A comparative evaluation of the analytical performances of Capillarys 2 Flex Piercing, Tosoh HLC-723 G8, Premier Hb9210, and Roche Cobas c501 Tina-quant Gen 2 analyzers for HbA_{1c} determination

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Abstract

Introduction: Haemoglobin A_{1c} (HbA_{1c}) is widely used in the management of diabetes. Therefore, the reliability and comparability among different analytical methods for its detection have become very important.

Materials and methods: A comparative evaluation of the analytical performances (precision, linearity, accuracy, method comparison, and interferences including bilirubin, triglyceride, cholesterol, labile HbA_{1c} (LA_{1c}), vitamin C, aspirin, fetal haemoglobin (HbF), and haemoglobin E (Hb E)) were performed on Capillarys 2 Flex Piercing (Capillarys 2FP) (Sebia, France), Tosoh HLC-723 G8 (Tosoh G8) (Tosoh, Japan), Premier Hb9210 (Trinity Biotech, Ireland) and Roche Cobas c501 (Roche c501) (Roche Diagnostics, Germany).

Results: A good precision was shown at both low and high HbA_{1c} levels on all four systems, with all individual CVs below 2% (IFCC units) or 1.5% (NGSP units). Linearity analysis for each analyzer had achieved a good correlation coefficient ($R^2 > 0.99$) over the entire range tested. The analytical bias of the four systems against the IFCC targets was less than ± 6% (NGSP units), indicating a good accuracy. Method comparison showed a great correlation and agreement between methods. Very high levels of triglycerides and cholesterol (≥ 15.28 and ≥ 8.72 mmol/L, respectively) led to falsely low HbA_{1c} concentrations on Roche c501. Elevated HbF induced false HbA_{1c} detection on Capillarys 2FP (> 10%), Tosoh G8 (> 30%), Premier Hb9210 (> 15%), and Roche c501 (> 5%). On Tosoh G8, HbE induced an extra peak on chromatogram, and significantly lower results were reported.

Conclusions: The four HbA_{1c} methods commonly used with commercial analyzers showed a good reliability and comparability, although some interference may falsely alter the result.

Keywords: Haemoglobin A_{1c}; capillary electrophoresis (CE); high-performance liquid chromatography (HPLC); affinity chromatography; immunoassay

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Introduction

Haemoglobin A_{1c} (HbA_{1c}), a major portion of the glycated haemoglobins, is formed by a nonenzymatic interaction of glucose with the N-terminal valine residue of the HbA β chain in two basic steps: first, glucose binds reversibly to haemoglobin (Hb) as an aldimine Schiff base (an intermediate, termed labile HbA_{1c} (LA_{1c}), or Hb pre-A_{1c}); and then, the aldimine is transformed *via* an Amadori rearrangement into an irreversible ketoamine (1). Because HbA_{1c} reflects a mean blood glucose level over 2–3 months (normal lifespan of red blood cells) with a low short-term variability, it is widely used in the management of diabetes to monitor long-term glycemic control and to assess the risk of developing complications (2,3). In the last several years, the American Diabetes Association (ADA) and other major organizations have endorsed the use of HbA_{1c} determination for diabetes screening and have suggested the value of 6.5% (48 mmol/mol) as a diagnostic cut-off (4,5).

Analytical methods for HbA_{1c} quantification have been available since the 1970s. Presently, a num-

ber of methods are used: the capillary electrophoresis (CE) or ion-exchange chromatography method separates and determines HbA_{1c} from other Hb fractions based on charge differences; the boronate affinity chromatography method separates and quantifies glycated Hb from the nonglycated Hb based on the cis-diol group; and the immunoassay method uses antibodies to recognize the structure of the N-terminal glycated amino acids of the Hb β chain for quantification (6). As different methods for HbA_{1c} determination exhibit different characteristics and performances, over the past years, major efforts have been made by the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to standardize HbA_{1c} determination. The IFCC reference system has been defined as the only valid anchor to implement a standardization of the measurement (7). However, variability between methods is still observed in the presence of other members of the haemoglobin family (e.g., LA_{1c}, fetal haemoglobin (HbF), and variants) or interfering substances in the samples (e.g., bilirubin, triglyceride, cholesterol, and drugs) (8-13).

Due to clinical requirements and management demands, the reliability of different methods used to measure HbA_{1c} and their potential interchange ability represent a key feature in clinical practice. Therefore, the aim of this study was to evaluate the performances (precision, linearity, and accuracy), concordance (method comparison), and influence of the most frequent analytical interferences (bilirubin, triglyceride, cholesterol, LA_{1c}, vitamin C, aspirin, HbF, and haemoglobin E (HbE)) using the four systems. The results of this study will be helpful for laboratorians to be aware of the limitations of the methods and to select the appropriate one that is less likely to have interference.

