



HbA1c Assays

Comparison of Hemoglobin Variants across Siemens Healthineers HbA1c Assays

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**Clinical
Brief**

Comparison of Hemoglobin Variants across Siemens Healthineers HbA1c Assays

Abstract

Background: According to the International Federation of Diabetes, it is estimated that 463 million people between the ages of 20–79 years around the world are living with diabetes. Nearly half of these people are unaware that they have the condition and are therefore at high risk of developing serious diabetes-related complications. The number of deaths resulting from diabetes and its complications in 2019 is estimated to be 4.2 million.¹ Along with a healthy diet and regular exercise, early diagnosis is important in delaying the onset of type 2 diabetes. Glycated hemoglobin (HbA1c) is a form of measurement of glycemic states that reflects the average blood glucose level over the preceding 8–12 weeks. HbA1c is formed by a nonenzymatic Maillard reaction between glucose and the N-terminal valine of the β -chain of HbA, whereby a labile Schiff base is formed and converted into the more-stable ketoamine (irreversible) via an Amadori rearrangement. Siemens Healthineers offers HbA1c assays on the following laboratory diagnostic systems: the Atellica® CH Analyzer, ADVIA® Chemistry Systems, Dimension® Integrated Chemistry Systems, and Dimension Vista® Intelligent Lab Systems.

Method: A hemoglobin variant study was performed on the following Siemens Healthineers assays: Atellica CH A1c_E, ADVIA Chemistry A1c_E, Dimension A1C, and Dimension Vista A1C. A minimum of 20 samples were tested according to CLSI protocol EP07-A2 for each of the following variants: HbA2, HbC, HbD, HbE, and HbS. Samples were obtained from the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Results: The hemoglobin variants study yielded the following overall mean %biases across tested variants:

Atellica CH A1c_E Assay: –4.61 to –0.14% bias

ADVIA Chemistry A1c_E Assay: –1.05 to –2.88% bias

Dimension A1C Assay: –2.27 to –0.17% bias

Dimension Vista A1C Assay: –1.08 to 0.79% bias

Background

Diabetes is a growing global problem. Over 420 million adults globally have diabetes mellitus,¹ a chronic disease caused by inadequate production of or cellular sensitivity to insulin, resulting in abnormally high blood glucose (hyperglycemia). While a healthy diet, regular exercise, and maintaining body weight are essential in delaying the onset of type 2 diabetes, early diagnosis and regular monitoring are important for long-term diabetes care. Glycemic states can be measured by fasting blood glucose, fructosamine, or glycated hemoglobin (HbA1c). Glycated hemoglobin (HbA1c) is a form of measurement of glycemic states. HbA1c is formed by a nonenzymatic Maillard reaction between glucose and the N-terminal valine of the β -chain of HbA, whereby a labile Schiff base is formed and converted into the more stable ketoamine (irreversible) via an Amadori rearrangement.

Mutations in the genes encoding the alpha and beta subunits of hemoglobin result in changes to the hemoglobin protein (Figure 1). Over 1000 variants have been recorded. The majority of these variants have no effect on the production, structure, or function of hemoglobin, while others are associated with diseases (e.g., HbS: sickle cell trait/sickle cell anemia; HbC [homozygous]: mild hemolytic anemia.)²

The most common variants worldwide (in order of approximate prevalence) are HbS, HbE, HbC, and HbD. However, exact prevalence varies widely from country to country, and even within different geographic areas within a country or region. The highest endemicity of HbS is found in Africa, although HbS is also highly prevalent worldwide due to migratory patterns. Prevalence of HbE is common in Southeast Asia, and ~30% of Southeast Asians living in the U.S. carry this variant. HbC can be found in up to 50% of West Africans and ~3% of African-Americans. HbD is carried by North Indians, Pakistanis, and Afghans and is found in approximately 2% of individuals from these regions and their descendants.

