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A high-throughput test for diabetes care: An evaluation of the next generation Roche Cobas c 513 hemoglobin A_{1C} assay

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ABSTRACT

Objectives: The level of glycated hemoglobin A (HbA_{1C}) in blood is the preferred marker for diabetes monitoring and treatment. Here, we evaluate the analytical performance of the Roche Diagnostics Cobas c 513, a stand-alone HbA_{1C} immunoassay analyzer.
Design and Methods: Performance was assessed with regards to imprecision, accuracy, linearity, method comparison against the Roche Cobas Integra 800 CTS, specimen stability, interference from common hemoglobin variants and hemoglobin F, and throughput.
Results: Within-run and between-run precisions were 0.5–0.7 and 0.8–1.3%CV, respectively. An average bias of −1.6% to proficiency survey samples was observed. The c 513 correlated well with the Integra (slope = 0.94, *y*-intercept = 0.50, and correlation coefficient = 0.998). The effect of hemoglobin variants on this assay was negligible while specimens containing ≥10% HbF demonstrated a negative bias. The c 513 instrument can process up to 340 samples per hour.
Conclusions: The c 513 is a precise, accurate, automated high throughput analyzer for measuring HbA_{1C} in large laboratories.

1. Introduction

Diabetes mellitus is a pathological condition that affects all age groups worldwide [1]. The percentage of glycated hemoglobin A_{1C} (HbA_{1C}) reflects the mean plasma glucose level over the previous 3–4 months for most individuals and can be used to diagnose type 2 diabetes [2] as well as monitor and treat type 1 and type 2 diabetes [3,4]. Immunoassay has become an accepted approach for the measurement of HbA_{1C}. Following the on-board detergent-induced lysis of whole blood samples, the amount of HbA_{1C} is quantified by the binding of an antibody to HbA molecules that are glycated at the N-terminus of their β-chain [5]. This amount is compared to the total concentration of Hb in the sample determined by absorbance values and a percent HbA_{1C} is calculated. In North America, HbA_{1C} is

Abbreviations: CAP, College of American Pathologists; CI, confidence interval; CLSI, Clinical Laboratory Standards Institute; CV, coefficient of variation; Hb, hemoglobin; HbA, adult hemoglobin; HbA_{1C}, glycated hemoglobin A_{1C}; HPLC, high-performance liquid chromatography; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; LoB, Limit of Blank; LoD, Limit of Detection; NGSP, National Glycohemoglobin Standardization Program; TE, total error.

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reported using the National Glycohemoglobin Standardization Program (NGSP) units (%) which can be converted to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) SI units (mmol/mol) using the following equation: IFCC = 10.93(NGSP)-23.50.

In this study, we evaluated the performance characteristics of the Roche Cobas c 513 (Roche Diagnostics, Basel, Switzerland), a stand-alone next generation immunoassay analyzer for HbA_{1C}. We assessed the imprecision, accuracy, analytical measuring range, and throughput of this instrument. Method comparisons studies were performed against a previous generation immunoassay analyzer (Roche Integra 800 CTS). Finally, we studied the effects of erythrocyte sedimentation and potential interference from hemoglobin variants.

2. Materials and methods

In addition to the c 513, this study employed the routine HbA_{1C} analyzers at Calgary Laboratory Services (Calgary, Canada; Roche Cobas Integra 800 CTS) and DynaLIFE$_{Dx}$ [Edmonton, Canada; Variant II Turbo 2.0 (BioRad Laboratories, Hercules, California)]. Calibrators and the Roche Tina-Quant® HbA_{1C} Gen.3 reagent were supplied by Roche. This test is based on the turbidimetric inhibition immunoassay for hemolyzed whole blood prepared either on-board (whole blood application) or manually by the operator (hemolysate application). The c 513 immunoassay is standardized according to the IFCC reference method for HbA_{1C} and is certified by the NGSP program to ensure alignment with the Diabetes Control and Complications Trial.