Materials and methods

Analyzers

Four systems were used to obtain HbA_{1c} measurements: a Capillarys 2 Flex Piercing (Capillarys 2FP) (Sebia, France) CE system, a Tosoh HLC-723 G8 (Tosoh G8) (Tosoh, Japan) (variant-mode) ion-exchange high-performance liquid chromatography (HPLC) system, a Premier Hb9210 (Trinity Biotech, Ireland) boronate affinity HPLC system, and a turbidimetric inhibition immunoassay (TINIA) system using the Tina-quant Gen2 assay on a Roche Cobas c501 (Roche c501) (Roche Diagnostics, Germany) instrument. All four systems were certified by the IFCC and NGSP.

The analyzers as well as the associated reagents used for this evaluation were used according to the manufacturers' instructions and calibrated only once according to their routine standard operating procedures prior to any sample analysis. Internal quality controls (both low and high levels) supplied by the manufacturers and purchased from Bio-Rad Laboratories, Hercules, CA, USA (740 Diabetes Controls, lot: 33861/33862) were measured along with the samples on the same day during this study. The same assessment test by the four systems was carried out on the same day by the same technician in our laboratory. The same assessment test by one system was carried out using the same batch accompanied with the same controls.

Samples

Whole blood samples (N = 157) and umbilical cord blood (N = 1) were collected in EDTA-containing tubes (2.0 mL, BD Diagnostics, Franklin Lakes, NJ, USA) and kept at 4 ± 2 °C to be analyzed within 24 h. For precision, method comparison, and analytical interference of Hb variants, the samples were kept at - 80 °C (14,15) and analyzed within 1 month. Whole blood samples were obtained from the Clinical Laboratory of Guangdong Provincial Hospital of Chinese Medicine. Umbilical cord blood was collected by clinicians at the Department of Obstetrics and Gynecology of the Third Affiliated Hospital, Guangzhou Medical University, from the umbilical cord vein attached to the placenta, which had been detached from a newborn. Whole blood samples (N = 151) were collected according to HbA_{1c} concentrations (3.0–15.0%; 9–140 mmol/mol) on a Premier Hb9210 instrument, with HbF < 1%, normal HbA_2 (2.5–3.5%), and without Hb vari-

ants on the Hb phenotype analysis by Bio-Rad Variant II (Bio-Rad, Japan) system using the beta thalassemia program. The Hb variants HbA/E (N = 6) (a substitution of lysine for glutamic acid at position 26 of the β chain) were collected from routine laboratory testing samples for thalassemia and confirmed by DNA sequencing of Hb α and β-chain gene (HBA_1, HBA_2 and HBB) at the Beijing Genomics Institute (BGI, Shenzhen, China).

This study was approved by the Research and Ethics committee of our institution, and all participants signed their consents prior to the study.

Precision

The precision was evaluated in accordance with the EP15-A2 (16) protocol of the Clinical and Laboratory Standards Institute (CLSI). Two whole blood samples at levels of 5.0% (31 mmol/mol) and 8.5% (69 mmol/mol) by Premier Hb9210 were divided into five aliquots per level and frozen at -80 ºC. One aliquot of each level was thawed daily, and the HbA_{1c} value was analyzed three times per day during a period of five consecutive workdays (N = 15 *per* level). Precision was evaluated as the coefficient of variation (CV), which is calculated from the data series mean and standard deviation. The formulas used to calculate CV were as follows:

$$S_{within} = \sqrt{\frac{\sum_{d=1}^{D}\sum_{i=1}^{n}(X_{di} - \overline{X}_d)^2}{D(n-1)}} \quad (1)$$

$$S_{total} = \sqrt{\frac{n-1}{n} \times S_{within}^2 + B} \quad (2)$$

$$B = \frac{\sum_{d=1}^{D}\left(\overline{X_d} - \overline{\overline{X}}\right)}{D-1} \quad (3)$$

$$\%CVwithin = Swithin/\overline{\overline{X}} \times 100\% \quad (4)$$

$$\%CVtotal = Stotal/\overline{\overline{X}} \times 100\% \quad (5)$$

where D is the total number of days (five), n is the total number of replicates *per* day (three), X_{di} is the result of replicate i for day d, $\overline{X_d}$ is the average of all results for day d, and $\overline{\overline{X}}$ is the average of all results.

Acceptable CV is recommended to be less than 3% for SI units and 2% for NGSP units (17-19).

Linearity

A test for linearity was carried out in accordance with the CLSI protocol EP6-A 8 (20). Linearity was investigated by preparing six different ratios of samples (5:0, 4:1, 3:2, 2:3, 1:4, and 0:5) from two samples with HbA1c results of 4.2% (22 mmol/mol) and 14.9% (139 mmol/mol) by Premier Hb9210 containing the same total Hb concentration (129 g/L). Each sample was assayed in duplicate, and the means were used to examine the linearity. The theoretical HbA_{1c} values (calculated from the ratios of the mixed packed-cells with high and low HbA_{1c} values) and the measured values were compared. Polynomial regression analysis was performed for first-, second-, and third-order polynomials.

