

Original Research

Performance Evaluation of a New Fluorescent-Based Lateral Flow Immunoassay for Quantification of Hemoglobin A1c (HbA1c) in Diabetic Patients

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Abstract

Background: Rapid hemoglobin A1C (HbA1c) level monitoring is essential in slowing the progression of diabetes. This need becomes challenging in low resources countries where the social burden of the disease is overwhelming. Recently, fluorescent-based lateral flow immunoassays (LFIA) gained wide attention for small laboratories and population surveillance. **Aim:** We aim to evaluate the performance of Finecare™ HbA1c Rapid Test, certified by CE, NGSP, and IFCC, for the quantitative measurement of hemoglobin A1C (HbA1c) along with its reader. **Methods:** A total of 100 (fingerstick and venepuncture whole blood) samples were analyzed by Wondfo Finecare™ HbA1c Rapid Quantitative Test and the results were compared with the reference assay Cobas Pro c503. **Results:** A strong correlation was observed between Finecare™/Cobas Pro c503 with fingerstick ($r > 0.93$, $p < 0.0001$) and venous ($r > 0.97$, $p < 0.0001$) blood samples. Finecare™ measurements showed excellent agreement and compliance with Roche Cobas Pro c503 as the mean bias was negligible; 0.05 (Limits-of-agreement: $-0.58-0.68$) with fingerstick and 0.003 (Limits-of-agreement: $-0.49-0.50$) with venous blood. Interestingly, a very small mean bias (0.047) was also shown between the fingerstick and the venepuncture data, indicating that the type of sample used does not affect the results and the high reproducibility of the assay. Finecare™ showed 92.0% (95% CI: 74.0–99.0) sensitivity and 94.7% (95% CI: 86.9–98.5) specificity compared to the Roche Cobas Pro c503 using fingerstick whole blood samples. Finecare™ showed 100% (95% CI: 86.3–100) sensitivity and 98.7% (95% CI: 92.8–100) specificity compared to the Cobas Pro c503 using venepuncture samples. Cohen's Kappa denoted excellent agreement with Cobas Pro c503; 0.84 (95% CI: 0.72–0.97) and 0.97 (95% CI: 0.92–1.00) using fingerstick and venous blood samples, respectively. Most importantly, Finecare™ showed a significant difference between normal, pre-diabetic, and diabetic samples ($p < 0.0001$). Similar results were obtained when an additional 47 samples (from different participants; mainly diabetic) were analyzed in a different lab using different Finecare™ analyzer and different kit lot number. **Conclusions:** Finecare™ is a reliable and rapid assay (5 min) which can be easily implemented for long-term monitoring of HbA1c in diabetic patients, particularly in small laboratory settings.

Keywords: serology; lateral flow immunoassay; LFIA; HbA1c; diabetes

1. Introduction

The prevalence of diabetes mellitus is significantly expanding at an alarming pace all over the globe. The worldwide burden of diabetes mellitus (DM) has increased from 30 million in 1985 to 382 million in 2014, and current trends indicate that these rates will continue to expand [1]. According to the most recent projections reported by the In-

ternational Diabetes Federation (IDF), the number of people living with diabetes mellitus will rise to 643 million by 2030 [2].

Glycated hemoglobin (HbA1c) serves as a reliable indicator of glycemic status in diabetic patients over a period of two to three months [3]. HbA1c is produced once hemoglobin is chemically linked to glucose [3]. Tradi-



