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Original article

Carryover impacts on biochemical analytes preceding with whole blood HbA1C samples on automated chemistry

Tatchapol Nanthakhan*

Department of Laboratory Medicine, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand

Abstract

Background: Automated clinical chemistry analyzers, employing robotic pipetting and cuvette washing systems, are the standard means for processing patient specimens. The pipetting and washing systems are designed to continuously sample patient specimens and clean sample probes and reaction cuvettes or vessels. An obvious concern is the potential for carryover of analyte from one patient specimen into another or more following patient specimens. Moreover, the sedimentation time of the blood before the sampling process may vary from lab to the other, depending on the device and protocol used, affecting the analysis outcomes.

Objectives: This study aimed to examine the continued effects of residue carried by the sample probe used in whole blood HbA1c analysis on other biochemical analyses performed by the automated system and determine the relationship of the HbA1c level with the sedimentation time of red blood cells.

Methods: This cross-sectional analysis was performed on an archive of blood samples of 100 type-2 diabetic (T2DM) patients collected from May to June 2023. Blood sedimentation time varied from 0 to 7, 15 to 30 min. The samples were analyzed for cumulative sugar levels (HbA1c) with the enzymatic assay, and sample carryover was assessed using Alinity C modules (Abbott Diagnostics, USA). The levels of HbA1c at the initial time point (0 min; T0) were compared with the levels at three different time points (7 min; T1, 15 min; T2, and 30 min; T3).

Results: The carried-over potassium, AST, ALT, ALP, and CPK values are 0.002 ± 0.068 mmol/L, - 0.040 ± 1.008 U/L, - 0.100 ± 1.454 U/L, -0.080 ± 1.550 U/L, and 0.060 ± 1.308 U/L, respectively. There is no significant difference in the HbA1c level in all comparison groups (T0-T1: 7.0 ± 1.3, T0-T2: 7.0 ± 1.3, T0-T3: 7.1 ± 1.3). The HbA1c values at the initial time point positively correlated with those found in three different 3 time periods (0 - 7 min (T1); $r = 0.9990$; $P < 0.001$, 0 - 15 min (T2); $r = 0.9985$; $P < 0.001$, and 0 - 30 min (T3); $r = 0.9986$; $P < 0.001$), respectively.

Conclusion: Our study demonstrates that carryover residues from 0, 7, 15, and 30 minutes do not interfere with the HbA1c level and other clinical chemistry test results performed by the automated analyzer. Additionally, HbA1c values were not affected by the sedimentation time of red blood cells (up to 30 min).

Keywords: Analytical laboratory error, carryover sample, HbA1c.

***Correspondence to:** Tatchapol Nanthakhan, Department of Laboratory Medicine, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok 10330, Thailand.
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Diabetes mellitus (DM) is a significant global health issue with a growing prevalence in Thailand, where it poses a substantial public health risk. Individuals with DM face a 2-4 times higher risk of vascular diseases and associated mortality. [1] Type 2 diabetes (T2DM) with insulin resistance is the most commonly found among DM patients. The American Diabetes Association (ADA) and the World Health Organization (WHO) have officially endorsed HbA1c as a biomarker for diagnosing and monitoring diabetes. [2] Recently, Thailand has established guidelines for diabetes management to assist with diagnosis and treatment. [3]

Blood glucose levels are essential for monitoring diabetes. A high blood glucose level, known as hyperglycemia, indicates the presence of diabetes; however, food consumption throughout the day can also raise blood glucose levels. To minimize the impact of recent food intake on glucose measurements, the HbA1c test, which measures glycated hemoglobin, can be used for diagnosing and monitoring diabetes. The HbA1c level reflects average glucose levels over the past two to three months, and an HbA1c level greater than 6.5% indicates diabetes.

