Effects of Hemoglobin C and S Traits on the Results of 14 Commercial Glycated Hemoglobin Assays

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Abstract

Glycated hemoglobin is widely used in the management of diabetes mellitus. At least 300,000 Americans with diabetes mellitus have the hemoglobin (Hb) C or S trait. The accuracy of HbA_{1c} methods can be adversely affected by the presence of these traits. We evaluated the effects of HbC and HbS traits on the results of 14 commercial HbA_{1c} methods that use boronate affinity, enzymatic, immunoassay, and ion exchange methods. Whole blood samples from people homozygous for HbA or heterozygous for HbC or HbS were analyzed for HbA_{1c}. Results for each sample type were compared with those from the CLC 330 comparative method (Primus Diagnostics, Kansas City, MO). After correcting for calibration bias by comparing results from the homozygous HbA group, method bias attributable to the presence of HbC or HbS trait was evaluated with a clinically significant difference being more than 10% (ie, 0.6% at 6% HbA_{1c}). One immunoassay method exhibited clinically significant differences owing to the presence of HbC and HbS traits.

Glycated hemoglobin, reported as HbA_{1c}, is a biochemical marker that is routinely used in the management of diabetes mellitus to monitor long-term glycemic control and assess the risk of developing complications.[1-3] The presence of hemoglobin (Hb) C or S trait has been shown to affect the accuracy of some HbA_{1c} assays.[4-9] In 2004, there were 23.5 million non-Hispanic blacks aged 18 years or older in the United States,[10] of whom at least 10% have HbC or HbS trait.[11] The prevalence of diabetes mellitus, diagnosed and undiagnosed, in non-Hispanic black men is 13.0% and in non-Hispanic black women is 16.3%.[12] This works out to between 305,000 and 383,000 people who have diabetes mellitus and HbC or HbS trait.

Because of the number of patients with diabetes mellitus who could potentially be affected by inaccurate HbA_{1c} results due to interference by the presence of HbC or HbS trait, we continued our earlier investigations and evaluated the effects of HbC and HbS traits on the results of 14 commercial HbA_{1c} methods that use ion exchange chromatography (6 methods), immunoassay (5 methods), boronate affinity (2 methods), and enzymatic (1 method) techniques. Nearly all of the methods evaluated in this study have not been previously evaluated in a rigorous manner for interference from the presence of HbC or HbS trait. This is the first time that we have evaluated an enzymatic assay for HbA_{1c} for the possibility of interference by Hb variants. We reexamined 2 ion exchange methods to see whether they currently have interference issues because we have previously shown there may be lot-to-lot variability in susceptibility to interference by Hb traits for some HbA_{1c} methods.[8,9]

Materials and Methods

Whole blood samples from people homozygous for HbA (n = 68) and heterozygous for HbC or HbS (n = 58 and n = 72, respectively) were collected in EDTA tubes. The clinical site for sample collection was located in an urban area with a high proportion of African American patients so there would be a good opportunity to identify samples with HbC or HbS traits. After routine clinical testing had been completed, Hb variants were identified by inspection of chromatograms obtained with a VARIANT analyzer (Bio-Rad Laboratories, Hercules, CA) using the Beta Thal Short Program run according to manufacturer's instructions. No additional confirmatory testing of samples containing Hb variants was performed. Aliquots of these samples that had HbA_{1c} results between 4% and 14% (0.04-0.14) were analyzed within 7 days by using the Afinion AS100 method (Axis Shield, Norton, MA) and stored at –70°C for analysis by the other methods. Not all samples were analyzed by every method. Studies with samples from human subjects were approved by the University of Utah Institutional Review Board (Salt Lake City).