tionally, high plasma glucose levels were used for DM diagnosis. Plasma glucose level is typically measured after fasting or two hours after an oral glucose (75 g) tolerance test in symptomatic patients [4]. Recently, the American Diabetes Association and the World Health Organisation (WHO) recommended the use of HbA1c ($\geq 6.5\%$) for DM diagnosis [5]. This was based on the fact that HbA1c can predict clinical outcomes of the disease. In this context, many studies showed that HbA1c strongly correlates with chronic microvascular complications of diabetes, including retinopathy, nephropathy, and neuropathy [6,7]. Most importantly, HbA1c levels have also been proven to be helpful in algorithms for calculating cardiovascular risk (CVD), along with gender, age, blood pressure, smoking status, and cholesterol [8–10], and thus may be a relevant biomarker to be considered in CVD prevention strategies [11]. HbA1c testing offers significant practical advantages while typically being more expensive than blood glucose testing, with an average net cost of 13.6 times that of a plasma glucose measurement [12]. HbA1c testing may be done at any time of day and does not need any specific pre-test preparation by the patient (such as overnight fasting) [12,13]. Therefore, monitoring HbA1c levels in diabetic patients in a timely and consistent manner helps in slowing the progression of the disease. However, this need becomes challenging in settings with low resources and an absence of laboratory infrastructure, which are also places where the societal impact of the illness is often overwhelming [14]. The current laboratory diagnostic techniques for HbA1c, such as cation-exchange HPLC, capillary electrophoresis, and affinity chromatography, involve expensive instruments, are laborious, and require a longer turnaround time [15].

Lateral flow immunoassays (LFIA) are attractive for small or point-of-care (POC) settings and population surveillance. They are rapid, inexpensive, simple to use, most importantly, rely on easily accessible samples such as whole blood from a fingerstick [16,17]. Finecare™ HbA1c Rapid Quantitative Test is a fluorescence immunoassay for the quantitative determination of HbA1c in human blood (venepuncture or fingerstick). In this study, we aimed to evaluate the performance of Finecare™ HbA1c Rapid Quantitative Test by using samples obtained by fingerstick and venepuncture. In addition, to compare the performance of Finecare™ HbA1c Rapid Quantitative Test with the reference technique, Cobas Pro c503 clinical chemistry analyzer from Roche Diagnostics.

2. Materials and Methods

2.1 Sample Collection and Ethical Approval

In collaboration with the Ministry of Health (MOH) in Jordan, Wondfo Biotech (Guangzhou, China) conducted two validation studies on Finecare™ HbA1c Rapid Quantitative Test; one was performed in a private referral laboratory (n = 100 samples) and the other was performed in a public health laboratory that belongs to the MOH (n = 47

samples), and the other was performed in a private referral laboratory (n = 100 samples). HbA1c was measured from collected fingerstick and matched venous blood samples for a total of 147 participants from both laboratories. Testing results were provided to our lab for analysis, and that data was unaccompanied by any patient identifications or private information other than the primary demographic data, including age and gender. Accordingly, an Ethical approval exemption (QU-IRB 1766-E/22) was granted by Qatar University.

2.2 Wondfo Finecare™ HbA1c Rapid Quantitative Test

Finecare™ HbA1c Rapid Quantitative Test is based on fluorescence immunoassay technology and measures the level of HbA1c in human blood using a sandwich immunodetection approach. According to the manufacturer's test leaflets and flyer, the Finecare™ HbA1c POC test, according to the manufacturer's test leaflets and flyer, is traceable to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method for measuring HbA1c and is certified by the National Glycohemoglobin Standardization Program (NGSP) as having documented traceability to the Diabetes Control and Complications Trial (DCCT) reference method [18,19]. The NGSP awards certification to manufacturers for successfully meeting specific performance criteria [20]. The test was carried out according to the manufacturer instructions. The LFA reaction time is 5 min, and the measuring range is 4.0–14.5%

2.3 Roche Cobas Pro c503 Reference Method

The Tina-quant Hemoglobin A1cDx assay is intended to diagnose diabetic patients. It is *in vitro* diagnostics assay to quantify hemoglobin A1c (mmol/mol) and % hemoglobin A1c in whole venous blood on the cobas pro c503 clinical chemistry analyzers. This approach is based on the turbidimetric inhibition immunoassay of blood samples that have been hemolyzed. The anti-HbA1c antibody forms a soluble complex with a single binding site on HbA1c. Polyhapten react with excess anti-HbA1c antibodies to generate an insoluble compound, which is evaluated by turbidimetry. The measuring range is 4.0–14.5%.