In principle, the HbA1c assay determines the enzymatic level, which involves utilizing an automated clinical chemistry analyzer to assess the amount of glycated hemoglobin. Other assays include immunoassay, high-performance liquid chromatography, and affinity chromatography, which can also be used to measure HbA1c levels. But they all require whole blood with ethylene diamine tetraacetic acid (EDTA). Despite this, an issue frequently encountered in high-throughput laboratories when analyzing HbA1c is the sedimentation of red blood cells. Hematological conditions of T2DM patients can affect both age and aggregation of red blood cells, increasing the impact on the results of HbA1c and other blood chemistry levels. [4, 5] Interlaboratory variation in processing protocol is a crucial factor, highlighting the need for standardized benchmarks for HbA1c analysis. While overall turnaround time is often determined by the instrument's throughput, carefully evaluate the analyzer's design and functionality and ensure that it functions accurately and efficiently. [6] Automated clinical chemistry analyzers often utilize robotic pipetting and cuvette washing systems that are specifically engineered to consistently collect patient specimens and sanitize sample probes and reaction cuvettes or vessels. However, carryover contamination may be possible, a transfer of the substance being analyzed from one patient sample to one or more subsequent patient samples. Addressing this issue may require corrective measures, such as internal programming to mitigate carryover between immunoassay and chemistry analyzers, which can substantially impact the turnaround time in certain systems. There are reported evaluation research studies on analytical performance carryover between immunoassay and chemistry analyzers in a clinical laboratory setting. A study evaluating the analytical performance of transfer between an immunoassay and a chemistry analyzer in a clinical laboratory has been reported. The analyzer was found to be highly efficient and free of contamination. of the sample and demonstrates that this instrument is suitable for routine clinical use. [7] This study aimed to investigate the impact of the carried-over residues from sample probes in the whole blood high-throughput HbA1c assays on HbA1c and other biochemical measurements. Additionally, the study evaluated the potential influence of red blood cell sedimentation, which can occur during sample wait times in a high-throughput laboratory setting, on HbA1c levels. Although the manufacturer rigorously tests analyzers to satisfy local and global regulatory requirements, laboratories must still verify the performance of instruments before reporting patient results.

Materials and methods

Sample study population

A sample study with type 2 diabetes (n = 100, 50 males and 50 females) was recruited at King Chulalongkorn Memorial Hospital (KCMH), Thai Red Cross Society, Thailand, from 1st May 2023 to 30th June 2023. Patient data was collected following the Declaration of Helsinki, and the Institutional Review Board Committee approved the study at the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB no.477/2023). After routine analysis, the leftover specimens from whole blood samples were used for tests in this study. The diagram displaying a study protocol is shown in **Figure 1.**

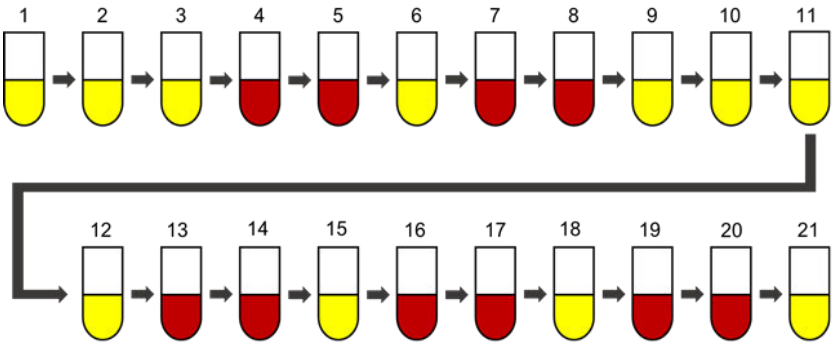

Figure 1. Specimen-to-specimen carryover study design per pSMILE carryover guideline. [8] A sample probe is used consecutively from the first sample to the last sample. Red: high-concentration specimen. Yellow: low-concentration specimen.

HbA1c level and clinical chemistry test (carryover study)

Carryover evaluation was conducted according to the pSMILE carryover guidelines. [8] According to the manufacturer's instructions, patient blood samples were analyzed for Hb1Ac and other clinical chemistry parameters using an Alinity C analyzer (Abbott Diagnostics, USA). Briefly, whole blood samples were aliquoted into 10 vials, designated as "High" specimens, for HbA1c analysis using an enzymatic assay. This 2-step assay involves protease cleavage of HbA1c at the beta hemoglobin chain, followed by oxidation of the total hemoglobin (THb) to methemoglobin using sodium nitrite and sodium azide. The HbA1c concentration is directly proportional to hemoglobin levels, and the ratio of HbA1c to THb is calculated as a percentage of HbA1c. Then, the amount of Glycated Hemoglobin (HbA1c) and Total Hemoglobin (THb) will be measured. Calculate the HbA1c to THb ratio as % HbA1c (National Glycohaemoglobin Standardization Program, NSGP) or in mmol/mol (International Federation of Clinical Chemistry, IFCC). Additionally, plasma samples were aliquoted into 10 vials, designated as "Low" specimens, for analysis of potassium (K), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and creatine kinase (CPK). The clinical chemistry analyzer utilizes photoelectric colorimetry to quantify specific chemical constituents within the samples.