Samples were analyzed by the following assays and instruments: Afinion AS100; *ultra*[2], Primus Diagnostics, Kansas City, MO; Diazyme reagents, Diazyme Laboratories, Poway, CA, on a Modular P analyzer, Roche Diagnostics, Indianapolis, IN; COBAS INTEGRA 800 generation 2, Roche Diagnostics; Microgenics reagents, Microgenics, Fremont, CA, on a Modular P analyzer; Olympus AU400 HbA_{1c}/THb (Olympus, Center Valley, PA); Pointe Scientific reagents, Pointe Scientific, Canton, MI, on an Olympus AU400 analyzer; VITROS 5,1 FS, Ortho Clinical Diagnostics, Rochester, NY; D-10 Short Program (3 minutes), D-10 Extended Program (6 minutes), VARIANT, VARIANT II, and VARIANT II TURBO, Bio-Rad Laboratories, Hercules, CA; and G7, Tosoh Biosciences, San Francisco, CA. All assays were performed according to the manufacturers' instructions. The CLC 330 boronate affinity method (Primus Diagnostics) was used as the comparative method in a National Glycohemoglobin Standardization Program (NGSP) Network Laboratory with in-house calibrator materials. This method has previously been shown to be unaffected by the presence of HbC and HbS traits.[13] Results for all methods were reported as NGSP HbA_{1c} equivalents.

For each method, results for each type of sample (homozygous HbA, heterozygous HbC, and heterozygous HbS) were compared with results from the CLC330 method. An overall test of coincidence of 2 least-square linear regression lines was performed with SAS software (SAS Institute, Cary, NC) to determine whether the presence of HbC or HbS trait produced a statistically significant difference ($P < .01$) in results relative to the comparative method. To determine whether the presence of HbC or HbS trait produced clinically significant effects on the HbA_{1c} results, we chose evaluation limits of 6% and 9% based on recommendations by the American Diabetes Association of an upper reference limit of 6% (0.06) and a general goal of 7% (0.07) and the Diabetes Control and Complications Trial conventional treatment group mean HbA_{1c} of approximately 9% (0.09). Deming regression analysis was performed to estimate the average bias at 6% (0.06) and 9% (0.09) HbA_{1c}. After correcting for possible calibration bias by comparing results from the homozygous HbA sample group, evaluation of method bias attributable to the presence of HbC or HbS trait was performed. A relative deviation of greater than 10% (ie, 0.6% at 6% HbA_{1c} and 0.9% at 9% HbA_{1c}) compared with the homozygous HbA sample group was used to define a clinically significant difference. This 10% relative difference has been used in previous studies.[7-9,14]

Results

Information on the number of samples analyzed by each method for each sample type and the average biases at 6% (0.06) and 9% (0.09) HbA_{1c} due to the presence of HbC and HbS traits are shown **Table 1**. We observed no clinically significant differences attributable to the presence of HbC or HbS trait for any assay except the Olympus AU400. The Olympus HbA_{1c} method demonstrated a clinically significant positive bias for HbC and HbS trait samples at both evaluation limits of 6% (0.06) and 9% (0.09). The package insert for this method indicates that samples containing HbC or HbS trait can have result elevations of 40% due to the presence of these variants. An abstract indicated that samples containing HbC and HbS traits showed maximum positive biases of 43% and 32%, respectively, when analyzed by the Olympus method compared with the VARIANT II method.[15] This information is consistent with our data.

Box plots for each combination of sample type and assay are shown **Figure 1**. The presence of HbC trait produced statistically significant differences ($P < .01$) for all methods except the Afinion AS100, D-10 Extended, *ultra*[2], and VARIANT II TURBO methods. The presence of HbS trait produced statistically significant differences only for the G7, Microgenics, Olympus, Pointe Scientific, and VARIANT II methods. One other interesting observation is that the G7, Microgenics, and Pointe Scientific methods showed an increased range of differences with the comparative method for samples containing HbS trait compared with the differences seen for samples homozygous for HbA and those containing HbC trait.

Discussion

Some previous studies have shown clinically significant effects on HbA_{1c} measurements by the presence of HbC or HbS trait with certain ion exchange methods, unlike the

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Table 1
Mean Differences Between Test and Comparative Methods for Samples Containing the HbC or HbS Trait*