Accuracy

Four samples used in the method comparison were validated by the Shanghai IFCC Reference Laboratory using the IFCC HPLC/CE reference method (21). The measurements of HbA_{1c} were made in triplicate. The relative bias was calculated by each of the four system values against the IFCC reference method value for each sample. The proficiency testing acceptance limit ± 6% of College of American Pathologists (CAP) was set as the accuracy limit (relative bias was calculated by NGSP units) (7).

Method comparison

The correlation between systems was assessed by analyzing 93 samples representing a range of HbA_{1c} values 4.0–13.0% (20–119 mmol/mol) by Premier Hb9210. The boronate affinity HPLC method with the Premier Hb9210 instrument was used as the comparative method.

Analytical interferences

Bilirubin, triglycerides/cholesterol, LA_{1c}, vitamin C, and aspirin

Interferences of bilirubin, triglycerides/cholesterol, LA_{1c}, vitamin C, and aspirin were evaluated in two native samples with HbA_{1c} results of 5.3% (34 mmol/mol) and 7.6% (60 mmol/mol) by Premier Hb9210. Hyperbilirubinemic or triglycerides/cholesterol-rich plasma and drugs (glucose, vitamin C, and aspirin) were prepared: bilirubin (< 10 µmol/L and 445.50 µmol/L; measured by Roche Modular P Chemistry Analyzer (Modular P), Roche Diagnostics, Germany), triglyceride (1.48, 10.21, and 19.10 mmol/L; measured by Roche Modular P), cholesterol (4.63, 10.29, and 10.90 mmol/L; measured by Roche Modular P), glucose (277.78 mmol/L; China Otsuka Pharmaceutical, Tianjin, China), vitamin C (ascorbic acid; 250 mg/mL; CSPC Ouyi Pharmaceutical, Shijianzhuang, China), and aspirin (100 mg; Bayer Healthcare, Milano, Italy). Then, various dilutions were prepared by mixing the original pool with plasma or isotonic saline solution (0.9%, Jiangxi pharmaceutical, Ganzhou, China).

Interferences of bilirubin and triglycerides/cholesterol were assessed by mixing washed red blood cells with various dilutions of hyperbilirubinemic or triglycerides/cholesterol-rich plasma to achieve final concentrations of 89.10, 178.20, 267.30, 356.40, and 445.50 µmol/L bilirubin; 2.04, 4.08, 6.12, 8.16, and 10.21 mmol/L triglycerides; 2.06, 4.12, 6.18, 8.23, and 10.29 mmol/L cholesterol; and 3.82 / 2.18, 7.64 / 4.36, 11.46 / 6.54, 15.28 / 8.72, and 19.10 / 10.90 mmol/L triglycerides/cholesterol.

Interference of LA_{1c} was examined by incubating samples with various dilutions of glucose solutions (5.65, 27.78, 55.56, 138.89, and 277.78 mmol/L) at 37 °C for 3 h, and the samples were mixed every 30 min prior to the assay (22). The amount of LA_{1c} formed was estimated using a Tosoh G8 analyzer (LA_{1c} can be separated from HbA_{1c} and others, and the percentage value is the percentage of total Hb as reported).

Interference of vitamin C or aspirin was performed by mixing blood samples spiked with various dilutions of vitamin C or aspirin solution (final concentrations: 25, 50, 100, 150, 200, and 250 mg/mL vitamin C; and 6.66, 13.32, 19.98, 26.64, and 33.30 mg/mL aspirin).

The measurements of HbA_{1c} were made in triplicate, and the relative bias of each specimen was calculated from the observed value against the baseline value.

HbF

Interference with HbF was evaluated by mixing 40 native samples representing a range of HbA_{1c} values (4–13%; 20–119 mmol/mol) with umbilical cord blood in order to obtain 6 groups with final HbF concentrations of 5–10% (N = 5), 10–15% (N = 5), 15–20% (N = 7), 20–25% (N = 10), 25–30% (N = 5), and 30–40% (N = 8). The HbF concentration was analyzed by the Tosoh G8 instrument as the HbF percentage (HbF can be separated from HbA_{1c} and others, and the percentage value is the percentage of total Hb as reported).

The measurements of HbA_{1c} were made in triplicate. The relative bias of each specimen was calculated from the observed value and the baseline value (NGSP units).

Hb variant

Interference with a Hb variant was tested in several samples containing the most frequent Hb variant HbA/E (N = 6). The boronate affinity HPLC method with the Premier Hb9210 instrument was used as the comparative method as it is not expected to be influenced by the presence of Hb variants (23). For each test method, the results were compared to those obtained using the comparison method.

Statistical analysis

Data analysis was performed using MedCalc version 14.8.1 (MedCalc Software, Ostend, Belgium). The method comparisons were performed by Passing-Bablok regression, and the differences between any two methods were presented in a Bland-Altman plot. For method comparison and analytical interferences, relative bias > ± 7% (calculated by NGSP units) was considered clinically significant (NGSP criterion) (7).