Assay imprecision for both the automated whole blood and manual hemolysate applications on the c 513 was determined following the Clinical Laboratory Standards Institute (CLSI) EP05-A3 [6] using Roche PreciControl HbA_{1C} (within run) or Bio-Rad Liquicheck Diabetes Control (between run) materials. Accuracy was assessed using stored proficiency testing samples from the College of American Pathologists (CAP; GH-1-15 2015 and GH-1-10 2016). The Limit of Detection (LoD) for both Hb and HbA_{1C} was determined based on the Limit of Blank and the standard deviation of a low concentration patient sample. The upper limit of linearity for both the Hb and HbA_{1C} procedures was verified by assaying a series of dilutions of a high Hb patient sample and the Roche HbA_{1C} calibrator [7]. A method comparison of the c 513 whole blood or hemolysate application to the Integra 800 CTS assay was performed using 40 fresh EDTA whole blood samples distributed across the reportable range of the HbA_{1c} assay retrieved after routine measurement. The optimal throughput of the c 513 was determined by running 250 patient samples retrieved after routine measurement in one run. The following times were recorded: first sample seen, last sample seen, first result out, and last result out. The time from first sample seen to first sample result and the time to complete all samples were calculated. These same patient samples were then run on the Integra 800 CTS and the same throughput metrics recorded and calculated. In addition, once placed into operation practical throughput was assessed by pulling patient test result volumes verified during the morning shift post running of quality control materials over 4 days. The effect of sedimentation of whole blood samples on the measurement of $\%HbA_{1C}$ was investigated by re-assaying 30 unmixed samples 24 h after the initial mixing. These samples ranged from 4.5 to 15.5 $\%HbA_{1C}$ (median value, 11.9 $\%HbA_{1C}$). The effect of potentially interfering hemoglobin variants on the HbA_{1C} measurement was investigated by assaying patient samples ($N = 6$–36) containing heterozygous HbS, HbC, HbE, HbD, or HbJ or elevated levels of HbF (6.8–29.9%) on the c 513, Integra 800 CTS, and Variant II Turbo 2.0. All calculations were performed using Microsoft Excel 2010 (Microsoft Corporation, Washington, USA).

3. Results

The imprecision of both the whole blood and hemolysate HbA_{1C} applications on the c 513 is shown in Table 1. Within run precision was comparable at both normal and pathological levels of HbA_{1C} for both the whole blood and hemolysate applications (0.5–0.7 %CV). Between run imprecision was greater for the hemolysate application (2.7–3.1 %CV) than for the whole blood application on the c 513 (0.8–1.3 %CV) at all levels of $\%HbA_{1C}$ tested. Accuracy assessed by running 25 stored CAP samples (5.0–12.1 $\%HbA_{1c}$) demonstrated an average absolute bias of −0.11 $\%HbA_{1C}$ and a percentage bias of −1.6% across the analytical measuring range [Deming linear regression: slope (95%CI) = 1.01 (0.98–1.04), y-intercept (95%CI) = -0.20 (−0.43-0.02), correlation coefficient = 0.998]. The LoDs were determined to be 0.42 and 0.031 mmol/L, for the Hb and HbA_{1C} assays, respectively. The upper limit of the Hb and HbA_{1C} assays were verified to be 17.0 and 1.72 mmol/L, respectively. Both assays were linear up until this point. Method comparisons between HbA_{1C} measured on the c 513 and Integra 800 CTS are shown in Fig. 1. Overall, the applications compared well (slopes = 0.93–0.94, and *y*-intercepts = 0.49–50 $\%HbA_{1C}$, correlation coefficients >0.99), however at $\%HbA_{1C}$ >10%, an increasingly negative bias was observed. To process 250 samples the c 513 requires 44 min, with the time from first sample read to first sample result being 14 min. In contrast, the time for the Integra 800 CTS to complete all the samples was 2.5 h. From this data, in 1 h, the c 513 can process approximately 340 samples, 3.4 times as many as the Integra 800 CTS. When practical throughput was assessed once the instrument was placed in operation, a median throughput volume of 309 samples/hour was observed.