HbA1c level test study

Whole blood samples were left undisturbed on the bench for three designated times: 7 min (T1), 15 min (T2), and 30 min (T3). T0 represented the samples processed immediately upon arrival at the facility. HbA1c analysis was performed using the Alinity C automated clinical chemistry analyzer (Abbott Diagnostics, USA). Results were compared across all time points, with data presented as correlation coefficients, slopes, and intercepts. Statistical acceptance criteria from the NGSP were applied with a maximum allowable bias of 5.0%.

Statistical analysis

Demographic data, including gender, age, and comorbidity, were presented as numbers and percentages. The Chi-square test was used to analyze the correlation between HbA1c levels at 0, 7, 15, and 30 minutes (red blood cell sedimentation time). Continuous data, including HbA1c levels in diabetic patients, were presented as mean, standard deviation (SD), and median (interquartile range). Statistically significant differences between HbA1c levels between each comparison group were determined using the Kruskal-Wallis test, $P < 0.05$ was considered statistically significant. Correlation between HbA1c levels at different time points was assessed using Pearson's correlation coefficient (r), slope, and intercept values. T-statistic was used to calculate the statistical significance of the correlation. Carryover effects of continuous sample probe use in HbA1c and clinical chemistry tests were evaluated, with a carryover value not exceeding the error limit (± 3SD of the Low-Low result) deemed statistically significant. Bland-Altman plot was used to assess the agreement between the HbA1c measurements obtained at time point 0 and those obtained at subsequent time points (7, 15, or 30 min). Each dot represents the difference between the patient sample at time point 0 and subsequent time points. The mean of the differences between measurements is

represented by a black line, with ±2SD from the mean difference indicated by parallel lines. Statistical analyses were performed using SPSS for Windows version 18.0 (SPSS Inc., Illinois, USA) and EP evaluator version 11.3.0.23 (Rhoads, Data Innovations)

Results

A total of 100 samples with T2DM patients were recruited from KCMH between 1 May and 30 June 2023. The study cohort comprised 50 males and 50 females, with a mean age of 64.0 ± 12.0 years (males: 61.9 ± 10.9 years; females: 66.1 ± 12.6 years). The age distribution was as follows: 30 - 39 years (3.0%), 40 - 49 years (7.0%), 50 - 59 years (23.0%), 60 - 69 years (29.0%), and over 70 years (38.0%). Comorbidities were prevalent among the study participants, with 54.0% presenting with diabetes, hypertension, and dyslipidemia. Diabetes combined with hypertension was observed in 30.0% of the patients, while diabetes combined with dyslipidemia accounted for 16.0% **(Table 1).**

The specimen carryover experiment assessed the impact of sample probe contamination in the HbA1c assay on the K, AST, ALT, ALP, and CPK test results. The observed carryover values were 0.002 mmol/L for K, - 0.040 U/L for AST, -0.100 U/L for ALT, 0.080 U/L for ALP, and 0.060 U/L for CPK. The corresponding error limits were ± 0.068 mmol/L, ± 1.008 U/L, ±1.454 U/L, ±1.550 U/L, and ±1.308 U/L, respectively **(Table 2).**

Analysis of HbA1c levels in 100 samples, with a range of 5.4% to 16.6%, revealed mean values of 7.0% (SD = 1.25, range 5.4% - 11.6%), 7.0% (SD = 1.3, range 5.5% - 11.6%), and 7.05% (SD = 1.26, range 5.5-11.6%) at 0, 7, and 15 minutes, respectively **(Figure 2A).** Bland-Altman analysis comparing HbA1c levels at 0 minutes with 7, 15, and 30 minutes showed mean differences of - 0.034, -0.066, and - 0.092, with limits of agreement (LOA) of -0.146 to 0.078, -0.197 to 0.065, and -0.228 to 0.044, respectively **(Figure 2B).**

Our analysis of the relationship between HbA1c levels and sedimentation time was correlated between baseline (0 min) and other sediment times (7,15,30 min) **(Figure 2C).**

Table 1. Demographic and characteristics data of the study group.