Results

Precision

The within-run CVs at low and high HbA_{1c} concentrations on all four systems were less than 2.0% and 1.2% (with IFCC units), or 1.3% and 1.0% (with NGSP units), respectively (Table 1). The total CVs at the low and high HbA_{1c} levels were less than 2.0% and 1.7% (with IFCC units), and 1.4% and 1.5% (with NGSP units), respectively (Table 1).

Linearity

The linear regression analysis for each of the four analyzers showed a line with a good correlation coefficient ($R^2 > 0.99$) over the entire range tested (Table 1).

Accuracy

The relative bias against the IFCC targets was less than ± 6% for all four systems tested (NGSP units), indicating a good accuracy of these methods (Table 2).

Method comparison

The correlation between the Capillarys 2FP and Premier Hb9210 analyzers, expressed in NGSP units, is described with the Passing-Bablok regression fit: Y = 1.07 (95% confidence interval (CI): 1.03 to 1.10) X – 0.31 (95% CI: - 0.51 to - 0.08), without significant deviation from linearity (P = 0.13) (Figure 1A). The Bland-Altman plot showed a mean absolute difference of 0.13% HbA_{1c} (Figure 1B). All samples had less than 7% relative difference.

The correlation between the Tosoh G8 and Premier Hb9210 analyzers, expressed in NGSP units, is described with the Passing-Bablok regression fit: Y = 1.02 (95% CI: 1.00 to 1.05) X – 0.01 (95% CI: - 0.23 to 0.10), without significant deviation from linearity (P = 0.46) (Figure 1C). The Bland-Altman plot showed a mean absolute difference of 0.13% HbA_{1c} (Figure 1D). All samples had less than 7% relative difference.

The correlation between the Roche c501 and Premier Hb9210 analyzers, expressed in NGSP units, is described with the Passing-Bablok regression fit: Y = 0.96 (95% CI: 0.93 to 1.00) X + 0.23 (95% CI: 0.00

Table 1. Precision and linearity of HbA_{1c} values analyzed by the four analyzers

Analyzers	Imprecision (mmol/mol)				Imprecision (%)				Linearity					
	CV Within-run, %		CV Total, %		CV Within-run, %		CV Total, %		HbA_{1c}, %		95% CI		R^2	Regression line equation
	Low	High	Low	High	Low	High	Low	High	low	high	A	B		
Capillarys 2FP	1.76	1.11	1.94	1.15	1.20	0.95	1.32	0.99	3.5	15.3	0.95–1.09	- 1.06–0.30	0.9978	y = 1.0193 x – 0.3812
Tosoh G8	0.71	0.32	1.33	0.61	0.47	0.39	0.87	0.53	3.6	14.9	0.88–1.11	- 0.67–1.62	0.9932	y = 0.9938 x + 0.4744
Premier Hb9210	0.98	0.84	1.41	1.61	0.65	0.62	0.93	0.68	4.2	14.9	0.87–1.11	- 0.75–1.79	0.9920	y = 0.9900 x + 0.5207
Roche c501	1.90	0.99	1.76	1.65	1.21	0.82	1.16	1.40	4.0	13.3	- 0.62–0.20	0.97–1.06	0.9990	y = 1.0107 x – 0.2094

Low - whole blood sample with 5.0% (31 mmol/mol) HbA_{1c} concentration. High - whole blood sample with 8.5% (69 mmol/mol) HbA_{1c} concentration. CV – coefficient of variation. Linearity was calculated using NGSP units (%). 95% CI – confidence intervals of 95%. R^2 – coefficient of correlation. The regression line equation is presented as y = A x + B. A – regression line slope. B - regression line intercept.

Table 2. Accuracy of HbA_{1c} values analyzed by the four analyzers

Sample number	HbA1c, %					Relative bias, %			
	Target value	Capillarys 2FP	Tosoh G8	Premier Hb9210	Roche c501	Capillarys 2FP	Tosoh G8	Premier Hb9210	Roche c501
1	5.4	5.4	5.7	5.2	5.2	0.0	5.6	-3.7	-3.7
2	6.1	6.4	6.2	6.0	6.2	4.9	1.6	-1.6	1.6
3	7.8	7.9	8.0	7.6	7.6	1.3	2.6	-2.6	-2.6
4	10.4	10.7	10.5	10.3	10.1	2.9	1.0	-1.0	-2.9

The CAP proficiency testing acceptance limit of ± 6% was set as the accuracy limit (relative bias was calculated by NGSP units).

to 0.45), without significant deviation from linearity (P = 0.08) (Figure 1E). The Bland-Altman plot showed a mean absolute difference of -0.04% HbA1c (Figure 1F). All samples had less than 7% relative difference.

