Diagnosing and Monitoring Diabetic Patients with a Siemens Healthineers End-to-end Solution for HbA1c Testing

Schimenes TV, Tyler A, Schaible C, Robinson C, Gisiora J, Navarro F, Urquhart J, Jones J.
Siemens Healthcare Diagnostics Inc., Newark, DE, U.S.

siemens-healthineers.com

SIEMENS Healthineers

Diagnosing and Monitoring Diabetic Patients with a Siemens Healthineers End-to-end Solution for HbA1c Testing

Abstract

Background: According to the International Federation of Diabetes, it is estimated 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] 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. 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. There has been a trend to perform HbA1c testing on chemistry systems and point-of-care (POC) analyzers. These systems offer simplicity and wide availability where HPLC, electrophoresis, and chromatography technology are not readily available. Siemens Healthineers offers HbA1c assays on a variety of laboratory diagnostic and POC systems: the Atellica® CH Analyzer, ADVIA® Chemistry Systems, Dimension® Integrated Chemistry Systems, Dimension Vista® Intelligent Lab Systems, and the DCA Vantage® Analyzer.

Method: A method comparison study was performed according to CLSI protocol EP09-A3 using 50 samples obtained from the National Glycohemoglobin Standardization Program (NGSP). The study compared the Atellica CH A1c_E Assay to the following assays: ADVIA Chemistry A1c_E, Dimension A1C, Dimension Vista A1C, and DCA Vantage HbA1c. Method comparison statistics are based on Deming regression. All the HbA1c assays are globally available.

Results: The method comparison study yielded the following Deming regression equations:

Atellica CH A1c_E = 0.986[ADVIA Chemistry A1c_E] + 0.030% HbA1c (r = 0.996)

Atellica CH A1c_E = 1.019[Dimension A1C] – 0.428% HbA1c (r = 0.991)

Atellica CH A1c_E = 1.020[Dimension Vista A1C] – 0.184% HbA1c (r = 0.996)

Atellica CH A1c_E = 0.972[DCA Vantage HbA1c] + 0.032% HbA1c (r = 0.994)

The mean %bias compared to NGSP is –0.84, 0.14, 3.26, –0.19, and 1.59 for the Atellica CH A1c_E, ADVIA Chemistry A1c_E, Dimension A1C, Dimension Vista A1C, and DCA Vantage HbA1c assays, respectively.

Conclusions: Siemens Healthineers offers an end-to-end solution for HbA1c testing, which includes POC as well as automated chemistry systems. All Siemens Healthineers HbA1c assays demonstrate close agreement across all systems and <3.5% mean bias to NGSP samples, enabling laboratories and physicians to be confident of results regardless of the system used.

Background

Diabetes is a growing global problem. Based on the 2019 estimates, by 2030 a projected 578.4 million, and by 2045, 700.2 million adults aged 20–79 years will be living with diabetes. Diabetes is 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.[2] 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. There has been a trend to perform HbA1c testing on chemistry systems and point-of-care (POC) analyzers. These systems offer simplicity and wide availability where HPLC, electrophoresis, and chromatography technology are not readily available. Siemens Healthineers offers HbA1c assays on a variety of laboratory diagnostic and POC systems: the Atellica CH Analyzer, ADVIA Chemistry Systems, Dimension Integrated Chemistry Systems, Dimension Vista Intelligent Lab Systems, and the DCA Vantage Analyzer.

Materials and Methods

The ADVIA Chemistry and Atellica CH A1c_E assays use an enzymatic method that specifically measures N-terminal fructosyl dipeptides on the beta-chain of HbA1c. The concentration 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 hemolyses the red blood cells, and sodium nitrite is used to convert hemoglobin to methemoglobin. The first reagent (R1) 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, methemoglobin is converted into the stable azido-methemoglobin in the presence of sodium azide, and the total hemoglobin concentration is measured at 478/805 nm. The second reagent (R2) containing fructosyl peptide oxidase (FPOX)', to define the term in the graphic 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 define the term in the graphic) to develop a color that is measured at 658/805 nm.