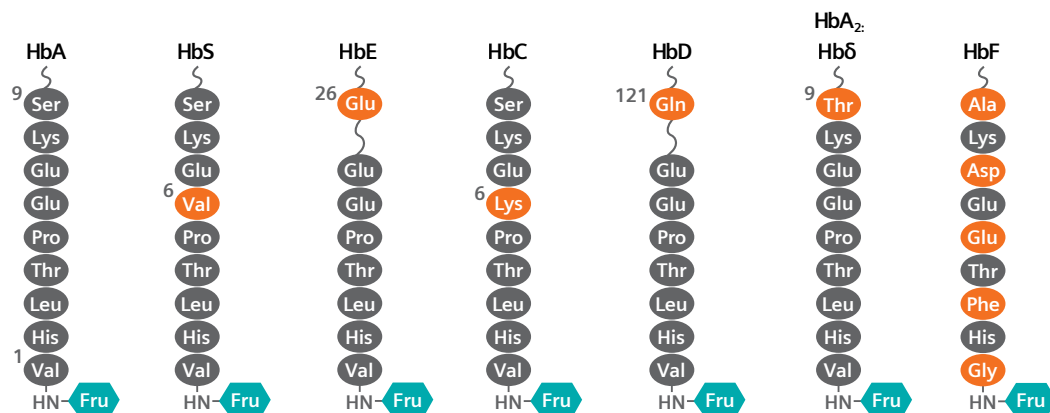


Figure 1. Common hemoglobin variants.^{3,4}

HbA₂ is a variant form of HbA. HbA₂ is composed of two α (alpha) subunits and two δ (delta) subunits. The δ subunits are similar to β subunits but vary at seven amino acids spread throughout the chain. The first eight N-terminal amino acids are identical, however, allowing for comparable glycation of valine as occurs on the β subunit of HbA_{1c}. Approximately 2% of the hemoglobin found in normal blood consists of HbA₂.

HbF is the fetal form of hemoglobin and is composed of two α subunits and two γ (gamma) subunits. There are two forms of the γ subunit: Type G has an N-terminal glycine (Gly) and type A has an N-terminal alanine (only the G type is shown in Figure 1). Both of these residues are available for glycation in approximately 80–85% of HbF molecules. In contrast to HbA, only about 40% of total glycation occurs at the N-terminal residue in the nonacetylated fraction. The γ N-terminal also glycates at approximately 25–33% the rate of HbA's terminal Val; thus a much lower percentage of HbF γ subunits are glyated when compared to HbA glycation at the same glucose concentration. HbF normally declines rapidly after birth, and by 1 year of age it accounts for only about 2% of hemoglobin.

The percentage of HbF can be much higher in individuals with certain hemoglobinopathies, such as sickle cell anemia and β thalassemias, as the persistence of fetal hemoglobin can help compensate for hemoglobin defects. In some individuals without other hemoglobinopathies, hereditary persistence of fetal hemoglobin into adulthood can result in hemoglobin consisting of 30% or more HbF. This elevation could impact HbA_{1c} levels, but patients and physicians would be aware of their status before testing.

Siemens Healthineers offers HbA_{1c} assays on a variety of laboratory diagnostic systems: the Atellica CH Analyzer, ADVIA Chemistry Systems, Dimension Integrated Chemistry Systems, and Dimension Vista Intelligent Lab Systems.

Materials and Methods

ADVIA Chemistry and Atellica CH A1c_E assays

The ADVIA and Atellica CH A1c_E assays are an enzymatic method that specifically measures N-terminal fructosyl dipeptides on the beta-chain of HbA1c. The concentrations of glycated hemoglobin and total hemoglobin are measured separately; these measurements are used to determine the %HbA1c (NGSP units) or the hemoglobin A1c/tHb ratio in mmol/mol (IFCC units). A pretreatment solution hemolyzes the red blood cells, and sodium nitrite is used to convert hemoglobin to methemoglobin (MetHb). The first reagent is added, and the protease hydrolyzes the N-terminal fructosyl dipeptide fragment from the glycated hemoglobin beta-chain to form fructosyl-valine-histidine (fructosyl-dipeptide). At the same time, methHb is converted into the stable azido-MetHb in the presence of sodium azide, and the total hemoglobin concentration is measured at 478/805 nm for the ADVIA assay (478/694 nm for the Atellica CH A1c_E Assay). The second reagent containing fructosyl peptide oxidase (FPOX) is added to convert the fructosyl-dipeptide to hydrogen peroxide, a byproduct of the enzymatic oxidation reaction that reacts with the chromagen 10-(carboxymethylaminocarbonyl)-3,7-bis(dimethylamino)-phenothiazine (DA-67) in the presence of horseradish peroxidase (POD) to develop a color that is measured at 658/805 nm.

Dimension and Dimension Vista A1C assays

The Dimension and Dimension Vista A1C assays measure both glycated hemoglobin and total hemoglobin. The HbA1c measurement is based on a turbidimetric inhibition immunoassay (TINIA) principle, and the measurement of total hemoglobin is based on a modification of the alkaline hematin reaction. Using the values obtained for each of these two analytes, the relative proportion of the total hemoglobin that is glycated is calculated and reported. Pretreatment to remove the labile fraction is not necessary as only the Amadori rearranged form of HbA1c is detected. All hemoglobin variants that are glycated at the beta-chain N-terminus and have epitopes identical to that of HbA1c are measured by this assay.

Total hemoglobin measurement:

A sample of whole blood is hemolyzed and simultaneously converted to a derivative that has a characteristic absorbance spectrum. The total hemoglobin concentration is measured at 405 nm and 700 nm.