Analytical interferences

Assessment of interferences is shown in Table 3. No interference was observed with Capillarys 2FP, Premier Hb9210, and Tosoh G8 at the following interfering substance concentrations tested: 445.50 µmol/L bilirubin, 10.21 mmol/L triglyceride, 10.29 mmol/L cholesterol, or 19.10 / 11.90 mmol/L triglycerides/cholesterol. On Roche c501 analyzer, no analytical interference of bilirubin was noticed for concentrations reaching 445.50 µmol/L; while, at concentrations of 15.28 / 8.72 and 19.10 / 11.90 mmol/L triglycerides/cholesterol, a significant bias was observed (relative bias > ± 7%).

A glucose concentration less than 277.78 mmol/L (LA_{1c} < 10.9%) did not interfere with HbA_{1c} quantification on any of the four analyzers. In addition, no significant bias was observed with the results analyzed by the Capillarys 2FP analyzer in the presence of vitamin C up to 250 mg/mL. In contrast, using the Premier Hb9210 and Tosoh G8 analyzers, vitamin C concentrations of more than 50 and 150 mg/mL, respectively, interfered with the HbA_{1c} measurements.

Moreover, the HbA_{1c} value was falsely increased only with the Tosoh G8 analyzer at an aspirin concentration exceeding 26.64 mg/mL in the samples.

Compared with the native sample, on the Capillarys 2FP analyzer, significant biases were observed in samples with 10–15% HbF (relative bias > ± 7%). On the Premier Hb9210 analyzer, the results were not affected in the two groups of samples with 5–10% and 10–15% HbF (relative bias < ± 7%). On the Tosoh G8 analyzer, the HbA_{1c} results were obtained by a correction via excluding the HbF peak from the total integrated area, resulting in an insignificant interference in the samples with HbF < 30% (relative bias < ± 7%). For all samples analyzed by the Roche c501 analyzer, the HbA_{1c} results were influenced by elevated HbF values > 5% (relative bias > ± 7% for all groups).

The Capillarys 2FP analyzer gave a perfect separation of the variant HbE; on the Tosoh G8 analyzer, an extra peak was present between the A_{1c} and A_0 peaks (Figure 2). A rather good agreement was noticed between the Capillarys 2FP/Roche c501 and Premier Hb9210 analyzers (relative bias < ± 7%); meanwhile, a significant negative bias of HbA_{1c} values was observed systematically on the Tosoh G8 system in comparison with the Premier Hb9210 analyzer (relative bias > ± 7%).

Discussion

The data presented here are comparative evaluations of the analytical performance of four commonly used systems: Capillarys 2FP, Tosoh G8, Premier Hb9210, and Roche c501 Tina-quant Gen2. Precision studies showed good performances, with CVs of the four HbA_{1c} assays well within the recommendations (below 3% for IFCC units and 2% for NGSP units) (17-19). The linearity was excellent over the clinical range of HbA_{1c} values. Accu-