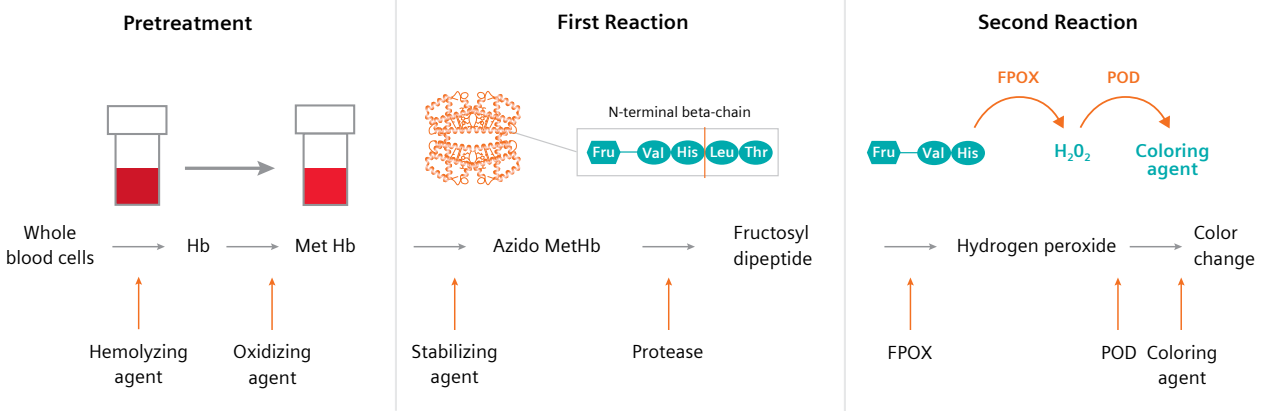

Figure 1. Reaction scheme of the ADVIA Chemistry and Atellica CH A1c_E assays.

Dimension Vista A1C Assay
The Dimension Vista A1C Assay measures both HbA1c and total hemoglobin. The HbA1c measurement is based on a turbidimetric inhibition immunoassay (TINIA) principle. Using the values obtained for each of these two analytes, the relative proportion of the total hemoglobin that is glycated is calculated and reported.

Total hemoglobin measurement: A sample of whole blood is added to a reaction vessel containing lysing reagent. This reagent lyses the red blood cells and simultaneously converts the released hemoglobin to a derivative that has a characteristic absorbance spectrum. An aliquot of the lysed whole blood is transferred from the reaction vessel to a cuvette, where total hemoglobin concentration is measured at 405 nm and 700 nm.

Hemoglobin A1c measurement: The same aliquot of lysed whole blood that is transferred from the reaction vessel to the cuvette for the Hb measurement is also used for the measurement of HbA1c. The cuvette contains an anti-HbA1c antibody in a buffered reagent. 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 to this cuvette. 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).

Dimension A1C Assay
The Dimension A1C Assay measures both HbA1c 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 added to the first cuvette containing lysing reagent. This reagent lyses the red blood cells and simultaneously converts the released hemoglobin to a derivative that has a characteristic absorbance spectrum. An aliquot of the lysed whole blood is transferred from the first cuvette to a second cuvette where total hemoglobin concentration is measured at 405 nm and 700 nm.

Hemoglobin A1c measurement: The same aliquot of the lysed whole blood that is transferred from the first cuvette to the second cuvette for the Hb measurement is also used for the measurement of HbA1c. The second cuvette contains an anti-HbA1c antibody in a buffered reagent. 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 to this cuvette. 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 3).

DCA Vantage HbA1c Assay
The DCA Vantage HbA1c Assay measures both the concentration of hemoglobin A1c specifically and the concentration of total hemoglobin, with the ratio between the two reported as percent hemoglobin A1c. All the reagents for performing both reactions are contained in the DCA® Hemoglobin A1c (HbA1c) cartridge. For the measurement of total hemoglobin, potassium ferricyanide is used to oxidize hemoglobin in the sample to methemoglobin. The methemoglobin then complexes with thiocyanate to form thiocyan-methemoglobin,
the colored species that is measured. The extent of color development at 531 nm is proportional to the concentration of total hemoglobin in the sample. For the measurement of specific HbA1c, an inhibition of latex agglutination assay is used (Figure 4). An agglutinator (synthetic polymer containing multiple copies of the immunoreactive portion of HbA1c) causes agglutination of latex coated with HbA1c specific mouse monoclonal antibody. This agglutination reaction causes increased scattering of light, which is measured as an increase in absorbance at 531 nm. HbA1c in whole-blood specimens competes for the limited number of antibody-latex binding sites, causing an inhibition of agglutination and a decreased scattering of light. The decreased scattering is measured as a decrease in absorbance at 531 nm. The HbA1c concentration is then quantified using a calibration curve of absorbance versus HbA1c concentration (Figure 4).