Variables	Diabetes type 2		
	Total	Male	Female
Sex (n)	100	50	50
Mean age (SD)	64.0 (11.9)	61.9 (10.9)	66.1 (12.6)
Age range, n (%)			
30 - 39	3 (3.0%)	1 (0.5 %)	2 (1.0 %)
40 - 49	7 (7.0%)	3 (1.5%)	4 (2.0 %)
50 - 59	23 (23.0%)	16 (8.0%)	7 (3.5 %)
60 - 69	29 (29.0%)	15 (7.5%)	14 (7.0%)
> 70	38 (38.0%)	15 (7.5%)	23 (11.5%)
Comorbidity, n (%)			
DM + HT	30 (30.0%)	8 (4.0%)	8 (4.0%)
DM + HLP	54 (54.0%)	8 (4.0%)	8 (4.0%)
DM + HT + HLP	54 (54.0%)	26 (13.0%)	28 (14.0%)

HT, hyperlipidemia; DM, diabetes; HLP, hyperlipidemia.

Table 2. Results of chemistry analytes of the study group.

Tests	Carryover	Error limit	Status
Potassium	0.002 mmol/L	± 0.068 mmol/L	pass
AST	- 0.040 U/L	± 1.008 U/L	pass
ALT	- 0.100 U/L	± 1.454 U/L	pass
ALP	- 0.080 U/L	± 1.550 U/L	pass
CPK	0.060 U/L	± 1.308 U/L	pass

Error limit (± 3SD of Low-Low results): The test passes if carryover does not exceed the error limit.