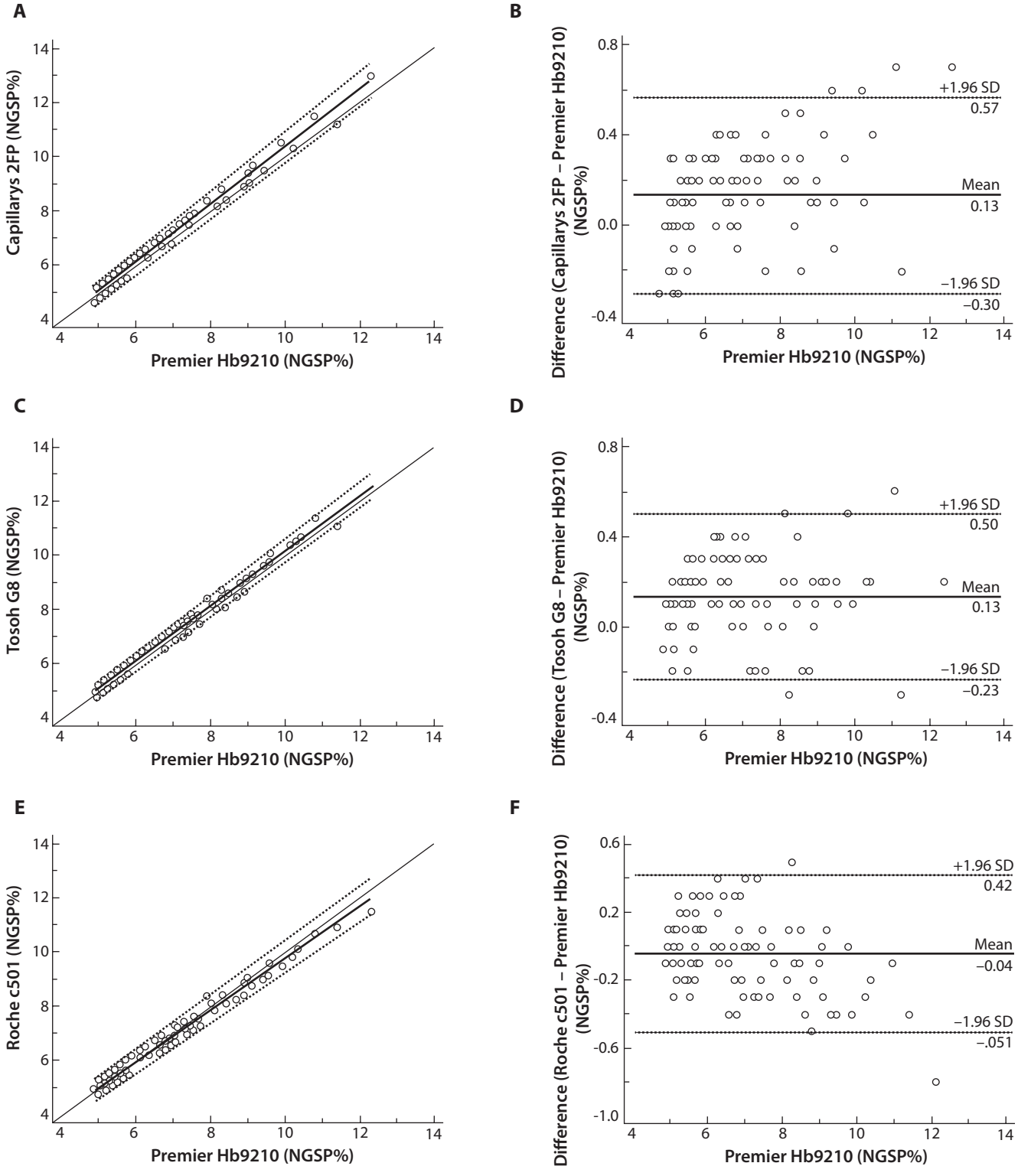

Figure 1. Comparison of HbA1c values obtained with four analyzer using Passing and Bablok (PB) regression analysis and Bland-Altman (BA) plot. (A) Comparison of Capillarys 2FP and Premier Hb9210 by PB. (B) Comparison of Capillarys 2FP and Premier Hb9210 by BA. (C) Comparison of Tosoh G8 and Premier Hb9210 by PB. (D) Comparison of Tosoh G8 and Premier Hb9210 by BA. (E) Comparison of Roche c501 and Premier Hb9210 by PB. (F) Comparison of Roche c501 and Premier Hb9210 by BA. In the Passing-Bablok regression analyses the dotted lines show the 95% confidence interval (CI); the gray lines represent the identity line (X = Y). In the Bland-Altman plots the solid lines show the mean difference, while the dotted lines show the mean difference ± 1.96 SD.

Table 3. Sample interferences for common interferents

Interferent	Relative bias from native sample, %							
	Capillarys 2 FP		TOSOH G8		Premier Hb9210		Roche c501	
	Low	High	Low	High	Low	High	Low	High
TBIL, µmol/L								
89.1	3.64	0.00	1.89	0.00	1.89	- 2.63	1.13	0.26
178.2	- 1.82	0.00	0.00	1.28	1.89	- 1.32	1.89	- 0.79
267.3	- 3.64	3.85	0.00	0.00	1.89	0.00	1.32	- 1.18
356.4	- 1.82	1.28	1.89	0.00	1.89	- 1.32	- 0.19	- 2.76
445.5	- 1.82	1.28	1.89	0.00	1.89	1.32	- 0.38	- 3.55
TG, mmol/L								
2.04	- 3.64	1.28	1.89	0.00	0.00	- 1.32	- 1.13	3.42
4.08	- 3.64	2.56	0.00	0.00	0.00	- 2.63	- 2.45	1.32
6.12	- 3.64	1.28	0.00	- 1.28	- 1.89	- 2.63	- 1.51	- 1.45
8.16	- 3.64	- 1.28	0.00	- 1.28	- 1.89	- 3.95	- 3.77	1.18
10.21	- 1.82	- 1.28	0.00	0.00	1.89	- 1.32	- 0.38	- 1.71
CHOL, mmol/L								
2.06	- 3.64	- 2.56	0.00	0.00	1.89	- 1.32	- 2.83	- 3.68
4.12	- 3.64	- 3.85	1.89	0.00	0.00	- 1.32	- 2.83	- 3.16
6.18	- 3.64	- 1.28	0.00	- 1.28	1.89	- 1.32	- 4.53	- 3.16
8.23	- 3.64	- 3.85	0.00	- 1.28	1.89	- 1.32	- 4.15	- 1.58
10.29	0.00	- 1.28	- 1.89	- 1.28	0.00	- 1.32	- 3.21	- 2.50
TG/CHOL, mmol/L								
3.82 / 2.18	- 3.64	- 1.28	1.89	0.00	3.77	- 1.32	- 1.70	- 3.55
7.64 / 4.36	0.00	- 1.28	0.00	1.28	1.89	- 2.63	- 2.83	- 3.42
11.46 / 6.54	0.00	0.00	1.89	- 1.28	1.89	- 1.32	- 4.15	- 3.42
15.28 / 8.72	0.00	2.56	1.89	0.00	3.77	- 2.63	**- 7.74**	- 5.13
19.10 / 11.90	0.00	1.28	1.89	0.00	3.77	- 2.63	**- 8.79**	- 5.26
Vit C, mg/mL								
25	/	/	/	/	3.8	4.8	/	/
50	1.92	0.00	3.70	1.23	**20.75**	**10.53**	4.53	-1.45
100	0.00	- 1.27	5.56	1.23	**50.94**	**25.00**	6.42	6.32
150	- 5.77	1.27	**14.81**	4.94	56.60	32.89	10.00	7.37
200	1.92	- 2.53	**24.07**	**7.41**	**52.83**	**34.21**	6.04	5.92
250	0.00	0.00	**22.22**	**8.64**	**50.94**	**32.89**	4.72	3.42
Aspirin (mg/mL)								
6.66	- 1.82	- 2.56	3.70	0.00	1.89	- 1.32	3.58	- 0.53
13.32	- 1.82	- 3.85	5.56	1.23	3.77	- 1.32	2.08	1.18
19.98	- 3.64	- 5.13	5.56	2.47	1.89	- 1.32	2.08	- 1.18
26.64	- 3.64	0.00	**9.26**	2.47	1.89	0.00	- 0.19	0.13
33.33	- 3.64	- 1.28	**11.11**	3.70	0.00	- 2.63	2.45	1.45
Glu (mmol/L)								
5.56	1.82	1.16	0.00	0.00	0.00	1.19	2.82	4.81
27.78	- 1.82	1.16	0.00	0.00	0.00	0.00	- 0.56	4.44
55.56	- 3.64	0.00	0.00	- 3.49	1.82	- 2.38	0.00	0.99
138.89	- 3.64	- 1.16	0.00	- 3.49	1.82	- 2.38	1.13	0.74
277.78	- 3.64	0.00	0.00	- 4.65	5.45	- 1.19	2.07	0.00

