

Validation by a Mass Spectrometric Reference Method of Use of Boronate Affinity Chromatography to Measure Glycohemoglobin in the Presence of Hemoglobin S and C Traits, Randie R. Little,^{1*} Hubert Vesper,² Curt L. Rohlfing,¹ Maria Ospina,² Sekineh Safar-Pour,³ and William L. Roberts³

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Glycohemoglobin (GHB) is routinely used both as an index of mean glycemia and as a measure of risk for the development and progression of diabetes complications (1, 2). Because the prevalence of diabetes is higher for non-Hispanic blacks than in non-Hispanic whites (3) and at least 10% of black Americans have either hemoglobin S (HbS) or HbC trait (4), it is probable that at least 150 000 Americans with diabetes have HbS or HbC trait.

Previous studies have shown that some GHB methods yield inaccurate results with samples heterozygous for HbS or HbC (5–9). Many of these interference studies have used boronate affinity chromatography as a reference method based on the assumption that this method should not be influenced by the presence of Hb variants (10). Because measurement of GHB by boronate affinity chromatography is based on the structure of the glycosylated component (cis-diol component of GHB), rather than differences in charge, it has been assumed that glycosylated

HbS and/or HbC are included in the glycosylated fraction and that GHB results obtained with boronate affinity methods, when these traits are present, are therefore equivalent to those obtained in homozygous HbA samples. However, there is no conclusive proof of this non-interference. Furthermore, recent comparisons between non-HPLC boronate affinity-based GHB methods and the CLC330 method have shown that these methods gave higher results for samples with HbC trait than the CLC330 (7, 8).

Here we compare the CLC330 boronate affinity HPLC method (Primus Corporation) with the IFCC Mass Spectrometry (MS) Reference Method for HbA_{1c} that is specific for HbA_{1c} (11). The first step in the procedure for the IFCC reference method (specifically measuring the β-N-terminal hexapeptide) is the cleavage of hemoglobin with the proteolytic enzyme endoproteinase Glu-C at position 6 from the N-terminal end of the β-chain. Because both HbS and HbC contain substitutions at this position on the Hb chain, there should be no hexapeptide formed from this enzymatic digestion of either of these hemoglobins. As expected, this reference method has previously been shown to produce no signal at the position of the N-terminal hexapeptide when purified HbS and HbC were analyzed (11). Therefore, only HbA hexapeptide glycation is measured with this method, and the presence of these traits does not interfere with measurement of HbA_{1c}. Assuming no difference in glycation rates for HbS and HbC vs HbA (12), the relationship between the IFCC MS method (which measures HbA_{1c} in relation to total HbA) and the CLC330 affinity HPLC (which measures total GHB in relation to total Hb) method should therefore