2.4 Statistical Method

Finecare™ and the reference technique, Cobas Pro c503, were compared using correlation and linear regression analysis. Because our data was not normally distributed, we estimated the spearman correlation coefficient (r), with r values of 0–0.39 indicating a weak correlation, 0.40–0.59 indicating a moderate connection, 0.6–0.77 indicating a high correlation, and 0.8–1 indicating a very strong correlation [21]. In addition, we assessed the area under the curve (AUC) of the Receiver-Operating Characteristic (ROC) curve, which measures the accuracy of a quantitative diagnostic test [22]. An AUC of 0.9–1.0 is denoted as excellent, 0.8–0.9 is denoted as very good, 0.7–0.8 is denoted

as good, 0.6–0.7 is denoted as sufficient, 0.5–0.6 is denoted as bad, and <0.5 is denoted as not useful. Moreover, we generated Bland-Altman plot, which is based on the quantification of the agreement between two quantitative measurements by studying the mean difference and constructing limits of agreement (LOA). Further, concordance analysis between Fineware™ and the reference methods Cobas Pro c503 was conducted, which includes the overall percent agreement (OPA), positive percent agreement (PPA), and negative percent agreement (NPA), accuracy, and Cohen's Kappa test. A Cohen's Kappa coefficient <0.40 suggests a poor agreement, 0.40–0.59 suggests a fair agreement, 0.60–0.74 suggests a good agreement and ≥ 0.75 suggests an excellent agreement [23]. All statistical tests were conducted using GraphPad Prism (GraphPad Software, Inc. Version 9, San Diego, CA, USA).

3. Results

3.1 Very Strong Correlation between Fineware™ and the Reference Method

We assessed the correlation between Fineware™ with the reference method, Roche Cobas Pro c503, using fingerstick and venous blood samples, as shown in Fig. 1. The correlation between Fineware™ employing fingerstick whole blood sample and Cobas Pro c503 was very high ($r = 0.93$, $p < 0.0001$). Likewise, Cobas Pro c503 and Fineware™ utilizing venous whole blood samples showed a very significant correlation ($r = 0.97$, $p < 0.0001$). The results of the linear regression study demonstrated that the built model could accurately predict the dependent variable between Fineware™ utilizing venous or fingerstick samples ($R^2 > 0.95$, $p < 0.0001$).

ROC curve analyses showed excellent performance for Fineware™ with an AUC of 0.994 and 0.998 using fingerstick and venous blood samples, respectively (Fig. 2).

3.2 A Very Strong Agreement between Fineware™ and the Reference Method by the Bland-Altman Plot

An alternative method for comparing Fineware™ and Roche Cobas Pro c503 using fingerstick and venous blood data and results agreement is by performing Bland-Altman plot analysis. Bland-Altman analysis aids in evaluating the agreement between two quantitative tests by graphically depicting the measurement variances by plotting the difference against the mean of the data [24,25]. Bland-Altman method computes the average difference between two methods of measurement and standard deviation (SD) of the difference, and calculates the '95% limit of agreement' (LOA) as the mean difference. The presentation of the '95% LOA' on the Bland-Altman plot permits a visual decision of how well two measurement methods are in concordance with each other. A smaller range between the LOA is commented as better compliance.

As shown in Fig. 3A,B, Fineware™ fingerstick and venous blood data showed excellent agreement and compli-

ance with Roche Cobas Pro c503 data as the mean bias are 0.05 (LOA: -0.58 – 0.68) and 0.003 (LOA: -0.49 – 0.5), respectively, which are very close to 0. Interestingly, a very small mean bias (0.047) was also observed between the fingerstick and the venepuncture data, indicating that the type of sample does not significantly affect the results, and that the assay has high reproducibility.

3.3 Fineware™ Results are Comparable to the Reference Method Cobas Pro c503

We evaluated the performance of Fineware™ using fingerstick and venous blood samples compared to Cobas Pro c503. As depicted in Fig. 4, there was no significant difference between the results obtained from Fineware™ and CobasPro c503 analytical analyser using the fingerstick or venous blood samples.