Low - whole blood sample with HbA_{1c} concentration of 5.3% measured by Premier Hb9210. High - whole blood sample with HbA_{1c} concentration of 7.6% measured by Premier Hb9210. TBIL - total bilirubin. TG – triglycerides. CHOL – cholesterol. Vit C - vitamin C. Glu - glucose. "/" - not performed. The bold results show relative bias > ± 7% (calculated by NGSP units). Relative bias > ± 7% was considered clinically significant (NGSP criterion).

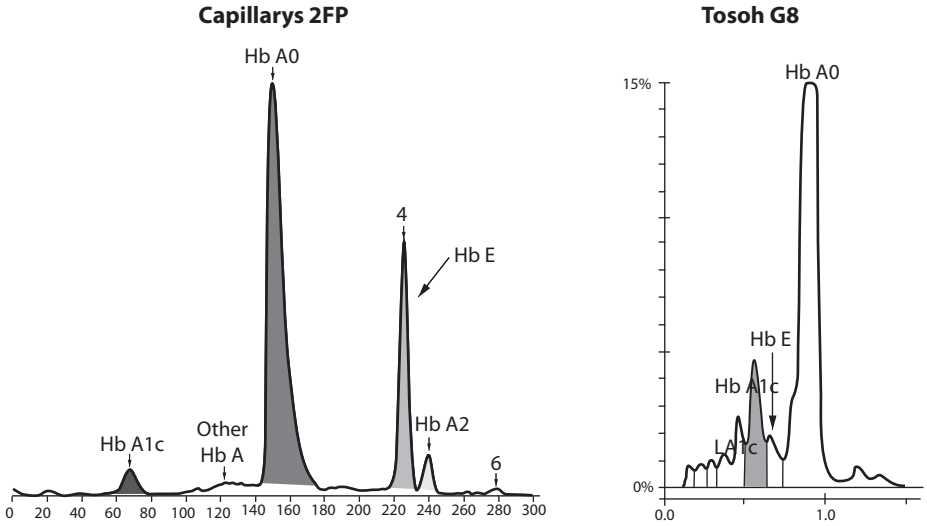

Figure 2. Detection of HbE with the Capillarys 2FP and Tosoh G8 analyzers. The arrow indicates the presence of the variant (HbE).

racy verification demonstrated a great consistency among the four systems tested in comparison with the IFCC values. The high analytical performances in terms of precision, linearity, and accuracy of all four systems were in accordance with previous studies (15,24-27) and manufacturer claims.

In the HbA$_{1c}$ comparison study, Passing-Bablock regression analysis highlighted a good correlation between any two methods. Moreover, the Bland-Altman plot showed that the HbA$_{1c}$ values between any two of the four systems were in good agreement.

