the presence of hemoglobin (Hb) C or S trait *(2–6)*. It has been estimated that there are at least 200 000 Americans with diabetes mellitus who also have either Hb C or S trait *(6)*. We have recently shown that the presence of Hb C or S trait does not affect the accuracy of GHb measurements made by the CLC 330 boronate affinity HPLC method *(7)*. We therefore evaluated the effects of Hb C and S traits on 11 commercial GHb methods, using the CLC 330 assay as the comparison method.

Whole blood samples from individuals homozygous for Hb A (n = 73) and heterozygous for Hb C or S (n = 46 and 76, respectively) were collected in EDTA-containing tubes. After routine clinical testing had been completed, Hb variants were identified by inspection of chromatograms obtained with a VARIANT analyzer (Bio-Rad Laboratories) and the Beta Thal Short program run according to the manufacturer's instructions. Aliquots of these samples that had 4–14% Hb A_{1c} were stored at 2–8 °C and analyzed within 10 days of collection except for aliquots for the HA8160 and HA8160 Beta Thal (BT) methods, which were shipped on dry ice and stored frozen until analysis. Not all samples were analyzed by each analytic method. This study was approved by the Institutional Review Board of the University of Utah.

Samples were analyzed by the following instruments/methods: A1c 2.2 Plus and G7 (Tosoh); A1cNow (Metrika); D-10, DiaSTAT, and VARIANT II (Bio-Rad Laboratories); Dimension RxL (Dade Behring); HA8160 HbA1c and HA8160 BT (Menarini Diagnostics); and PDQ (Primus). All of these methods were used according to the manufacturers' instructions and have been certified by the National Glycohemoglobin Standardization Program (NGSP). The CLC 330 GHb analyzer (Primus) was used as the comparison method in an NGSP Network Laboratory with in-house calibrator materials and assigned values. Results for all methods are reported as NGSP Hb A_{1c} equivalents.

For each test method, results obtained for each type of sample (homozygous Hb A, heterozygous Hb C, and heterozygous Hb S) were compared with those obtained by the CLC 330 comparison method. An overall test of coincidence of two least-squares linear regression lines was performed with SAS software (SAS Institute) to determine whether the presence of Hb C or S trait caused a statistically significant difference ($P < 0.01$) in results relative to the comparison method. Deming regression analysis was performed to determine whether the presence of Hb C or S trait produced a clinically significant effect on GHb results. Given recommendations by the American Diabetes Association of an upper reference limit of 6% and an action limit of 8%, we chose Hb A_{1c} evaluation limits of 6% and 9%. After correcting for possible calibration bias by comparing results from the homozygous Hb A sample group, we evaluated method bias attributable to the presence of Hb C or S trait, with a clinical significant difference being >10% (i.e., 0.6% at 6% Hb A_{1c} and 0.9% at 9% Hb A_{1c}).

The presence of Hb C trait produced statistically significant differences ($P < 0.01$) for all methods tested except for the DiaSTAT, HA8160, and PDQ methods. The presence of Hb S trait produced statistically significant differences for all methods except for the D-10 and PDQ methods. Box-plots for each combination of sample type and method are shown in Fig. 1. We observed no clinically significant interference attributable to Hb C or S trait with the A1c 2.2 Plus, Dimension RxL, G7, HA 8160, HA 8160 BT, and PDQ methods (Table 1). The presence of both Hb C and S traits produced clinically significant positive biases for the A1cNow and VARIANT II methods at 6% and 9% Hb A_{1c}, respectively. Hb C trait produced a clinically significant negative bias for the D-10 method at 9% Hb A_{1c}. Hb S trait produced a clinically significant positive bias at 6% Hb A_{1c} for both the DiaSTAT and DS5 methods. Several methods, including the D-10, DiaSTAT, Dimension RxL, DS5, HA8160, and VARIANT II, showed increased scatter when samples containing Hb C or S trait were tested compared with that seen for samples homozygous for Hb A.