3.4 Fineware™ Showed a Low Level of False Positives and False Negatives Comparable to the Reference Method

According to the current recommendations, patients with an HbA1c level $\geq 6.5\%$ would get intensive treatment. To assess the clinical applicability of Fineware™, the HbA1c results were compared with those of the reference method using a cut-off point of 6.5% for HbA1c using fingerstick and venous blood samples, as shown in Table 1A. Using fingerstick whole blood, 4 samples showed false positive results (4%), and two samples (2%) showed false negative results using Fineware™, as shown in Table 1A. Whereas using venous blood samples, only one sample showed a false positive result (1%), and none showed false negative results (0%) using Fineware™, as shown in Table 1A. Similarly, as shown in Table 1B, only 3 fingerstick whole blood samples showed false positive results (3%) when compared to venous blood. In contrast, only 2 fingerstick whole blood samples showed false negative results (2%) when compared to venous blood samples.

3.5 Fineware™ Demonstrates High Sensitivity and Specificity Comparable to the Reference Method

As shown in Table 2, Fineware™ demonstrated 100% (95% CI: 86.3–100) sensitivity and 98.7% (95% CI: 92.8–100) specificity compared to the reference method using venous blood samples. In addition, Fineware™ showed lower sensitivity of 92.0% (95% CI: 74.0–99.0) and a specificity of 94.7% (95% CI: 86.9–98.5) compared to the reference method using fingerstick whole blood. Moreover, we evaluated the sensitivity and specificity of Fineware™ using fingerstick compared to venous blood samples. As expected, using fingerstick whole blood showed a lower sensitivity of 92.3% (95% CI: 74.9–99.1) and a specificity of 95.9% (95% CI: 88.6–99.2) compared to venous blood.

The concordance assessment between the reference method Cobas Pro c503 and Fineware™ using venous and fingerstick whole blood samples is reported in Table 2. The OPA, PPV, and NPV between Cobas Pro c503 and