Hemoglobin A1c measurement:

The same aliquot of the lysed whole blood used for the Hb measurement is also used for the measurement of HbA1c. Hemoglobin A1c in the sample reacts with anti-HbA1c antibody to form a soluble antigen-antibody complex. A polyhapten reagent containing multiple HbA1c epitopes is then added. The polyhapten reacts with excess (free) anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex. The rate of this reaction is measured turbidimetrically at 340 nm and blanked at 700 nm and is inversely proportional to the concentration of HbA1c in the sample.

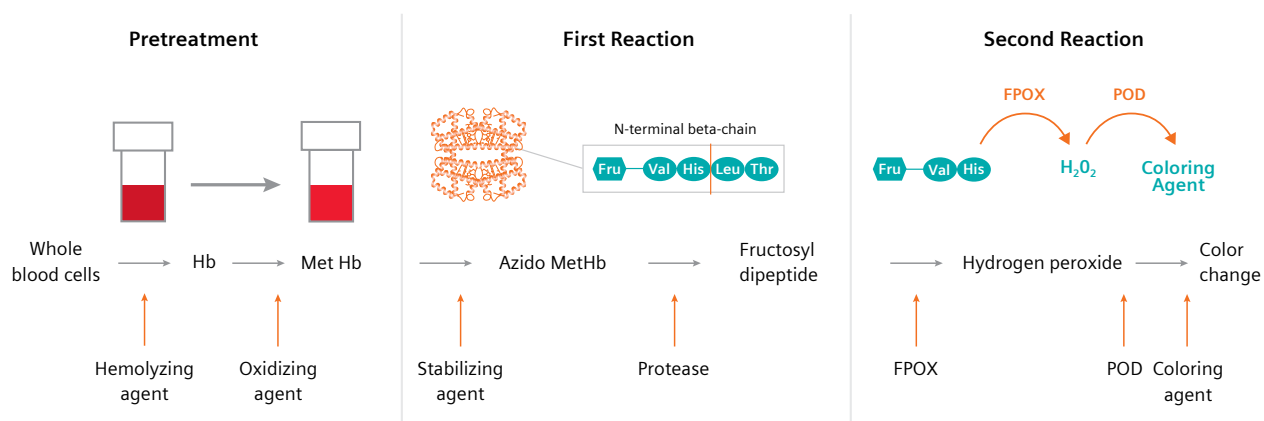


Figure 2. Reaction scheme of the ADVIA Chemistry and Atellica CH A1c_E assays.

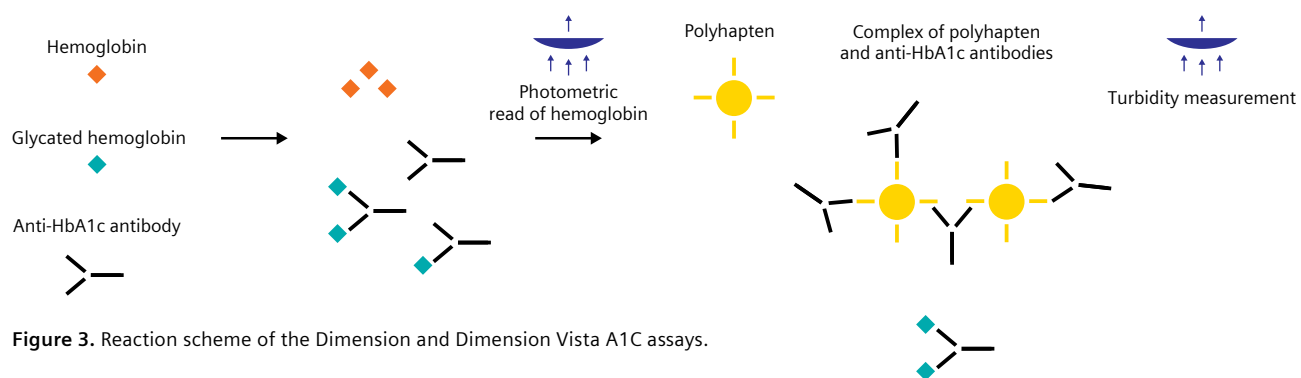


Figure 3. Reaction scheme of the Dimension and Dimension Vista A1C assays.

Results

Variant study

A hemoglobin variant study was performed on the following Siemens Healthineers assays: Atellica CH A1c_E, ADVIA Chemistry A1c_E, Dimension A1C, and Dimension Vista A1C assays. A minimum of 20 samples for each of the following variants were tested according to CLSI protocol EP07-A2: HbA2, HbC, HbD, HbE, and HbS.

Samples were obtained from the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Note that not all of the same samples were run on each platform.

