Elimination of HbS and HbC Variant Interference in the HbA1c Method on the ADVIA Chemistry Systems

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Abstract

Background: The first generation method for the determination of HbA1c on the ADVIA® Chemistry Systems (HbA1c) overrecovers samples with heterozygous hemoglobin variants S and C. HbS and HbC variants are present in as many as 10% of African Americans, and a lesser percentage of persons with Eastern Mediterranean, Indian or Saudi Arabian ancestry. Hence, there is a need to offer a test that gives accurate results in these populations. We report here an improved method that is accurate in the determination of HbA1c and is not affected by the presence of either HbS or HbC variants. This improvement was achieved by incorporation of a protease in the reagent to liberate the same glycated pentapeptide regardless of the hemoglobin variants present in whole blood. Furthermore, an option to automate sample pretreatment is now offered.

Methods: Precision was evaluated on multiple instruments and was tested over a period of 10 days. Accuracy and the effect of hemoglobin variants was assessed by analyzing HbA, HbC and HbS samples on ADVIA® 1200, ADVIA 1650, ADVIA 1800, and ADVIA 2400 chemistry systems and two HPLC systems (Tosoh G7 and Primus Ultra 2). Interference samples were prepared by spiking whole blood with the relevant materials.

Results: Within-run CVs ranged from 1.1% to 2.4% and total CVs ranged from 1.9% to 4.0%. The sample population used for the accuracy study included 40 HbA, 10 HbC and 10 HbS samples which had been assayed using the NGSP reference method (HbA samples) or the Primus Ultra 2 (HbC and HbS samples). A1c concentrations ranged from 4.6% to 12.5%. The 95% confidence interval for the average bias for all samples was from –0.59% to 0.72% HbA1c. The 95% confidence interval for the average bias for the HbC samples was from –0.20% to 0.81% HbA1c while that for the HbS samples was from –0.64% to 0.49% HbA1c. No significant interferences were observed with bilirubin (up to 60 mg/dL), triglycerides (up to 1000 mg/dL) and labile HbA1c (up to 1500 mg/dL glucose).

Conclusion: The improved ADVIA Chemistry Systems Hemoglobin A1c method (A1c) is precise and shows excellent agreement with the NGSP reference method. Samples with the hemoglobin variants HbC or HbS can be accurately assayed on the ADVIA Chemistry Systems.

Background

Numerous studies have shown that the current HbA1c method on ADVIA 1200, ADVIA 1650, ADVIA 1800, and ADVIA 2400 chemistry systems substantially overrecovers samples with heterozygous hemoglobin variants S and C. HbS and HbC variants are present in as many as 10 percent of African Americans and a lesser percentage of persons with eastern Mediterranean, Indian or Saudi Arabian ancestry.[1,2] This overrecovery is shown in Figure 1.

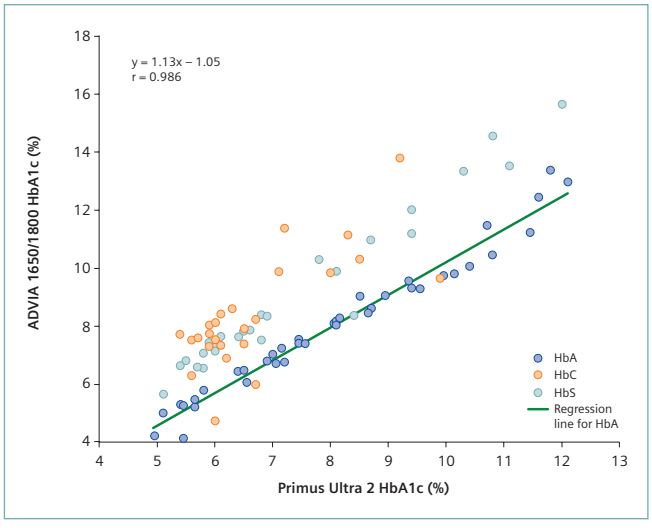

Figure 1.
Comparison of the ADVIA Chemistry first generation HbA1c method with the Primus Ultra 2 HPLC system HbA1c method

The overrecovery bias for HbS and HbC variant hemoglobins is a result of a single point mutation that changes the glutamic acid in position 6 of the β-chain of HbA to a lysine in HbC and valine in HbS (Table 1). Thus, the different affinities of the glycated N-terminal beta subunits of HbA, HbC and HbS for a mouse antibody produced using a synthetic peptide immunogen containing the same amino acid sequence as HbA is not unexpected. These differing affinities are most likely due to the conformational changes resulting from replacing the acidic glutamic acid in HbA with the basic amino acid lysine in HbC and the hydrophobic amino acid valine in HbS.