Our results have several similarities to previous studies. For example, one immunoassay method, the Dimension RxL, exhibited no clinically significant effect with either Hb C or S trait, but another immunoassay method, the A1cNow, exhibited a clinically significant positive bias with samples containing both Hb C and S traits. The Unimate and Cobas Integra immunoassay methods have been shown to exhibit a positive bias with both Hb C and S traits, whereas the DCA 2000, Tina-quant, and SYNCHRON CX 7 methods exhibit no clinically significant bias with either Hb C or S trait *(3, 4, 6)*. Likewise, several ion-exchange methods, including the A1c 2.2 Plus, G7, and HA8160, were not affected by either Hb C or S traits. The DiaSTAT, DS5, and VARIANT II all exhibited a clinically significant positive bias with samples containing Hb S trait. It has previously been shown that some ion-exchange methods, including the Diamat, HA8140, and VARIANT, have a positive bias with samples containing Hb S trait *(4, 6)*. It is noteworthy that two ion-exchange methods in the present study exhibited a clini-

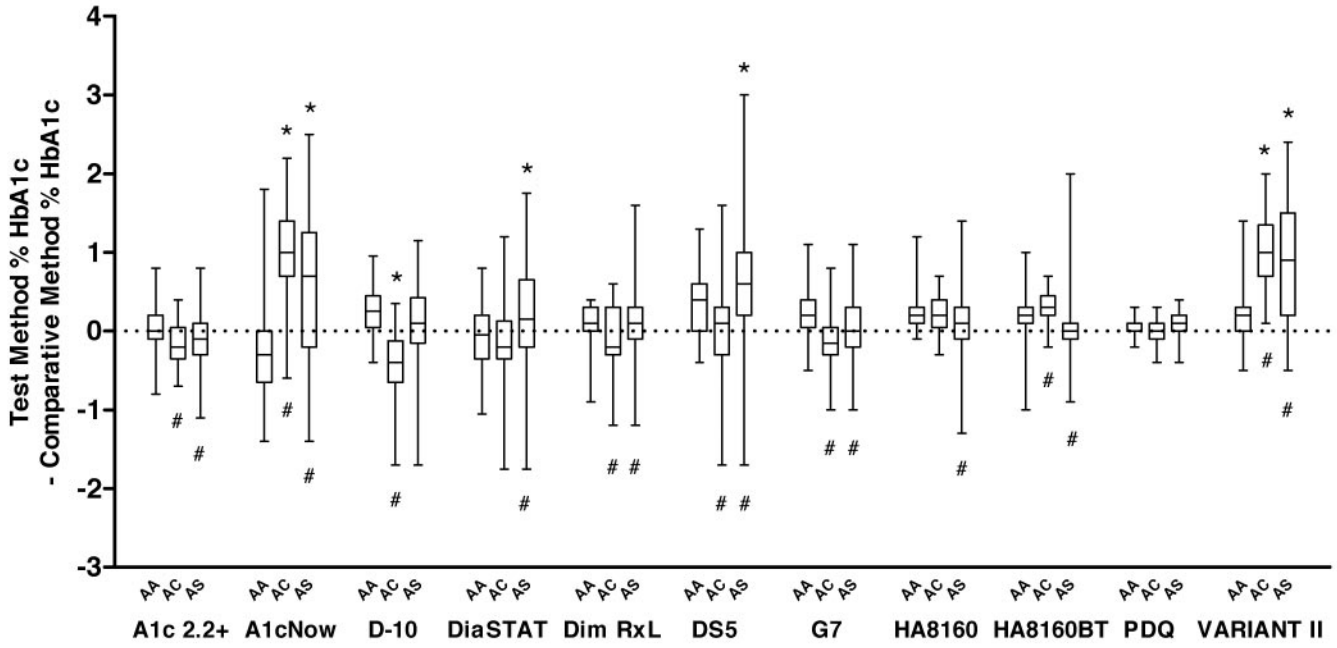

Fig. 1. Box-plots summarizing the absolute differences between each test method and the comparison method for each Hb phenotype.

