Siemens Healthineers Enzymatic Hemoglobin A1c Calibrator* Commutability and Uncertainty

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Abstract

Background: In 2014, the World Health Organization estimated 422 million adults were living with diabetes globally, and 1.6 million deaths directly related to diabetes occurred in 2015. 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 Hemoglobin A, whereby a labile Schiff base is formed and converted into the more stable ketoamine (irreversible) via an Amadori rearrangement. Commutability and low uncertainties of product calibrators are desirable attributes. Commutability assures that results obtained during value transfer are reasonably free from matrix effects. Uncertainties determined for a product calibrator estimate the expected dispersion of the result. The uncertainty of the product calibrator determines the measurement variability to guide the decision maker in distinguishing between that measurement variability and significant clinical effects.

Method: A commutability study of the ADVIA® Chemistry Systems Enzymatic Hemoglobin A1c assay calibrators versus the Designated Comparison Method (DCM) performed by National Glycohemoglobin Standardization Program (NGSP) and estimation of uncertainty of the product calibrator's assigned values for %HbA1c and mmol/mol HbA1c, with uncertainty reported in percent.

Results: The evaluation of commutability of the product calibrators using 76 whole blood samples tested within the NGSP network yielded the following regression equation:

ADVIA Chemistry A1c_E assay = 1.013 [NGSP] - 0.04 HbA1c (r=0.987)

The product calibrators fall within the 95% confidence interval.

The %HbA1c values assigned to the two level product calibrators have an estimated 0.12 to 0.24 %HbA1c (0.76 to 2.16 mmol/mol) expanded total uncertainty in percent (k=2).

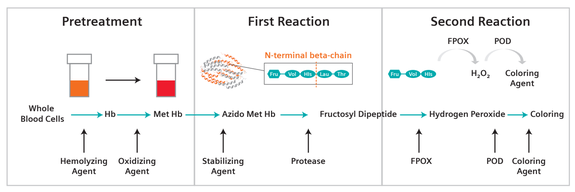
Conclusion: ADVIA® Chemistry Systems Enzymatic Hemoglobin A1c assay calibrators are commutable to the NGSP DCM and support a fully commutable traceability chain from the IFCC Reference Method to the patient result. The expanded total uncertainty from the manufacturing process is sufficient to maintain traceability to the IFCC Reference Method and for meeting the bias requirements of the manufacturer method certification from NGSP.

Materials and Methods

ADVIA Chemisty and Atellica CH A1c_E Assays

The ADVIA Chemistry and Atellica CH A1c_E assays are an enzymatic method that specifically measures N-terminal fructosyl dipeptides on the beta-chain of HbA1c. The concentration of glycated hemoglobin (A1c_E) and total hemoglobin (tHb_E) are measured separately; these measurements are used to determine the %HbA1c (NGSP units) or the A1c_E/tHb_E ratio in mmol/mol (IFCC units). A pretreatment solution hemolyzes the red blood cells, and sodium nitrite is used to convert hemoglobin (Hb) 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 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 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.



NGSP Designated Comparison Method

The NGSP Designated Comparison method is an ion-exchange HPLC method using Bio-Rex 70 resin with calibration based on results from the Diabetes Control and Complications Trial (DCCT).

Results

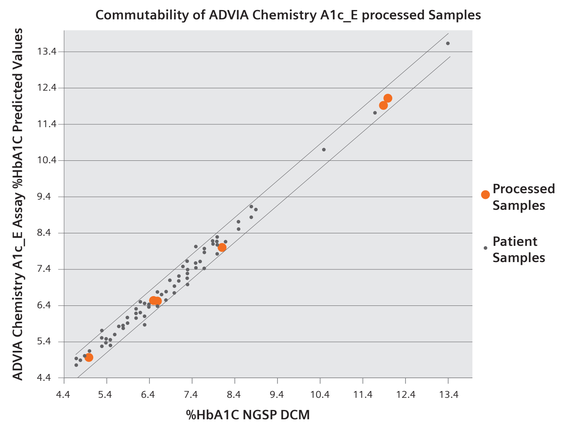
Commutability

Commutability was determined in accordance with CLSI EP32-R by using a collection of 76 single donor whole blood patient samples, the product calibrator, and 6 spiked samples tested on both the ADVIA Chemisty A1c_E assay (test assay) and the NGSP Reference Method (control assay). First, the ADVIA Chemistry A1c_E assay values were placed on the Y-axis and the NGSP values are on the X-axis for the donor samples to perform linear regression. Then, the pooled variance was calculated to determine the variance ratio needed for Orthogonal (Deming) Regression. Finally, a Deming Regression was performed on the donor samples. The predicted ADVIA Chemistry A1c_E assay results (y-axis) were plotted versus the actual NGSP results (x-axis), shown in Figure 2.