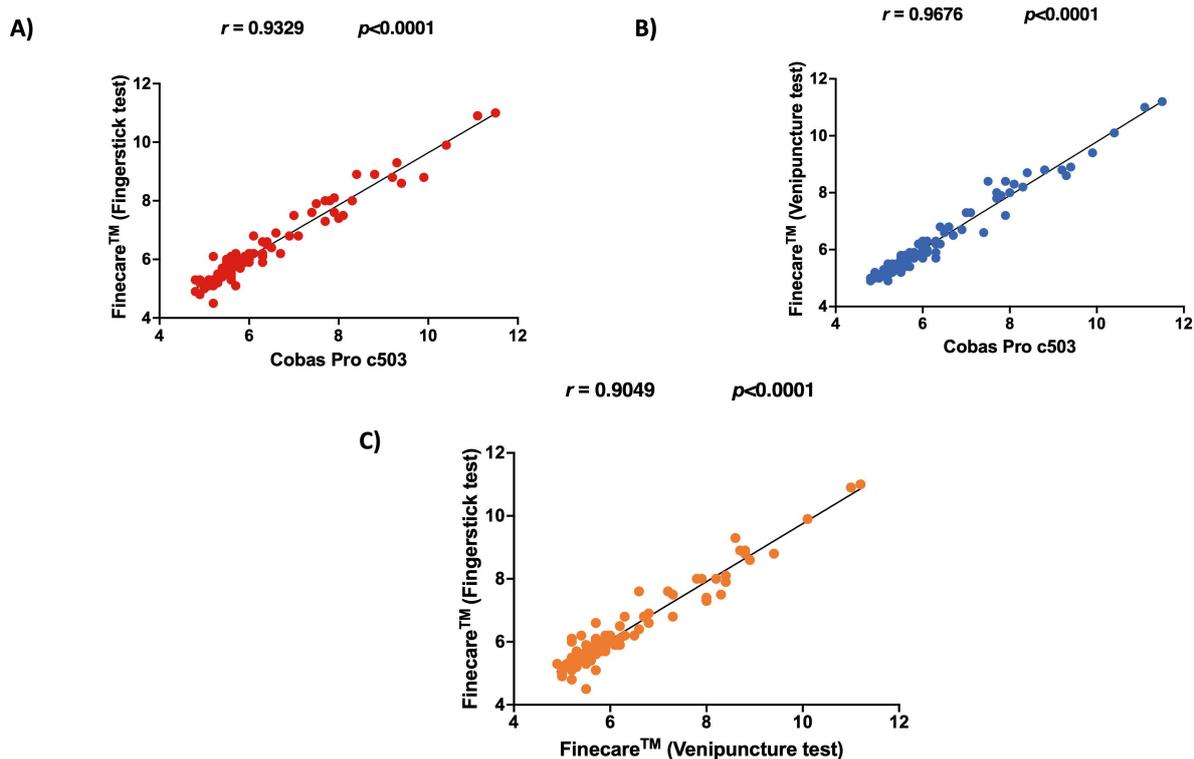


Fig. 1. Pairwise correlation and linear regression analysis for each assay. (A) Correlation plot of Finecare™ with Cobas Pro c503 using fingerstick blood sample; (B) Correlation plot of Finecare™ with Cobas Pro c503 using venous whole blood sample; and (C) Correlation plot of Fingerstick blood sample with venous whole blood sample using Finecare™ machine. Spearman correlation coefficient (r) and p-value were indicated. The coefficient of determination (R^2) was calculated to be 0.95, 0.97, 0.94 for (A–C), respectively. n = 100.

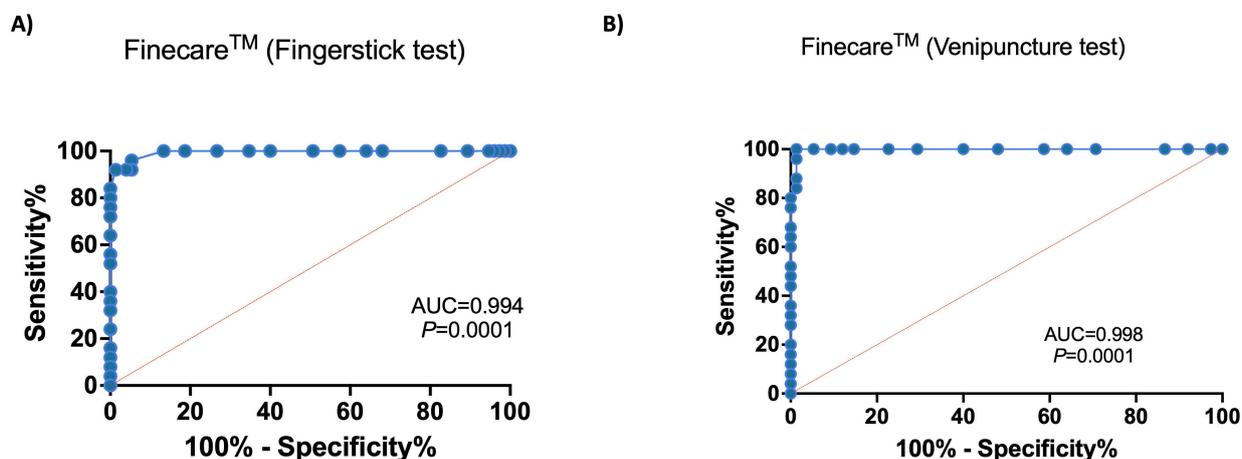


Fig. 2. ROC curve for Finecare™. Finecare™ showed excellent performance (AUC > 0.9) compared to the reference method using fingerstick (Fig. 2A) and whole blood (Fig. 2B) samples. AUC of 0.9–1.0 is considered excellent. AUC, the area under the curve; ROC, Receiver Operating Characteristic.

Finecare™ using venous blood were 99.0% (95% CI: 94.6–100), 96.2% (95% CI: 78.1–99.4), and 100% (95% CI: 95.1–100), respectively. Whereas, using fingerstick whole blood, the OPA, PPV, and NPV were 94.0% (95% CI: 87.4–97.8), 85.2% (95% CI: 68.8–93.8), and 97.3% (95%

CI: 90.4–99.3), respectively. Most importantly, Cohen’s Kappa coefficient denoted excellent agreement between Finecare™ and Cobas Pro c503 ($\kappa > 0.84$) using fingerstick and venous whole blood samples.