Assay Principle/Method	HbC Trait			HbS Trait		
	No. of Samples	6% (0.06) HbA_{1c}	9% (0.09) HbA_{1c}	No. of Samples	6% (0.06) HbA_{1c}	9% (0.09) HbA_{1c}
Boronate affinity						
Afinion AS100	40	–0.06	–0.12	39	–0.10	–0.11
ultra[2]	58	0.00	–0.09	71	0.03	–0.09
Enzymatic						
Diazyme	27	–0.19	–0.53	28	–0.14	–0.24
Immunoassay						
COBAS INTEGRA 800 generation 2	27	–0.28	–0.55	28	–0.08	–0.21
Microgenics	28	0.30	0.07	42	0.08	–0.37
Olympus AU400	27	2.28†	3.57†	28	1.36†	2.25†
Pointe Scientific	31	0.59	0.20	43	0.32	–0.16
VITROS 5,1 FS	31	–0.28	–0.24	43	–0.25	0.15
Ion exchange						
D-10 short	27	–0.10	–0.24	28	–0.02	–0.38
D-10 extended	27	0.09	–0.29	28	–0.08	–0.41
VARIANT	30	–0.45	–0.38	42	0.08	–0.24
VARIANT II	31	0.21	0.29	41	0.52	0.47
VARIANT II TURBO	31	0.17	0.14	43	0.04	–0.13
G7	31	–0.38	–0.29	43	–0.36	–0.82

Hb, hemoglobin.
* Deming regression analysis was performed using the CLC 330 (Primus Diagnostics, Kansas City, MO) as the comparative method. The average differences (%) of 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 between the method of interest and the comparative method for homozygous HbA samples was subtracted from that calculated for samples containing the HbC or HbS trait. Afinion AS100, Axis Shield, Norton, MA; *ultra*[2], Primus Diagnostics, Kansas City, MO; COBAS INTEGRA 800 generation 2, Roche Diagnostics, Indianapolis, IN; Microgenics, Microgenics, Fremont, CA; Olympus AU400, Olympus, Center Valley, PA; Pointe Scientific, Pointe Scientific, Canton, MI; VITROS 5,1 FS, Ortho Clinical Diagnostics, Rochester, NY; D-10 Short Program (3 min), D-10 Extended Program (6 min), VARIANT, VARIANT II, and VARIANT II TURBO, Bio-Rad Laboratories, Hercules, CA; and G7, Tosoh Biosciences, San Francisco, CA.
† Clinically significant differences (>0.6% or >0.9% HbA_{1c} at 6% or 9% HbA_{1c}, respectively) were found.