Hemoglobin	Epitope Sequence
HbA	Glucose-val-his-leu-thr-pro-**glu**-glu-lys-ser-ala-val-thr-ala-leu-try-gly-lys-val---
HbC	Glucose-val-his-leu-thr-pro-**lys**-glu-lys-ser-ala-val-thr-ala-leu-try-gly-lys-val---
HbS	Glucose-val-his-leu-thr-pro-**val**-glu-lys-ser-ala-val-thr-ala-leu-try-gly-lys-val---

Table 1. The epitope sequences for HbA, HbC and HbS

Cleaving the glycated hemoglobin at the carboxy side of proline five makes the HbC and HbS epitopes identical to HbA and thus in theory should eliminate overrecovery of the HbC and HbS. Cleavage of the peptide can be accomplished by adding proline-specific endopeptidase (PSE) into the A1c Agglutinator reagent. This study evaluated how addition of PSE affected HbA1c overrecovery for HbC and HbS variants.

Materials and Methods

Precision was evaluated according to CLSI document EP05-A2[3] with the exception that the number of days of analysis was 10 instead of 20. The method comparison study and the analysis of HbC and HbS variant samples were completed according to CLSI document EP9-A2.[4] HbA samples were tested on the ADVIA Chemistry systems and the Tosoh G7. HbC and HbS samples were tested on the ADVIA Chemistry systems and the Primus Ultra 2. Tosoh G7 and Primus Ultra 2 values were provided by the University of Missouri, Columbia, MO. Interference samples were prepared as described in CLSI document EP7-A2.[5] Labile HbA1c samples were prepared as described in the FDA's *Review Criteria for Assessment of Glycohemoglobin (Glycated or Glycosylated Hemoglobin) In Vitro Diagnostic Devices.*[6]

Results

						Within-Run		Total	
ADVIA System	Sample	Days	Runs	N	Mean (% HbA1c)	SD	CV (%)	SD	CV (%)
1200	Diabetic Control 1	10	20	40	5.63	0.14	2.4	0.14	2.6
	Diabetic Control 2	10	20	40	9.29	0.17	1.8	0.24	2.6
	Whole Blood Pool	10	20	40	5.41	0.12	2.2	0.16	3.0
1650/	Diabetic Control 1	10	20	40	5.67	0.13	2.3	0.17	3.0
1800	Diabetic Control 2	10	20	40	9.35	0.10	1.1	0.18	1.9
	Whole Blood Pool	10	20	39*	5.38	0.08	1.5	0.22	4.0
2400	Diabetic Control 1	10	20	39*	5.77	0.07	1.3	0.17	3.0
	Diabetic Control 2	10	20	39*	9.52	0.14	1.5	0.23	2.5
	Whole Blood Pool	10	20	39*	5.49	0.11	2.0	0.16	2.8

Table 2.
Precision estimates for the improved Hemoglobin A1c method (A1c) on the ADVIA 1200, ADVIA 1650, ADVIA 1800 and ADVIA 2400 Chemistry systems. The maximum total CV was 4.0% and the maximum within-run CV was 2.4%.

*One replicate was an obvious outlier and was deleted from the data set.

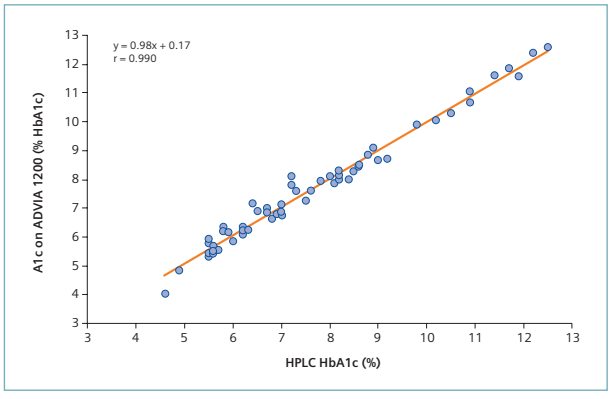

Figure 2.
A comparison of the improved Hemoglobin A1c method (A1c) on the ADVIA 1200 and HPLC (N = 60 which includes 40 HbA, 10 HbC and 10 HbS samples).