The *horizontal line inside* each *box* is the median difference between the test and comparison methods. The *upper* and *lower limits* of each *box* correspond to the 25th and 75th percentiles of the differences, respectively. The *upper* and *lower error bars* represent the maximum and minimum differences between the test and comparison methods. Differences from Hb AA that are statistically significant ($P < 0.01$) are indicated (#), as are clinically significant differences (*).

cally significant bias with samples containing Hb C trait. The D-10 method exhibited a negative bias, whereas the VARIANT II exhibited a positive bias. If a method demonstrates a positive bias attributable to Hb variants, then overly rigorous blood glucose control may be instituted with a concomitant increase in hypoglycemic episodes. If a method demonstrates a negative bias, then more rigorous blood glucose control may not be instituted, and the patient may have poorer glycemic control than is optimal.

We have previously examined the effects of Hb C and S traits on the A1c 2.2 Plus and VARIANT II ion-exchange methods *(4, 6)*. Neither method exhibited a clinically significant effect with either variant Hb in these earlier reports. In our present study, the A1c 2.2 Plus method exhibited no clinically significant effects with either variant Hb, but the VARIANT II method exhibited clinically significant effects for both Hb C and S traits. We have previously noted that another ion-exchange GHb method may intermittently show effects with Hb S trait samples, and we speculated that it may be attributable to variabil-

Table 1. Mean differences between the test methods and the comparison method for samples containing either Hb C or S trait.[a]

		Hb C trait			Hb S trait		
Method	**Assay principle**	**n**	**6% Hb A_{1c}**	**9% Hb A_{1c}**	**n**	**6% Hb A_{1c}**	**9% Hb A_{1c}**
A1c 2.2 Plus	Ion exchange	17	−0.08	−0.40	75	−0.22	−0.53
A1cNow	Immunoassay	39	+1.17[b]	+1.35[b]	62	+1.35[b]	+1.73[b]
D-10	Ion exchange	45	−0.43	−1.11[b]	76	+0.14	−0.22
DiaSTAT	Ion exchange	37	−0.11	−0.09	75	+0.79[b]	+0.46
Dimension RxL	Immunoassay	35	−0.06	−0.37	48	+0.03	+0.39
DS5	Ion exchange	43	−0.13	−0.72	74	+0.90[b]	+0.71
G7	Ion exchange	46	−0.29	−0.49	76	−0.16	−0.53
HA8160	Ion exchange	46	+0.02	−0.22	76	−0.24	−0.83
HA8160 BT	Ion exchange	46	+0.17	+0.04	75	−0.05	−0.49
PDQ	Boronate affinity	27	+0.01	−0.08	67	+0.01	+0.07
VARIANT II	Ion exchange	46	+0.82[b]	+0.91[b]	76	+1.25[b]	+1.28[b]

[a] Deming regression analysis was performed with CLC 330 as the comparison method. The mean differences (%) between the comparison method and each of the other methods at clinical decision cutoffs of 6% and 9% were calculated for each Hb trait. To correct for intermethod calibration differences, the mean difference for homozygous Hb A samples was subtracted from that calculated for samples containing Hb C or Hb S trait.

[b] Difference was clinically significant (>0.6% or >0.9% Hb A_{1c} at 6% and 9% Hb A_{1c}, respectively).

ity from lot to lot in mobile phase or column packing material *(4)*. It appears that intermittent interferences from Hb C and S traits with some, but not all, ion-exchange GHb methods continues to be an issue.

In summary, some current GHb methods show clinically significant interferences with samples containing Hb C or S trait. These interferences are not necessarily consistent within method types, and with ion-exchange methods may vary over time with changes in column or reagent lots.