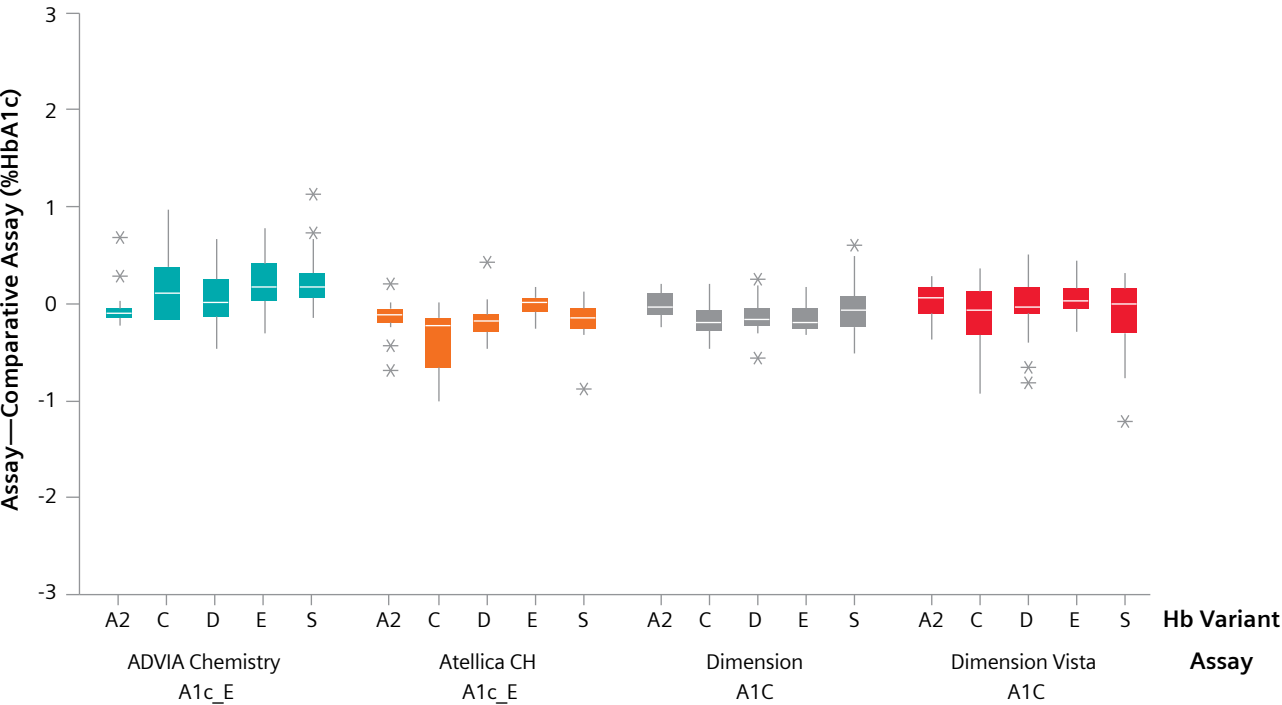


Figure 4. Box plot summarizing the absolute differences between each test method and the comparison method (NGSP reference method) for each variant type.

Table 1. Overall mean %biases of each test method and the comparison method (NGSP reference method) for each variant type.

Hb Variant	ADVIA Chemistry A1c_E					Atellica CH A1c_E					Dimension A1C					Dimension Vista A1C				
	A2	C	D	E	S	A2	C	D	E	S	A2	C	D	E	S	A2	C	D	E	S
n	20	45	24	20	25	20	37	27	21	20	23	20	20	20	22	25	53	31	29	29
Overall Mean %Bias	-1.05	1.02	0.18	2.70	2.88	-2.40	-4.61	-2.71	-0.14	-1.99	-0.17	-2.60	-2.09	-2.27	-0.94	0.63	-1.08	0.07	0.79	-0.31

Conclusion

Siemens Healthineers offers several hemoglobin A1c assays that demonstrated little to no significant interference across all systems and variants tested. The overall mean bias was <5% across all systems and variant types.

References:

1. IDF diabetes atlas. 9th edition. Available from: <http://www.diabetesatlas.org>
2. Rhea JM, et al. Am J Clin Pathol. 2014;141:5-16.
3. <https://www.uniprot.org/uniprot/P68871#sequences>
4. <https://www.uniprot.org/uniprot/P02042>

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An estimated 5 million patients globally benefit every day from our innovative technologies and services in the areas of diagnostic and therapeutic imaging, laboratory diagnostics, and molecular medicine, as well as digital health and enterprise services.

We are a leading medical technology company with over 120 years of experience and 18,000 patents globally. Through the dedication of more than 50,000 colleagues in 75 countries, we will continue to innovate and shape the future of healthcare.

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