To test the effect of erythrocyte sedimentation prior to aspiration, left over patient specimens were measured 24 h after their initial run time without prior mixing. Of the samples tested, 50% ($N = 15$; median 10.5 $\%HbA_{1C}$, range 4.5–12.4 $\%HbA_{1C}$) produced results with a mean absolute bias of −0.36 $\%HbA_{1C}$ (range −0.68 to 0.36 $\%HbA_{1C}$) and a mean percent bias of −3.4% (range −6.7 to 4.8%). For the remaining 50% ($N = 15$; median 12.9 $\%HbA_{1C}$, range 11.3–15.5 $\%HbA_{1C}$), a "*>Test*" flag was issued by the instrument for the HbA_{1C} measurement as the test results obtained were greater than the linearity of the application (1.61 mmol/L) [5]. The effect of interference from hemoglobin variants on the measurement of $\%HbA_{1C}$ by the c 513 is shown in Table 1. Overall, the c 513 and Integra 800 CTS produced very similar results (mean percent biases of −0.5 to 1.2%). Comparable results between the c 513 and the Variant II Turbo 2.0 were obtained for samples heterozygous for HbS, HbD, HbE, HbC, and with HbF ≤10% (mean percent biases: −6.4-(-2.9) %). Significant biases were observed between the c 513 and the Variant II Turbo 2.0 for samples heterozygous for HbJ or with HbF >10% (mean percent

Table 1
Analytical performance of the Roche Cobas c 513 HbA_{1C} assay.

Assay Precision				
Whole Blood				
Within Run Precision				
Control Material		Normal	Pathological	
Mean $\%HbA_{1C}$		5.6	10.6–10.8	
%CV		0.5	0.7	
N		20	20	
Between Run Precision				
Control Material		Level 1	Level 2	Level 3
Mean $\%HbA_{1C}$		5.4	9.1–9.3	13.8–14.4
%CV		1.3	0.8	1.0
N		20	20	20
Hemolysate				
Within Run Precision				
Control Material		Normal	Pathological	
Mean $\%HbA_{1C}$		5.6	10.6–10.8	
%CV		0.6	0.5	
N		20	20	
Between Run Precision				
Control Material		Level 1	Level 2	Level 3
Mean $\%HbA_{1C}$		5.4	9.1–9.3	13.8–14.4
%CV		2.8	2.7	3.1
N		20	20	20

Interference of hemoglobin variants				
Hb Variant	HbA_{1C} range (%)	N	Mean % Bias	
			Roche Cobas Integra 800 CTS	Bio-Rad Variant II Turbo 2.0
HbS trait	4.7–10.0	36	0.1	−5.6
HbD trait	4.9–9.6	17	−0.5	−2.9
HbE trait	5.2–12.6	15	−0.4	−6.4
HbC trait	5.0–7.7	7	0.0	−4.1
HbJ trait	5.1–6.8	7	1.2	52.3
HbF ≤ 10%	4.7–11.3	15	0.1	−4.7
>10%	4.2–7.2	11	−0.5	−13.7

biases: 52.3 and −13.7%, respectively).

4. Discussion

The quality goals of HbA_{1C} are driven by clinical requirements: a difference of 0.5 $\%HbA_{1C}$ between successive patient samples indicates a clinically significant change in glycemic control [8]. To meet this, total error (TE) targets are 3 or 6% based on biological variation [9] or analytical goals [10,11], respectively. As a component of TE, HbA_{1C} assays have a target between run precision of <2 $\%HbA_{1C}$ [10,11]. Reflecting the manufacturer's claims [5], the whole blood application on the c 513 met this precision goal (Table 1). In contrast, the hemolysate application did not meet this target nor the manufacture's claim (0.6–1.0% CVs), likely reflecting the manual steps required in the procedure. When compared to similar commercially available HbA_{1C} immunoassays [Beckman Coulter Inc. AU systems (Brea, California), Siemens Healthcare Dimension Vista (Erlanger, Germany], Ortho Clinical Diagnostics Vitros 4600, 5600 (Raritan, New Jersey)], the %CVs observed with the c 513 are superior and more in line with the top performing assays on the market [eg. Abbott Diagnostics Architect *c* System (Santa Clara, California) and Tosoh G8 Automated HPLC (Tokyo, Japan)] [12]. The systematic bias observed in our accuracy experiment (−1.6%) is outside of the target bias set by biological variation (1.1 and 1.5%)[9,11] but within that allowed by total allowable analytical error (2.0%). Combined together, the TE for the measurement of HbA_{1C} on the c 513 is 3.5–4.5%, values higher than the desirable TE determined by biological variation [9], but well within the analytical performance required by CAP [11,12].