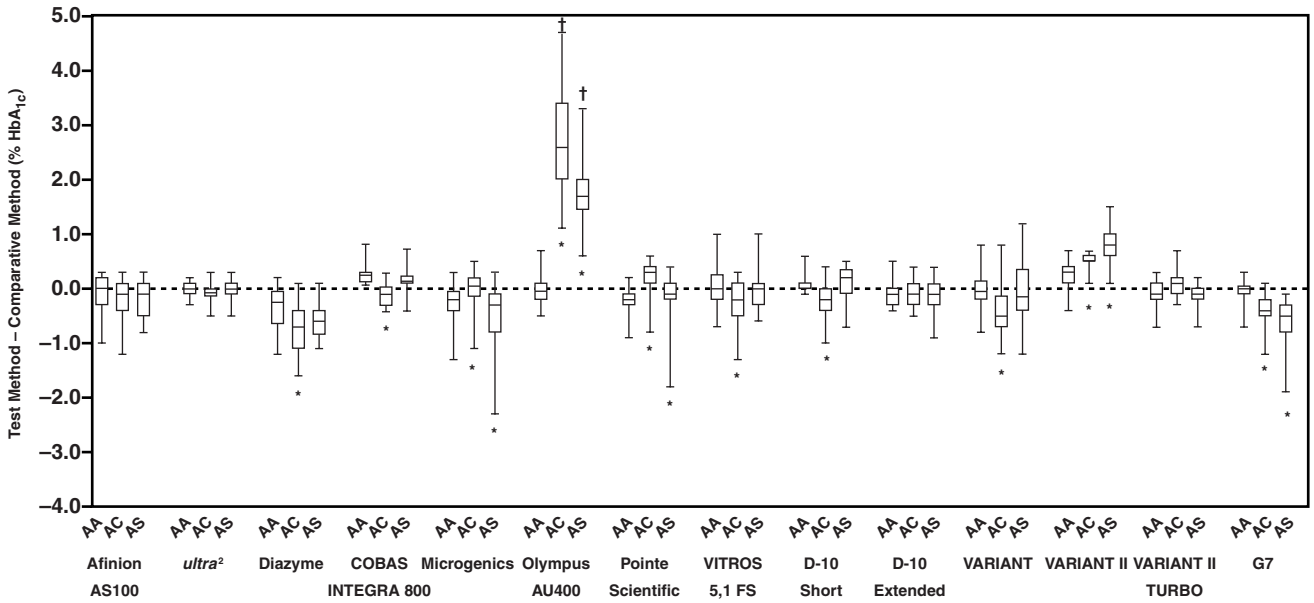

Figure 1 Box plots summarizing the absolute differences between each assay and the comparative method for each hemoglobin phenotype. The horizontal line inside each box is the median difference between the test and comparative methods. The upper and lower limits of each box correspond to the 25th and 75th percentiles of the differences, respectively. The upper and lower bars represent the maximum and minimum differences between the test and comparative methods. Differences from those obtained for homozygous hemoglobin A results that are statistically significant ($P < .01$) are indicated (*), as are clinically significant differences (†). Afinion AS100, Axis Shield, Norton, MA; *ultra*[2], Primus Diagnostics, Kansas City, MO; COBAS INTEGRA 800 generation 2, Roche Diagnostics, Indianapolis, IN; Microgenics, Microgenics, Fremont, CA; Olympus AU400, Olympus, Center Valley, PA; Pointe Scientific, Pointe Scientific, Canton, MI; VITROS 5,1 FS, Ortho Clinical Diagnostics, Rochester, NY; D-10 Short Program (3 min), D-10 Extended Program (6 min), VARIANT, VARIANT II, and VARIANT II TURBO, Bio-Rad Laboratories, Hercules, CA; and G7, Tosoh Biosciences, San Francisco, CA.

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present study in which all of these methods showed clinically nonsignificant effects. The G7 ion exchange method showed statistically but not clinically significant negative biases for samples with HbC and HbS traits, which is in agreement with the findings of a previous study.[14] However, in the present study, the net negative bias for the G7 method with samples containing the HbS trait estimated from Deming regression analysis at the 9% (0.09) HbA_{1c} evaluation limit was close to the limit of –0.9%. The VARIANT ion exchange method for HbA_{1c} has been previously reported to exhibit positive bias with samples containing the HbS trait.[8,9] In the present study, it exhibited a slight positive bias at the 6% (0.06) evaluation limit that was not clinically significant. The VARIANT II method has been previously shown to exhibit positive clinically significant biases for samples containing both HbC and HbS traits, but our results in the present study show a net positive bias with VARIANT II that is *not* clinically significant.[9,14] We have not yet evaluated the newly released VARIANT II NU reagents. Differences between the results obtained in this study and previous studies are consistent with lot-to-lot variability or intentional improvements in the response of ion exchange methods to interference by Hb variants.

Of the 5 immunoassays evaluated in the present study, the COBAS INTEGRA 800 method exhibited negative net biases estimated from Deming regression analysis for HbC and HbS traits at both evaluation limits, whereas the Olympus method exhibited positive net biases for both traits at both limits. The previous reagents used on the COBAS INTEGRA 800 had shown a clinically significant positive bias with samples containing the HbC or HbS trait.[4,7,8] The data from the current generation 2 assay on the COBAS INTEGRA 800 agree with another recent study indicating no clinically significant bias for either trait.[16]

For all immunoassay methods examined in this study, only 1, the Olympus method, demonstrated clinically significant effects from HbC and HbS traits. For both HbC and HbS traits, HbA_{1c} was overestimated, which could lead to overly rigorous glycemic control with consequent hypoglycemia. Attention to the effects of Hb variants on the results of HbA_{1c} methods is necessary to ensure accurate results for people who have an Hb variant *and* diabetes mellitus. Furthermore, for methods that do not allow the operator to identify the presence of an Hb variant, including boronate affinity, enzymatic, and immunoassay, if the method is affected to a clinically significant extent by a particular Hb variant that will be encountered in the patient population being tested, it may be necessary to determine the Hb phenotype of each patient with diabetes mellitus. If a patient with an Hb variant that interferes with the routine HbA_{1c} method is identified, all samples from that patient will need to be tested by an alternative method that is not prone to interference by the particular Hb variant. Alternatively, instead of determining the Hb phenotype of each patient, a comment could be added to each HbA_{1c} result indicating that the possibility of an Hb variant interference should be considered if the HbA_{1c} result is not consistent with the mean plasma glucose level estimated by the patient's self-monitoring results or clinical estimates of glycemic control.

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