Table 1A. Cross tabulation between the two Finecare™ testing methods: venepuncture whole-blood and fingerstick compared to the reference method. n = 100.

(A) Cut-off: 6.5%		Reference Method Cobas Pro c503		
		Positive	Negative	Total
Finecare™ (fingerstick)	Positive	23	4	27
	Negative	2	71	73
	Total	25	75	100
Finecare™ (venepuncture)	Positive	25	1	26
	Negative	0	74	74
	Total	25	75	100

Table 1B. Cross tabulation between fingerstick and venepuncture whole-blood samples using Finecare™. n = 100.

(B) Cut-off: 6.5%		Finecare™ (venepuncture)		
		Positive	Negative	Total
Finecare™ (fingerstick)	Positive	24	3	27
	Negative	2	71	73
	Total	26	74	100

* Positive: Diabetic ($\geq 6.5\%$), Negative: Normal ($< 6.5\%$).

Table 2. Concordance assessment between Finecare™ fingerstick, venepuncture test, and Cobas Pro c503. N = 100.

Reference	Test	Overall Percent Agreement (OPA)	Sensitivity	Specificity	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)	Accuracy/Efficiency	Cohen's Kappa Coefficient
Cobas Pro c503	Finecare™ (fingerstick)	94.0% (87.4–97.8)	92.0% (74.0–99.0)	94.7% (86.9–98.5)	85.2% (68.8–93.8)	97.3% (90.4–99.3)	94.0% (87.4–97.8)	0.84 (0.72–0.97)
Cobas Pro c503	Finecare™ (venepuncture)	99.0% (94.6–100)	100% (86.3–100)	98.7% (92.8–100)	96.2% (78.1–99.4)	100% (95.1–100)	99.0% (94.6–100)	0.97 (0.92–1.00)
Finecare™ (venepuncture)	Finecare™ (fingerstick)	95.0% (88.7–98.4)	92.3% (74.9–99.1)	95.9% (88.6–99.2)	88.9% (72.4–96.1)	97.3% (90.4–99.3)	95.0% (88.7–98.4)	0.87 (0.76–0.98)

Table 3. Cross tabulation between the Finecare™ (venepuncture) and Reference Method Cobas Pro c503 (venepuncture).

Cut-off: 6.5%		Reference Method Cobas Pro c503		
		Positive	Negative	Total
Finecare™	Positive	20	0	20
	Negative	5	22	27
	Total	25	22	47