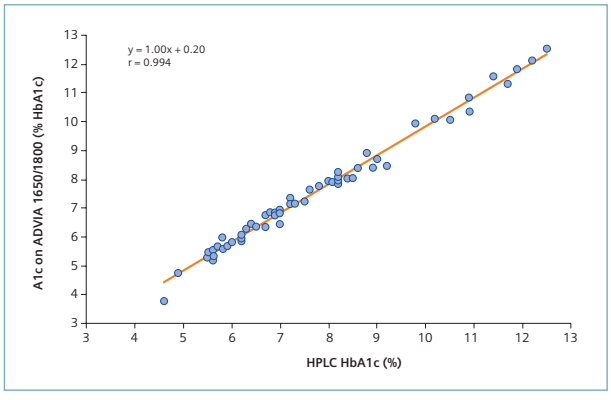

Figure 3.
A comparison of the improved Hemoglobin A1c method (A1c) on the ADVIA 1650/1800 and HPLC (N = 60 which includes 40 HbA, 10 HbC and 10 HbS samples).

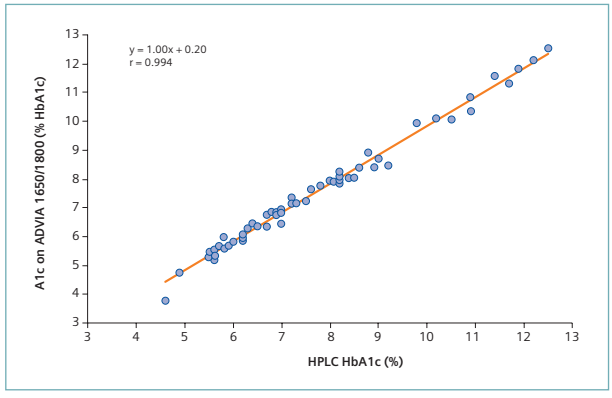

Figure 4.
A comparison of the improved Hemoglobin A1c method (A1c) on the ADVIA 2400 and HPLC (N = 60 which includes 40 HbA, 10 HbC and 10 HbS samples).

Hemoglobin Variant	Primus Ultra 2	ADVIA 1200	ADVIA 1650/1800	ADVIA 2400
HbC	5.6	5.7	5.3	5.7
HbC	5.8	6.2	5.6	6.0
HbC	5.8	6.4	6.0	6.4
HbC	7.3	7.6	7.1	7.3
HbC	5.5	5.9	5.5	5.9
HbC	7.2	8.1	7.2	7.5
HbC	6.5	6.9	6.4	6.6
HbC	7.2	7.8	7.3	7.5
HbC	6.4	7.2	6.4	6.7
HbC	5.5	5.8	5.3	5.6
HbS	5.6	5.6	5.2	5.5
HbS	11.7	11.9	11.3	11.3
HbS	6.2	6.4	5.9	6.2
HbS	6.2	6.3	5.9	6.3
HbS	10.9	11.0	10.4	10.6
HbS	7.0	6.8	6.5	6.8
HbS	6.7	7.0	6.3	6.8
HbS	8.2	8.3	7.9	8.2
HbS	8.9	9.1	8.4	8.9
HbS	4.6	4.1	3.8	4.0

Table 3.
A comparison of the HbA1c results for HbC and HbS variant samples measured by the improved Hemoglobin A1c method (A1c) on ADVIA Chemistry systems and the Primus Ultra 2 HPLC system.

			Low Pool		High Pool	
Interferent	Interferent Concentration	ADVIA System	HbA1c (%)	Interference (%)	HbA1c (%)	Interference (%)
Bilirubin	60 mg/dL	1200	5.5	–2.0	9.4	–1.5
		1650/1800	5.5	–6.0	9.6	–2.3
		2400	5.8	–3.8	9.3	–4.7
Triglycerides (Avian)	1000 mg/dL	1200	5.5	–0.7	9.4	1.0
		1650/1800	5.4	–4.9	9.6	–0.5
		2400	5.4	1.5	9.5	–0.9
Labile HbA1c	1500 mg/dL glucose (5 hours at 37°C)	1200	5.6	–2.5	–	–
		1650/1800	5.2	3.5	–	–
		2400	5.1	–4.0	–	–

Table 4.
Interference studies: there was minimal assay interference for bilirubin, triglycerides and labile A1c for the improved Hemoglobin A1c method (A1c).

References

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