Method comparisons between HbA_{1C} measured on the c 513 or the Integra 800 CTS demonstrate close agreement between the platforms, particularly for values < 10% (Fig. 1). However for values ≥ 10 $\%HbA_{1C}$, an increasingly negative bias was observed that can exceed 0.5 $\%HbA_{1C}$. To a physician interpreting a HbA_{1C} test result following a change from the Integra 800 CTS to the c 513, this negative bias could be erroneously interpreted as an improvement in glycemic control.

Roche Diagnostics has indicated that whole blood samples do not need to be mixed prior to loading onto the c 513 (*personal communication*). Supporting this, for 50% of the samples tested the percent bias introduced by erythrocytes sedimentation (up to 24 h) is well within the total error of this assay. However, 50% ($N = 15$) of samples tested could not produce a result after sitting for 24 h. Those samples unable produce results typically had an initial $\%HbA_{1C}$ measurement higher than those that did produce results (median 12.9, range 11.3–15.5 $\%HbA_{1C}$ vs. median 10.5, range 4.5–12.4 $\%HbA_{1C}$). In order to improve workflow and prevent instrument error flags, samples are gently inverted 10 times prior to loading on the instrument. This protocol accommodates samples that may have sat for

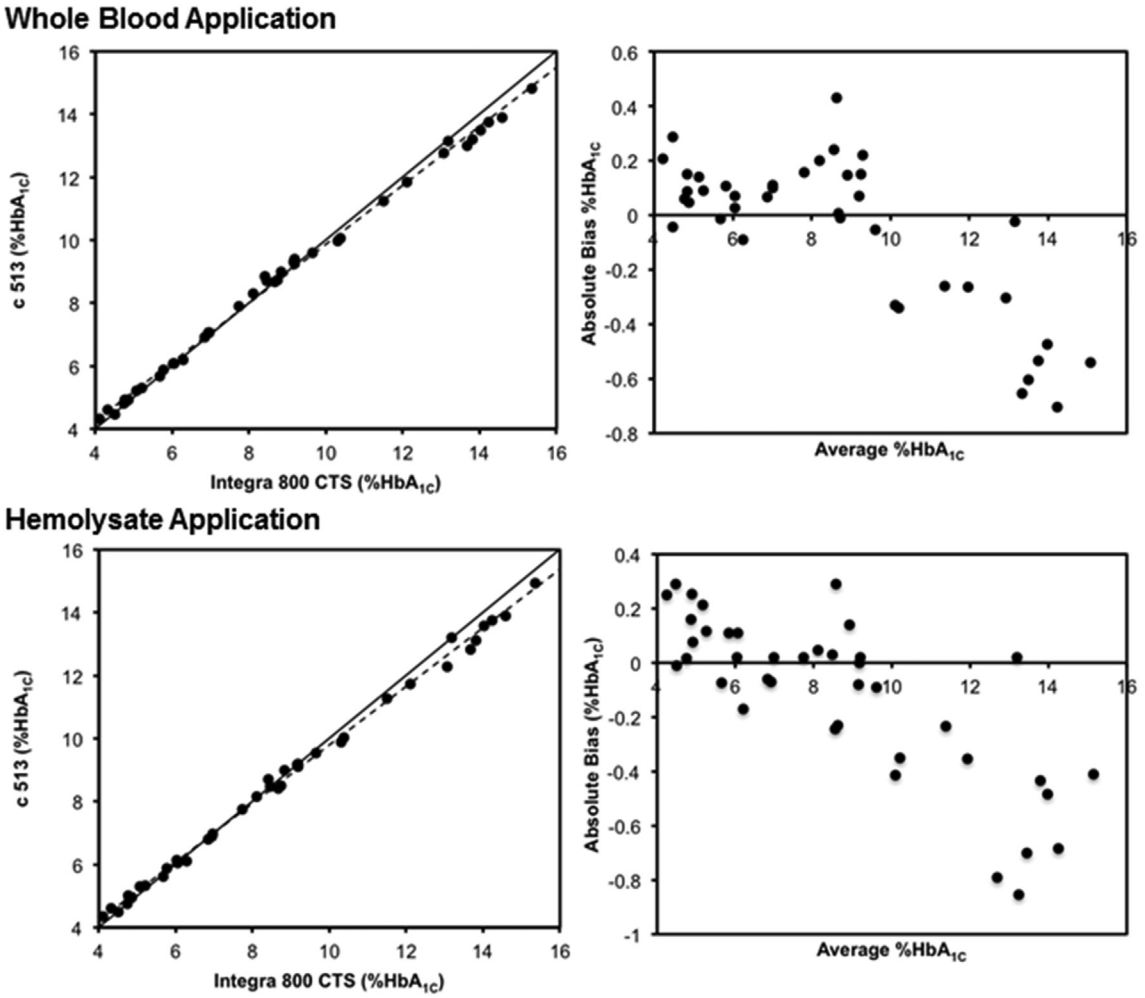

Fig. 1. Method comparisons of HbA_{1C} quantification by the c 513 and the Integra 800 CTS. Whole blood application: (*left*) Deming regression [$N = 40$, range 4.4–14.8 $\%HbA_{1C}$, slope (95% CI) = 0.94 (0.92–0.95), *y*-intercept (95%CI) = 0.50 (0.36–0.63), correlation coefficient = 0.998], (*right*) Bland-Altman plot. Hemolysate application: (*left*) Deming regression [$N = 40$, range 4.4–15.0 $\%HbA_{1C}$, slope (95%CI) = 0.93 (0.91–0.95), y-intercept (95%CI) = 0.49 (0.35–0.64), correlation coefficient 0.998], (*right*) Bland-Altman plot. In both Deming regression plots, the line of unity is indicated as a solid line.

longer than 24 h and/or have high hematocrits. In addition, samples must not be centrifuged prior to loading on the instrument as this will create an error in sample aspiration and produce incorrect results.