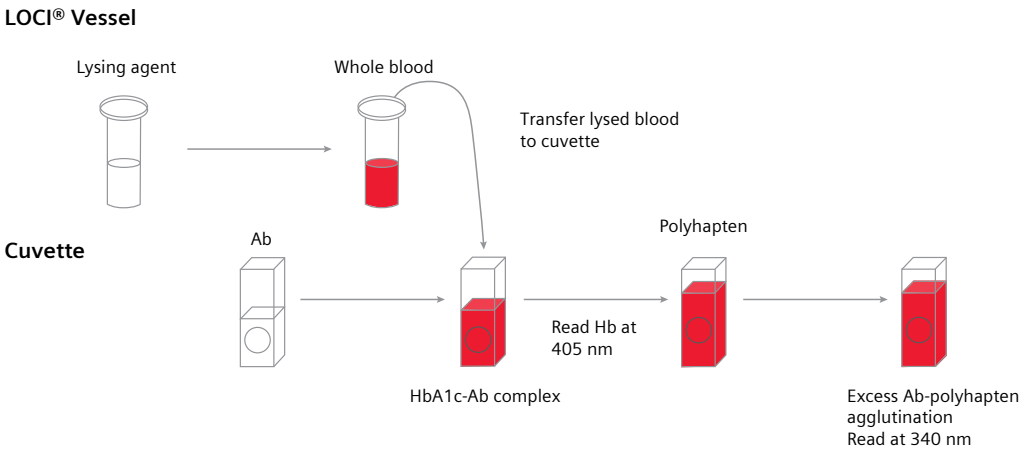

Figure 2. Reaction scheme of the Dimension Vista A1C Assay.

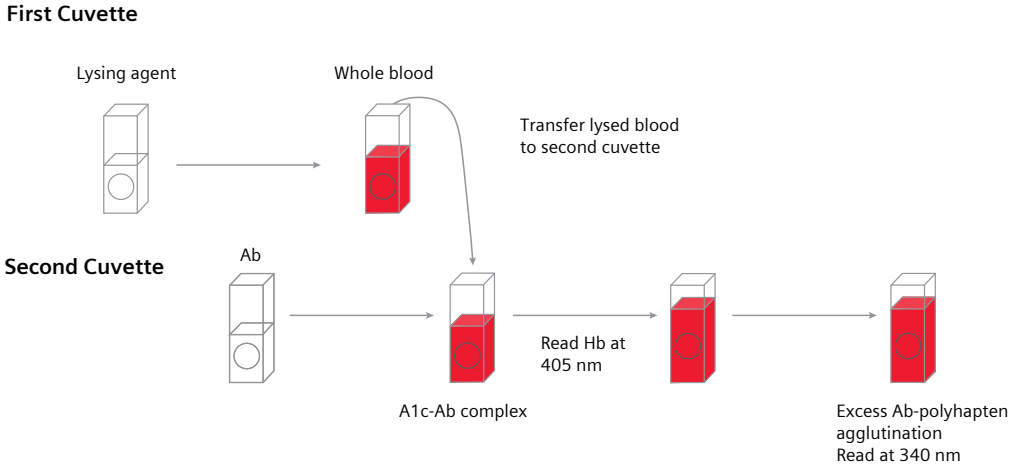

Figure 3. Reaction scheme of the Dimension A1C Assay.

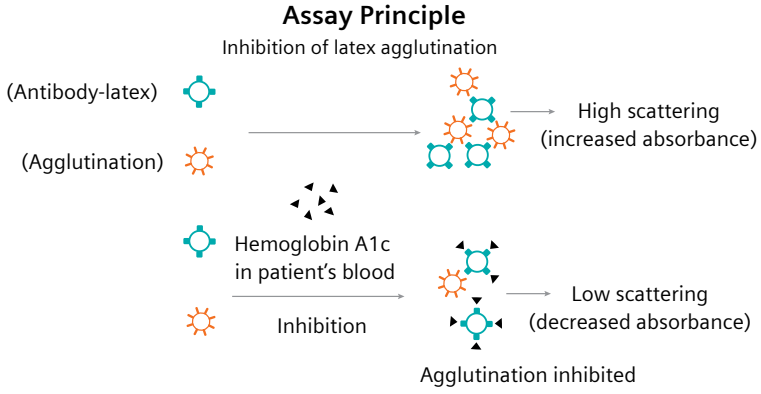

Figure 4. Reaction scheme of the DCA Vantage HbA1c Assay.

Results

Method comparison

Each assay was compared to the Atellica CH A1c_E Assay and an NGSP Secondary Secondary Reference Lab (SRL) by evaluating 50 samples using Deming regression analysis (Table 1); linear regression with first replicate results was used. Each assay correlates well with the Atellica CH A1c_E Assay and the NGSP SRL.

Data was analyzed in accordance with CLSI EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples. The mean %bias compared to NGSP is –0.84, 0.14, 3.26, –0.19, and 1.59 for the Atellica CH A1c_E, ADVIA Chemistry A1c_E, Dimension A1C, Dimension Vista A1C, and DCA Vantage HbA1c assays, respectively (Figure 6).

Table 1. Regression summary.

