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Glycohemoglobin (gHb) is a biochemical marker of long-term glycemic control that is highly correlated with complications of diabetes mellitus (1). The presence of hemoglobin (Hb) variants can adversely affect the accuracy of some gHb methods, depending on the variant [for recent reviews, see Refs. (2, 3)]. Previous studies have shown that some gHb methods yield inaccurate results with samples heterozygous for Hb C or S (4–7). In 2000, there were an estimated 26 million black or African Americans over age 17, of whom at least 10% had either Hb C or S trait (8–11). The prevalence of diabetes is estimated to be 5.1% of the adult population, with rates for non-Hispanic blacks 1.6 times greater than in non-Hispanic whites (12). It is therefore probable that at least 200,000 Americans with diabetes have either Hb C or S trait. We investigated the measurement of gHb in specimens containing Hb C or S trait, using eight commercial gHb methods that are currently in clinical use.

Whole blood samples from individuals homozygous for Hb A (n = 43) and heterozygous for Hb C or S (n = 43 and 61, respectively) were collected in EDTA tubes. Hb variants were identified by their characteristic chromotograms obtained with a VARIANT analyzer (Bio-Rad Laboratories) operated according to the manufacturer’s instructions and using a Beta Thal Short program. Aliquots of these samples, containing 4–14% Hb A1c, were stored at 2–8°C and analyzed within 10 days of collection. This study was approved by the Institutional Review Board of the University of Utah Health Sciences Center.

The CLC 330 gHb analyzer (Primus Corporation) was operated at the University of Missouri. This method was chosen as the comparative method for this study because results from boronate affinity chromatography are not expected to be influenced by the presence of Hb variants (13–16). The following methods/instruments were evaluated: Cobas INTEGRA 700 Hemoglobin A1c whole blood application (Roche Diagnostics Corporation), Glyco-Tek affinity column method (Helena Laboratories), Glycosal HbA1c (Provalis Diagnostics), HA8140 analyzer (Menerini Diagnostics), Nycocard HbA1c (Primus Corporation), Synchro CX7 (Beckman Coulter, Inc.), and VARIANT II HbA1c and VARIANT Total GHB analyzers (Bio-Rad Laboratories). All of these methods are certified by the National Glycohemoglobin Standardization Program (NGSP; June 2001) except for the Glyco-Tek method (17). CLC 330 HPLC analyses were performed in a NGSP Network Laboratory, using in-house calibrator materials and assigned values. For all other methods, samples were analyzed following the manufacturers’ instructions.

For each test method, results for each group of samples (Hb AA, Hb AC, Hb AS) were compared with results from the comparative method (CLC 330). An overall test for coincidence of two least-squares linear regression lines was performed using SAS software (SAS Institute Inc.) 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, using EP Evaluator release 3 software (David G. Rhoads, Kennett Square, PA), to determine whether the presence of Hb C and S traits produced clinically significant effects. Given the Diabetes Control and Complications Trial Reference Method upper reference limit of 6% and the American Diabetes Association goal and action limits of 7% and 8%, respectively, 6% and 9% Hb A1c were chosen as important evaluation limits. Given the need to clearly distinguish the difference
among 6%, 7%, and 8% Hb A1c, a >10% deviation (0.6% at 6% Hb A1c and 0.9% at 9% Hb A1c) compared with the Hb AA sample group was used to define a clinically significant difference.

The presence of Hb C produced statistically significant differences for all methods tested except the HA8140, Nyocard, and Synchron CX7 methods. The presence of Hb S trait produced statistically significant differences for all methods except the Nyocard and Synchron CX7 methods. Box plots for each group of samples and for each method are shown in Fig. 1. No clinically significant interference attributable to Hb AC or Hb AS was seen with the Nyocard, Synchron CX7, VARIANT GHb, and VARIANT II methods (Table 1). Both Hb AC and Hb AS produced clinically significant positive interferences for the Cobas INTEGRA method. The presence of Hb AC produced a clinically significant positive interference for the Glyco-Tek method at both 6% and 9% Hb A1c and a borderline clinically significant positive interference for the Glycosal method at 6% Hb A1c. The HA8140 method showed clinically significant interference from Hb AS at 6% Hb A1c, but not from Hb AC. The increased scatter observed with the Cobas INTEGRA and VARIANT GHb methods for samples containing Hb C or S trait compared with Hb AA samples was also noteworthy.

The Cobas INTEGRA immunoassay method showed clinically significant positive interferences with samples containing either Hb C or S trait. These variants result from a substitution of the glutamic acid at position 6 on the β chain by either a lysine (Hb C) or valine (Hb S) residue (5). With immunoassay methods, these amino acid substitutions at position 6 may alter the shape of the protein sufficiently to change its binding characteristics with the reagent antibody (16). A similar effect has been reported previously for the Roche Unimate immunoassay method (6). The package insert for the Cobas INTEGRA method indicates that specimens containing Hb S and Hb C variants may yield higher than expected gHb results. In our study, all samples with Hb C or S trait had results that were at least 1% Hb A1c higher than the comparison method. Because of this positive bias and the fact that this bias is highly variable, ranging from 1% to 6% Hb A1c in our study, samples with Hb C or S trait should not be tested by this method. The Cobas Integra, like all immunoassay and affinity-chromatography methods, does not indicate when a variant Hb is present; therefore, the Hb phenotype should be determined before testing if the patient is a member of an ethnic group with a high prevalence of these variants. If patients with Hb C or S trait are identified, an alternative method that is not subject to interference should be used. This approach may or may not be feasible depending on the ability of the laboratory and/or clinician to keep track of patients with Hb C or S trait and the costs associated with determining the Hb phenotype.