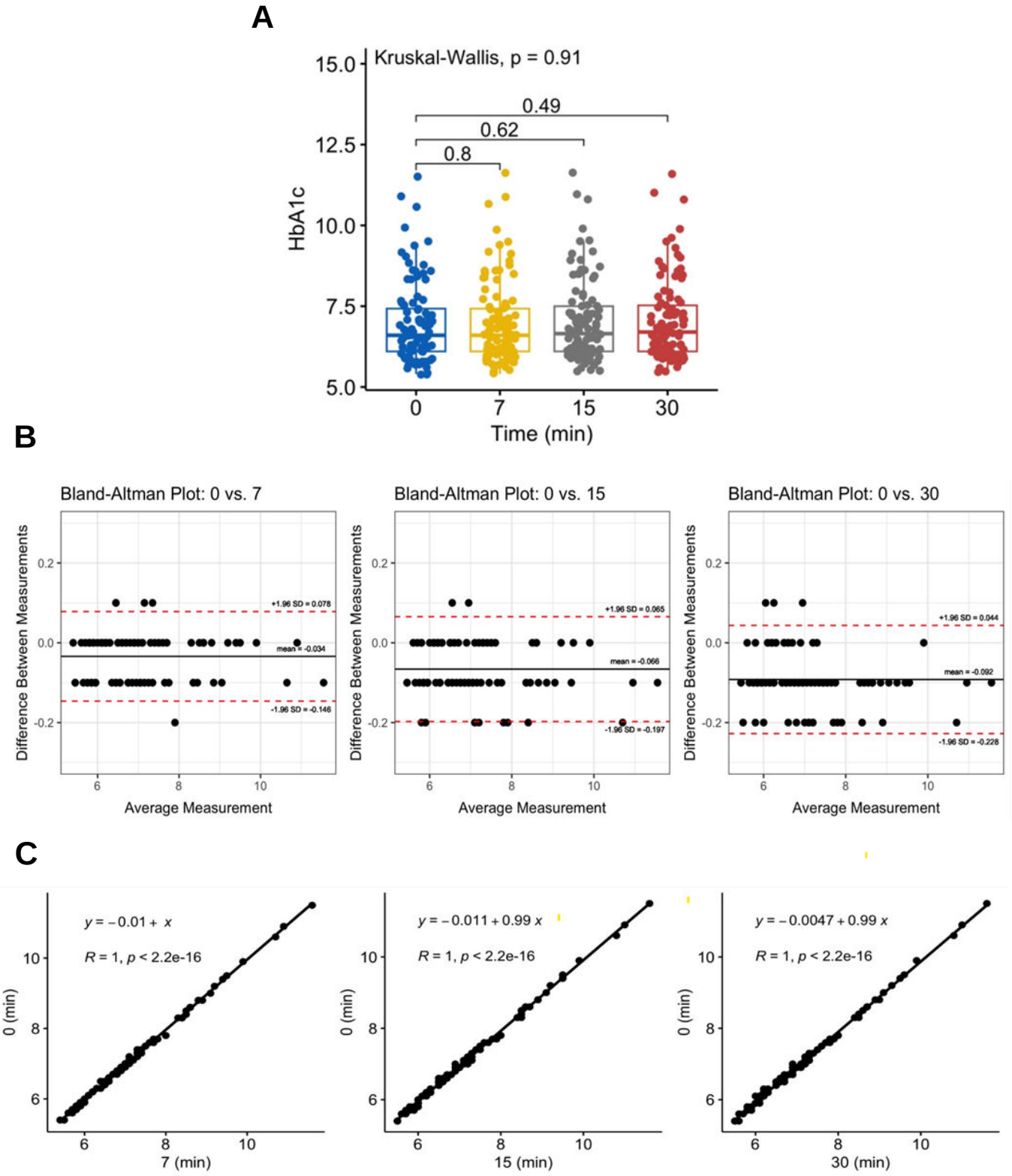

Figure 2. A Box-and-whisker plot displays the HbA1c levels of blood samples subjected to varying sedimentation times. A Kruskal-Wallis test was performed to assess statistical significance across all time points, followed by pairwise comparisons between each time point and time point 0 **(A).** Data are presented as the difference between measurement and average measurement of HbA1c levels **(B).** Bland-Altman regression analysis of HbA1c values between baseline (0 min) and other sediment times (7,15,30 min). Red dashed lines represent regression lines, and solid black lines represent identity lines. HbA1c values were correlated between baseline (0 min) and other sediment times (7,15,30 min) **(C)**.

Discussion

This study investigated the potential impact of sample-to-sample carryover and red blood cell sedimentation time on HbA1c measurement in high-throughput laboratory settings. The results indicated that HbA1c levels and other clinical chemistry measurements (e.g., potassium, AST, ALP, and CPK) were within acceptable ranges and showed no statistically significant difference. Furthermore, comparisons of HbA1c levels between samples processed immediately (time point 0) and those subjected to various sedimentation time points revealed no statistically significant differences among the groups. Therefore, neither carryover contamination from the sample probe nor erythrocyte sedimentation time significantly affected HbA1c or other clinical chemistry measurements within a 30-minute timeframe. HbA1c levels in T2DM patients can be reliably measured within this timeframe using the evaluated high-throughput assay protocol with an Alinity C analyzer. Furthermore, interlaboratory variations, such as sample waiting times due to fluctuating workloads, can influence red blood cell sedimentation, which is an important factor to consider for accurate HbA1c measurements. This study provides a benchmark for evaluating the impact of sedimentation and optimizing protocols for HbA1c analysis in various high-throughput settings, even those with analyzers different from the Alinity C analyzer.

The findings in this study align with those of Blijenberg BG*, et al*. [9], who also reported minimal specimen-related carryover on the ABBOTT Spectrum analyzer. As for RBC sedimentation, high serum cholesterol or hypercholesterolemia in T2DM was reported to affect RBC aggregation, but an elevation of fibrinogen often accompanies it. [5] The life span of RBCs in blood circulation was also reported to cause heterogeneity in the level of HbA1c in both healthy and DM individuals. [4] As a result, while this study and others demonstrated the robust performance of these HbA1c analyzers, it is important to acknowledge the potential for carryover and implement precautions in laboratory protocols, regardless of the analyzer or configuration.

The limitations of this study include the exclusive use of T2DM patient samples, a limited range of sedimentation times of red blood cells, and a limited selection of chemistry analytes. The number of samples used in the carryover assay, while consistent with the pSMILE validation protocol, could be increased to evaluate carryover effects further. Therefore, further research could expand upon these findings by examining the carryover effects in various types of DM patients, investigating a wider range of sedimentation times, and evaluating carryover across different clinical chemistry analytes. Moreover, it is important to note that this study cannot definitively rule out the possibility of carryover or red blood cell sedimentation issues in all high-throughput laboratory settings. Further investigations are needed to comprehensively assess carryover and sedimentation time in automated systems and establish best practices for mitigating their potential effects.

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Conflict of interest statement

The authors has completed and submitted the International Committee of Medical Journal Editors Uniform Disclosure Form for Potential Conflicts of Interest. None of the authors disclose any conflict of interest.

Data sharing statement

Data sharing statement. All data generated or analyzed during the present study are included in this published article. Further details are available for noncommercial purposes from the corresponding author on reasonable request.

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