The measurement of HbA$_{1c}$ by the Capillarys 2FP, Tosoh G8, and Premier Hb9210 analyzers was not subjected to common interferences such as bilirubin and triglycerides/cholesterol, and these findings confirmed the results presented previously (15,24,25,28). However, very high levels of triglycerides/cholesterol led to a falsely low HbA$_{1c}$ value in the immunoassay using the Roche c501 system. Furthermore, it seems that samples with a low HbA$_{1c}$ value are more susceptible to interference. According to the manufacturer's claim, no interference is observed with lipemic samples containing triglycerides concentrations less than 15.52 mmol/L.

LA$_{1c}$ is an intermediate molecule, and its concentration is associated with blood glucose concentra-

Table 4. HbA$_{1c}$ values for HbE on the Capillarys 2FP, Tosoh G8, Premier Hb9210, and Roche c501 systems

Sample	HbA$_{1c}$, %				Relative bias from Premier Hb9210, %		
	Premier Hb9210	Capillarys 2FP	Tosoh G8	Roche c501	Capillarys 2FP	Tosoh G8	Roche c501
1	5.5	5.6	4.2	5.4	1.8	**-23.6**	-1.8
2	5.0	4.8	4.2	5.0	-4.0	**-16.0**	0.0
3	5.3	5.3	4.7	5.6	0.0	**-11.3**	5.6
4	5.1	5.2	4.1	5.2	2.0	**-19.6**	2.0
5	5.6	5.9	4.6	5.7	5.4	**-18.0**	1.8
6	5.6	5.3	4.6	5.4	-5.4	**-18.0**	-3.6

The boronate affinity HPLC method with the Premier Hb9210 instrument was used as the comparative method. The bold results show the relative bias > ± 7% (calculated by NGSP units). Relative bias > ± 7% was considered clinically significant (NGSP criterion).

tions. The isoelectric points of LA_{1c} and its stable counterpart are similar, which may lead to little or no separation between them by some methods that rely on molecular charge for separation. In this study, no effect was reported on the quantification of HbA_{1c} by the two methods based on the principle of molecular charge. The current study showed that treatment with glucose up to 277.78 mmol/L *in vitro*, much higher than the concentration reported previously (15,24,29) and the manufacturer's claims, did not interfere with the HbA_{1c} measurement, suggesting that the blood LA_{1c} contents may not affect the quantification of HbA_{1c} by the four tested methods.

In our study, for the concentration of vitamin C or aspirin at a dose higher than clinical relevance, it is presumed that the concentration of vitamin C or aspirin in patient samples does not affect HbA_{1c} results tested by the four analyzers in clinical practice. Chronic ingestion of aspirin or vitamin C in high doses may promote acetylation of HbA_{1c} chains or inhibit Hb glycation, thereby affecting HbA_{1c} results (13,30-33). Thus, the effects of aspirin and vitamin C on HbA_{1c} assays are uncertain, and they may be of a biological nature rather than an analytical interference, although this needs to be investigated in a future study.

With the Capillarys 2FP instrument, at an increased proportion of HbF greater than 10%, which is in excess of that reported by Jaisson *et al*. (24), the separation of HbF and HbA_0 could not be accomplished, resulting in a false result. As the HbF amount was excluded from the total integrated amount through using the Tosoh G8 instrument software, it may not interfere with the HbA_{1c} measurement at a concentration < 30%, in agreement with a previous evaluation and NGSP reports (7,8). Elevated HbF > 15%, in agreement with boronate affinity methods reported by the NGSP, can affect the HbA_{1c} results using the Premier Hb9210 system, which might be due to a lower glycation rate of HbF compared with that of HbA (7). The HbA_{1c} concentrations can be misestimated with the Roche c501 instrument at a higher HbF level (> 5% in our study). However, there are no manufacturer claims of HbF interference. According to the NGSP, HbF levels > 10–15% can interfere with the Tina-quant Gen2 assay (7). Elevated HbF levels can occur under some pathological conditions such as beta-thalassemia, delta/beta-thalassemia, or a hereditary persistence of HbF (HPFH). The four instruments would lead to inaccurate results using different concentrations of HbF; therefore, it is important for laboratories to consider this fact in areas of a high prevalence of thalassemia or HPFH.

Over 1000 different Hb variants have been discovered. Quantification of HbA_{1c} in the presence of an Hb variant is an analytical challenge in the clinical laboratory. The most common Hb variants worldwide in descending order of prevalence are HbS, HbE, HbC, and HbD. As the second most prevalent hemoglobinopathy worldwide, HbE is mostly found in the Far East and Southeast Asia; thus, it has been included in the present study (11). A good agreement of HbA_{1c} concentrations was observed between the Capillarys 2FP, Roche c501, and Premier Hb9210 analyzers; therefore, it was concluded that the HbA1c measurements on these three systems were not affected by the presence of the HbE variant, confirming the results reported previously (9,10,15,23-25,28). However, on the Tosoh G8 instrument, the presence of HbE induces an extra peak on the chromatogram, which is not reportable as shown previously (10,23).

In summary, the four HbA_{1c} methods commonly used with commercial analyzers showed a good comparability and reliability, although some interference may impede the results.

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Potential conflict of interest

None declared.

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