Tested Assay	Comparison Assay (y)	n	r	Slope	y-intercept
Atellica CH A1c_E	ADVIA Chemistry A1c_E	50	0.996	0.986	0.030
Atellica CH A1c_E	DCA Vantage HbA1c	50	0.994	0.972	0.032
Atellica CH A1c_E	Dimension A1C	50	0.991	1.019	-0.428
Atellica CH A1c_E	Dimension Vista A1C	50	0.996	1.020	-0.184

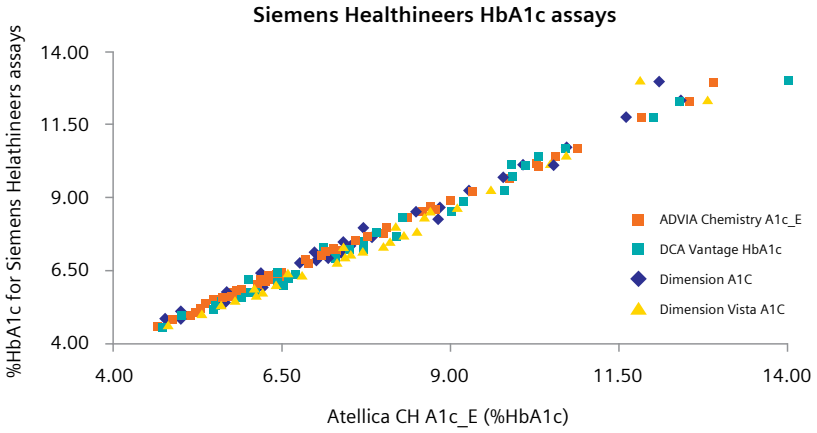

Figure 5. Correlation plot: Siemens Healthineers HbA1c assays versus Atellica CH A1c_E Assay.

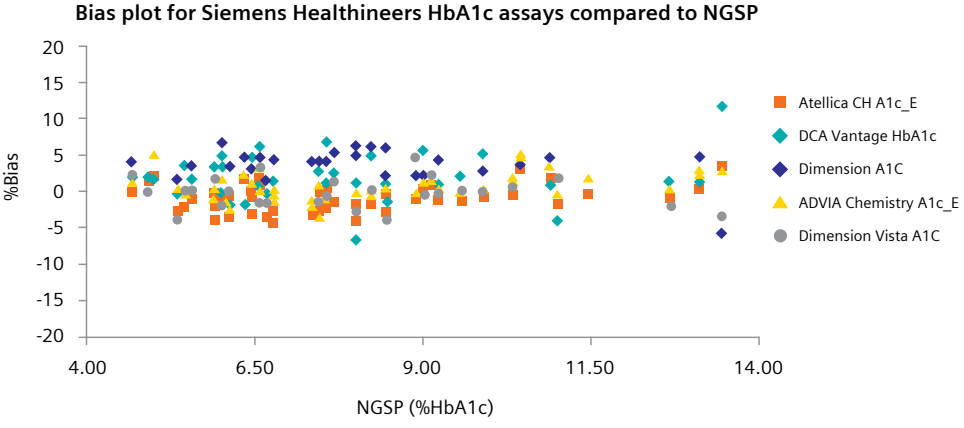

Figure 6. Bias plot: Siemens Healthineers assays versus the NGSP Secondary Reference Lab.

Conclusion

Siemens Healthineers offers an end-to-end solution for HbA1c testing, which includes POC as well as automated chemistry systems. All Siemens Healthineers HbA1c assays demonstrate close agreement across all systems and <3.5% mean bias to NGSP samples, enabling laboratories and physicians to be confident of results, regardless of the system used.

References:

1. The International Diabetes Federation. IDF diabetes atlas. Ninth edition, 2019. Available from: https://www.idf.org/e-library/epidemiology-research/diabetes-atlas.html
2. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329:977-86.

At Siemens Healthineers, our purpose is to enable healthcare providers to increase value by empowering them on their journey toward expanding precision medicine, transforming care delivery, and improving patient experience, all made possible by digitalizing healthcare.

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.

Product availability may vary from country to country and is subject to varying regulatory requirements. Please contact your local representative for availability.

Siemens Healthineers Headquarters
Siemens Healthcare GmbH
Henkestr. 127
91052 Erlangen, Germany
Phone: +49 9131 84-0
siemens-healthineers.com

Published by
Siemens Healthcare Diagnostics Inc.
Laboratory Diagnostics
511 Benedict Avenue
Tarrytown, NY 10591-5005
USA
Phone: +1 914-631-8000

Published by Siemens Healthcare Diagnostics Inc. · Order No. 30-21-DX-785-76 · 05-2021 · ©Siemens Healthcare Diagnostics Inc., 2021