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Effects of Oral Contraceptives and Hormone Replacement Therapy on Markers of Cobalamin Status, *Bettina Riedel,[1*] Anne-Lise Bjørke Monsen,[2] Per Magne Ueland,[3] and Jørn Schneede*[3] ([1] Laboratory of Clinical Biochemistry, Section of Clinical Pharmacology, and [2] Department of Pediatrics, Haukeland University Hospital, Bergen, Norway; [3] Locus for Homocysteine and Related Vitamins, University of Bergen, Bergen, Norway; * address correspondence to this author at: Laboratory of Clinical Biochemistry, Section of Clinical Pharmacology, Haukeland University Hospital, 5021 Bergen, Norway; fax 47-55-97-4605, e-mail bettina.marie.riedel@helse-bergen.no)

Low serum concentrations of vitamin B_{12} (cobalamin) have been observed in users of oral contraceptives (OCs) *(1)*, in women during pregnancy *(2)*, and in men treated with high doses of ethinylestradiol for prostate cancer *(3)*. Similar effects of hormone replacement therapy (HRT) have been noticed by some *(4)* but not all investigators *(5)*.

Serum cobalamin has low diagnostic accuracy as a marker of vitamin B_{12} status *(6)*. Because of the long-term consequences of cobalamin deficiency [see Ref. *(7)* for a review], it is important to investigate whether decreased total cobalamin in OC or HRT users is associated with other evidence of impaired cobalamin status, such as increased plasma concentrations of methylmalonic acid (MMA) and total homocysteine (tHcy) and decreased plasma concentrations of holo-transcobalamin (holoTC) *(8)*.

We performed a cross-sectional study on 264 female healthcare students and workers. All participants gave written informed consent, and the study was approved by the Regional Ethical Committee and by the Norwegian Social Science Data Services. Study groups were OC users (n = 54) and controls (n = 81; age range, 18–40 years) and HRT users (n = 51) and controls (n = 78; age range, 41–65 years). The most frequently used OCs were triphasic combination tablets containing the synthetic estrogen ethinylestradiol and the progestogens levonorgestrel or drospirenon [Trinordiol™ (Wyeth-Lederle) or Trionetta™ or Yasmin™ (Schering AG)]. The most commonly used HRTs were either tibolone (Livial™; Organon) or the naturally occurring estradiol in combination with the progestogen noretisterone (Trisekvens™, Kliogest™, or Activelle™; Novo Nordisk A/S). We did not collect data on menstrual cycle or menopause. Detailed characteristics of the study population are given in Table 1 of the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue4/.

Venous blood samples were collected over 6 months on a single occasion from each of the 264 consecutive nonfasting individuals. Blood samples used for tHcy measurements were immediately placed on ice, and EDTA-plasma was separated within 2 h after collection. For serum, blood was allowed to clot at room temperature for 30 min before centrifugation. The samples were kept at −80 °C until analysis, and the maximum storage time was 2.5 years.

Serum creatinine was analyzed by the Jaffe alkaline picrate method, and plasma MMA, plasma tHcy *(9)*, serum folate, whole-blood cell folate *(10, 11)*, serum cobalamin *(12)*, and plasma holoTC *(13)* were measured with the cited methods. A more detailed description of the methods is given in the online Data Supplement.

We used SPSS 10.0 for Windows NT 4.0 (SPSS Inc.) for statistical analyses, if not otherwise indicated. Mean values (range) are given for gaussian-distributed variables and median values (range) for variables showing skewed distributions. Means were compared by the Student *t*-test and medians by the Mann–Whitney *U*-test. Categorical data were compared between groups with the Fisher exact test. Logistic regression analysis was used to assess whether OC and HRT use independently affected plasma concentrations of MMA and tHcy after adjustment for age, serum creatinine, serum folate, number of cigarettes smoked, vitamin supplementation, and dietary factors, and results are reported as odds ratios (ORs) with 95%