The 95% prediction intervals are added to the plot along with the processed sample results; the product calibrator and internal controls.

Linear regression analysis of ADVIA Chemistry
A1c_E assay = 1.013 [NGSP] - 0.04 HbA1c (r=0.987)

Figure 2:
Deming Regression of ADVIA Chemistry A1c_E Assay vs NGSP Reference Method



Results

Uncertainty

Uncertainty was determined in accordance with CLSI EP32-R and ISO 17511. The aggregate of significant sources of uncertainty were compiled after reviewing each step in the traceability chain. Estimates of uncertainty were calculated for two product calibrator lots and the largest total uncertainty is reported.

Total uncertainty = $\sqrt{U_1^2+U_2^2+U_3^2}$

Expanded total uncertainty = 2 x Total Uncertainty

U1 represents the uncertainty of the IFCC Calibrators as reported in Jeppsson, J-O, et al., Approved IFCC Reference Method for the Measurement of HbA1c in Human Blood, Clin Chem Lab Med, 40:78-89 (2002), page 83.

U2 represents the cumulative uncertainty of the secondary standards whereby the manufacturing and value assignment processes are the largest sources of uncertainty.

U3 represents the cumulative uncertainty of the product calibrators whereby the manufacturing and value assignment processes are the largest sources of uncertainty.

The value assignment protocol for the secondary and product calibrators uses a Latin Square approach whereby 3 instruments, 3 reagent lots, and 3 days are used to generate 9 curves for a total of 45 replicates per calibrator level.

Figure 3:
ADVIA Chemistry A1c_E Calibrator Traceability Chain for Hemoglobin A1C

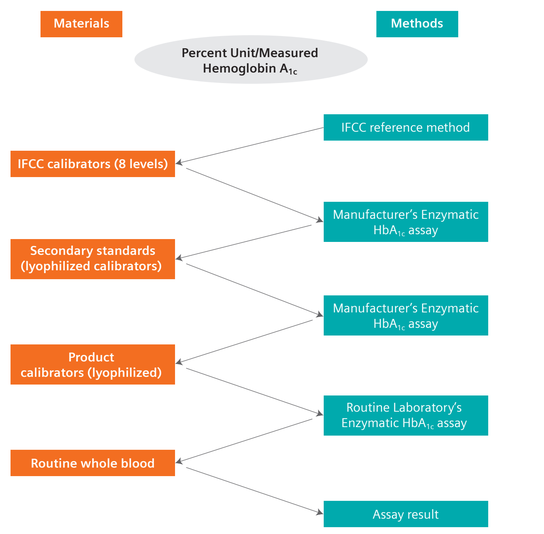


Table 1:
Reported Uncertainties

		A1c_E Calibrator Normal	A1c_E Calibrator Abnormal
Approximate Calibrator Concentration	Common Units (%)	5.2	11.5
Expanded Total Uncertainty	Common Units (k = 2) (%)	0.12	0.24
Approximate Calibrator Concentration	SI Units (mmol/mol)	33	102
Cumulative Total Uncertainty	SI Units (k = 2) (mmol/mol)	0.76	2.16
Expanded Total Uncertainty	(k = 2) (% of %HbA1c)	2.3	2.12

Conclusion

ADVIA® Chemistry Systems Enzymatic Hemoglobin A1c assay calibrators are commutable to the NGSP DCM and support a fully commutable traceability chain from the IFCC Reference Method to the patient result. The expanded total uncertainty from the manufacturing process is sufficient to maintain traceability to the IFCC Reference Method and for meeting the bias requirements of the manufacturer method certification from NGSP.

References:

1. The International Diabetes Federation. IDF Diabetes Atlas. Eighth edition; 2017. 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.

* Product availability may vary from country to country and is subject to varying regulatory requirements.

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