The Glyco-Tek method uses a boronate affinity resin to measure total gHb. One possible explanation for the overestimation of gHb with Hb AC specimens is charge interactions between the lysine residue at position 6 of the Hb C β chain and the boronate resin. Why this effect is

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**Table 1. Average differences from the comparison method for samples containing either Hb C or S trait.**

<table>
<thead>
<tr>
<th>Method</th>
<th>Assay principle</th>
<th>6% Hb A1c</th>
<th>9% Hb A1c</th>
<th>6% Hb A1c</th>
<th>9% Hb A1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobas Integra</td>
<td>Immunoassay</td>
<td>2.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glyco-Tek</td>
<td>Boronate affinity</td>
<td>1.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>Glycosal</td>
<td>Boronate affinity</td>
<td>0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.72</td>
<td>0.37</td>
<td>0.55</td>
</tr>
<tr>
<td>HA8140</td>
<td>Ion exchange</td>
<td>0.22</td>
<td>0.28</td>
<td>0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.57</td>
</tr>
<tr>
<td>Nyocard</td>
<td>Boronate affinity</td>
<td>0.23</td>
<td>0.07</td>
<td>−0.13</td>
<td>−0.13</td>
</tr>
<tr>
<td>Synchron CX7</td>
<td>Immunoassay</td>
<td>−0.52</td>
<td>−0.27</td>
<td>−0.41</td>
<td>−0.19</td>
</tr>
<tr>
<td>Variant II</td>
<td>Ion exchange</td>
<td>0.42</td>
<td>0.65</td>
<td>0.57</td>
<td>0.43</td>
</tr>
<tr>
<td>Variant GHb</td>
<td>Boronate affinity</td>
<td>0.59</td>
<td>0.86</td>
<td>0.40</td>
<td>0.66</td>
</tr>
</tbody>
</table>

<sup>a</sup> Deming regression analysis was performed using the CLC 330 as the comparison method. The average differences (%) of each of the other eight 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.

<sup>b</sup> Clinically significant (>0.6% or >0.9% Hb A1c at 6% and 9% Hb A1c, respectively) differences were found.
seen for the Glyco-Tek method and not for the Nycocard, VARIANT GHB, and CLC 330 affinity methods is uncertain. The Glycosal method, which also uses the boronate affinity principle, shows a clinically significant positive interference only at 6% Hb A1c and performs adequately at 9% Hb A1c. These results indicate that there are differences between boronate affinity methods with respect to accuracy with samples containing Hb C trait. Each boronate affinity method should be carefully evaluated for interference by common variant Hbs that may be encountered before being used for patient samples that contain these variants.

Overestimation of gHb by the HA8140 ion-exchange method with samples containing Hb S trait was clinically significant only at 6% Hb A1c. One explanation may be that Hb A0 and Hb S0 are not well resolved from each other. This could lead to underestimation of Hb A0 and consequent overestimation of the percentage of Hb A1c. A previous report on the HA8140 method indicated that for Hb C, S, and D traits, the abnormal Hb eluted after the Hb A0 peak was identified as a variant and did not interfere with measurement of Hb A1c (18). Our results suggest that there may be some lot-to-lot variability in the column resin and/or mobile phase that could affect the chromatographic separation. A similar effect has been observed for other ion-exchange methods (7).

It is noteworthy that all clinically significant interferences resulting from the presence of Hb C and S traits were positive. The resulting overestimation of gHb could lead to an overly aggressive treatment regimen with a consequent increase in hypoglycemic episodes. Although this hypothesis still requires formal testing in a clinical outcome study, it seems likely that, given the magnitude of the effects observed in the Glyco-Tek method for Hb C trait (median difference of 1.4% Hb A1c) and in the Cobas INTEGRA method for both Hb C and S traits (median differences of 2.7% Hb A1c and 1.9% Hb A1c, respectively), some increase in the frequency of hypoglycemic episodes will occur.

In conclusion, the presence of Hb C or S trait can produce clinically significant differences in gHb results for some methods. For all of the methods examined in this study that had clinically significant effects, the percentage of Hb A1c was overestimated. Attention to the effects of Hb variants on the results of gHb methods is necessary to ensure accurate results for people who have both a Hb variant and diabetes mellitus. In addition, more detailed information about the effects of common Hb variants, including Hb C and S traits, should be included in the package inserts for each commercially available gHb method. Ideally, mean bias estimates would also be provided at clinically useful concentrations of Hb A1c (e.g., 6% and 9%). On the basis of the results from this study and a previous one (7), the following methods appear to be suitable for gHb determinations in diabetic patients who have either Hb C or S trait: Bayer DCA 2000, Beckman Synchroon CX7, Bio-Rad Variant GHB, Bio-Rad Variant II, Primsys Nycocard, Roche Tina-Quanti II, and Tosoh A1c 2.2 Plus methods. It is important to note, however, that with some methods, the degree of interference from Hb variants may be reagent and/or column lot dependent.

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References