Due to differences in amino acid sequence, Hb variants have the potential to introduce significant bias into HbA_{1C} results depending on the methodologies employed. This can lead to misdiagnosis of diabetes mellitus in patients. Clinically insignificant differences in $\%HbA_{1C}$ values were seen between the c 513 and Integra 800 CTS for all hemoglobin variants tested, and for heterozygous HbS, C, D, and E samples run on the Variant II Turbo 2.0 (Table 1) [13]. In contrast, in samples heterozygous for HbJ a large bias of 52.3% was observed between the c 513 and the Variant II Turbo 2.0. The inability of ion-exchange HPLC methods to accurately quantitate HbA_{1C} in heterozygous HbJ samples has been observed previously and is the result of a negative interference in the HbA_{1C} peak on the chromatogram [14]. Immunoassays can reliably quantitate HbA_{1C} in samples from patients heterozygous for HbJ [15].

Unlike the other variants tested, HbF is a unique as it does not reflect a point mutation but rather a different Hb subfraction comprised of 2 α and 2 γ chains. In our study, when HbF comprised ≤10% of the Hb in a patient sample, limited bias was observed between $\%HbA_{1C}$ results obtained on the c 513, Integra 800 CTS, and the Variant II Turbo 2.0 (Table 1). In samples with HbF >10%, similar results were obtained on the c 513 and the Integra 800 CTS, but these results demonstrated a mean percent bias of −13.7% (absolute bias of −0.67 $\%HbA_{1C}$) when compared with those from the Variant II Turbo 2.0. This negative interference was proportional to the amount of HbF in the patient sample as the measured $\%HbA_{1C}$ decreased by −0.06%HbA_{1C}/%HbF (percent bias of −1.0%/%HbF) in samples with HbF >10%.

In summary, our data supports the routine use of the c 513 for $\%HbA_{1C}$ quantification by the clinical laboratory. The assay is precise and accurate and the instrument is capable of testing >7500 specimens in 24 h. While care must be taken to ensure correct sample

processing is followed, this instrument is a good solution for large references labs with HbA_{1C} volumes of 1500 per day or higher.

Declaration of competing interest

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