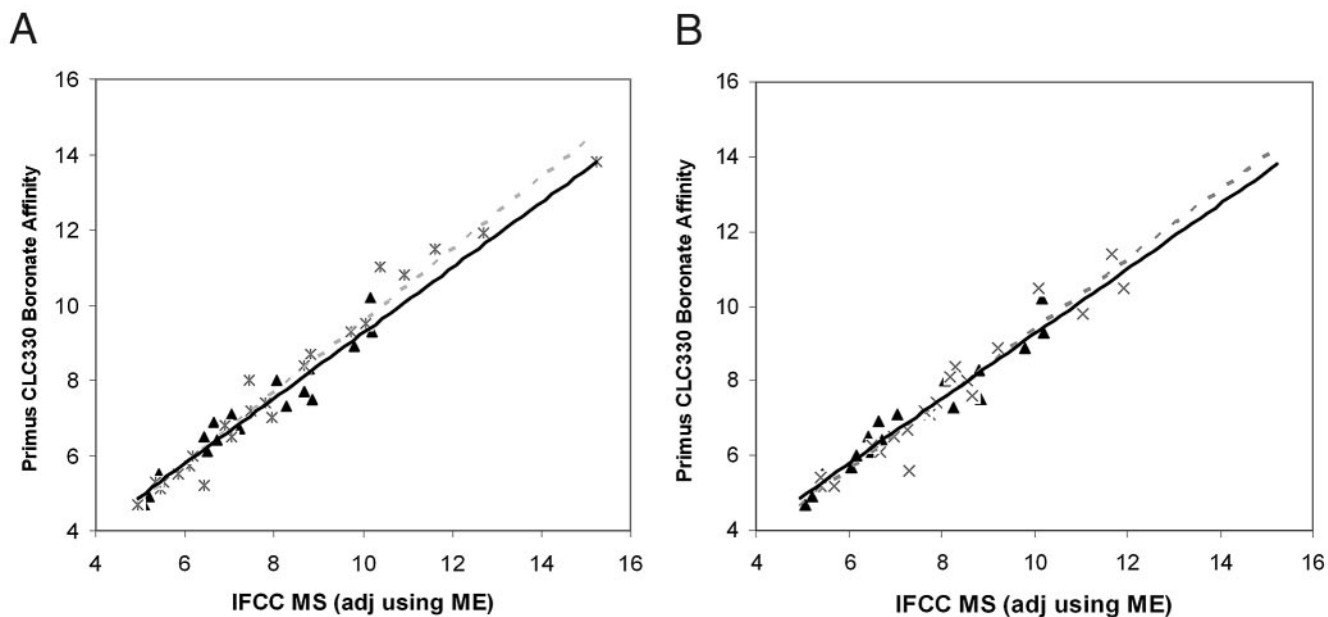


Fig. 1. Relationship between HbA_{1c} measured by the IFCC MS and CLC330 HPLC methods.

Results are calibrated to NGSP HbA_{1c} values. Results for samples containing HbAS [A; * and dashed line ($y = 0.95x + 0.04$; $r = 0.98$)] or HbAC [B; × and dashed line ($y = 0.93x + 0.03$; $r = 0.96$)] are compared with results for HbAA samples [▲ and solid line ($y = 0.87x + 0.58$; $r = 0.97$)]. *adj using ME*, adjusted using master equation.

be the same regardless of whether the samples contain HbAS, HbAC, or HbAA.

To confirm this hypothesis, we collected and analyzed 63 samples containing HbAA ($n = 20$), HbAS ($n = 23$), or HbAC ($n = 20$) by both the CLC330 HPLC and IFCC MS methods. This study was approved by the Institutional Review Board of the University of Utah Health Sciences Center. Statistical analyses were performed with SAS and Excel. IFCC MS results were converted to the currently reported National Glycohemoglobin Standardization Program/Diabetes Control and Complications Trial (NGSP/DCCT) equivalent HbA_{1c} number scale (13) by use of a previously developed master equation (14) so that absolute differences in results between methods could be compared. Results from the CLC330 HPLC method were expressed as NGSP HbA_{1c} equivalents. The relationships between the two methods for samples containing each of these traits were determined by linear regression analysis (Fig. 1).

To determine whether the presence of HbS or HbC trait caused a statistically significant difference in results (vs HbAA), we performed an overall test of coincidence of least-squares linear regression lines (SAS Institute). As in previous studies, HbA_{1c} concentrations of 6% (the upper limit of normal) and 9% were also used as clinical evaluation limits (7–9). The results show that the relationships between the two methods when GHB is measured in samples containing HbAS or HbAC are not statistically significantly different from the relationship in HbAA ($P = 0.59$ and 0.71 for HbS and HbC, respectively). After we subtracted the differences between the two methods in HbAA samples, the average differences between MS and boronate affinity were -0.03% and -0.15% GHB at 6% GHB and 0.23% and 0.04% GHB at 9% GHB for HbAS and HbAC, respectively.

We conclude that CLC330 boronate affinity GHB results for samples heterozygous for HbS and HbC are comparable to those of the IFCC reference method, which has been shown to have no interference. These data support the contention that the CLC330 boronate affinity HPLC assay is not affected by the presence of HbS or HbC trait and is therefore an appropriate comparative method for

the study of HbS and HbC interference with GHB methods.

This work was supported in part by the ARUP Institute for Clinical & Experimental Pathology.

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