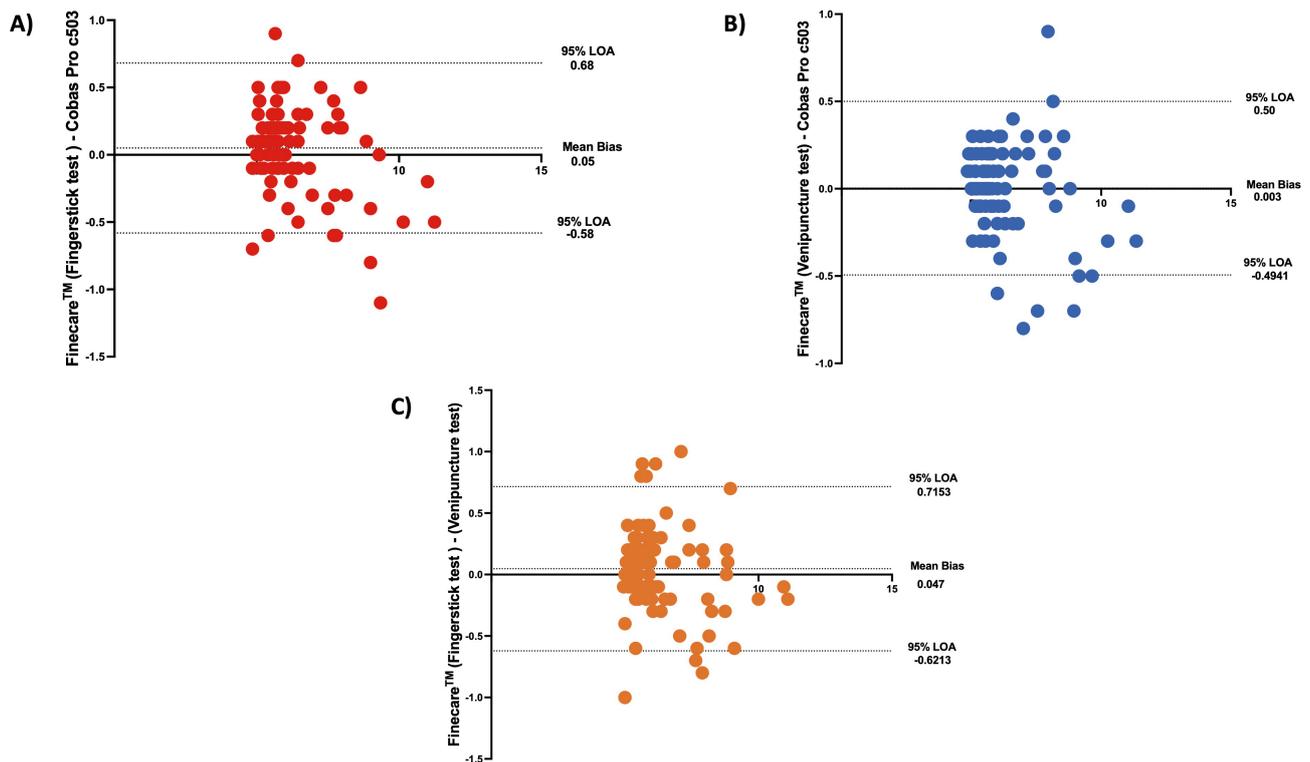


Fig. 3. The bland-Altman plot. Dotted lines present mean bias and agreement limits. The bias is represented by the gap between the X axis and the mean bias dotted line. The mean bias was calculated to be 0.05, 0.003, 0.047 for (A–C), respectively. LOA, Limit of Agreement. n = 100.

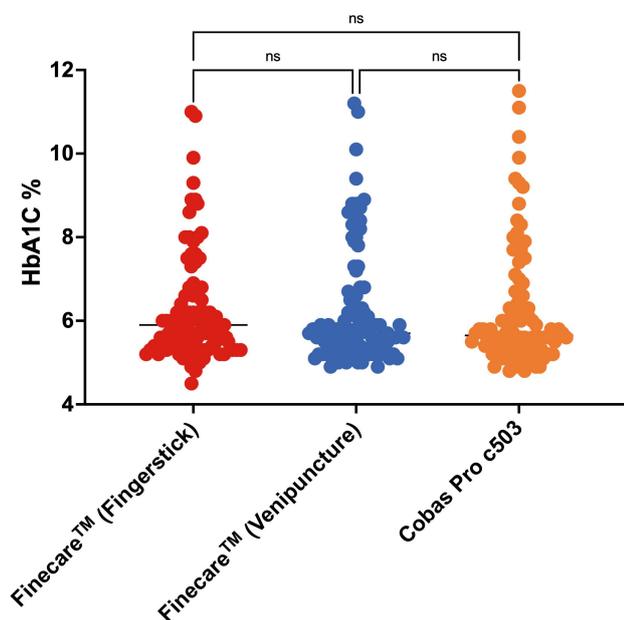


Fig. 4. Distribution of numerical results obtained from fingerstick whole blood and venous blood using Finecare™ machine and the reference method; Cobas Pro c503. The Nonparametric Kruskal-Wallis test was used to compare the differences between groups. ns, non-significant. n = 100.

3.6 Finecare™ Test could Distinguish between the Pre-Diabetic and Diabetic Groups Similar to the Reference Method

We classified the participants into three groups according to the American Diabetes Association (ADA): no diabetes (HbA1c < 5.7%), pre-diabetes (HbA1c 5.7–6.5%), diabetes (HbA1c ≥ 6.5%). Similar to the reference method, Finecare™ showed a significant difference between the normal, pre-diabetic, and diabetic samples ($p < 0.001$), as shown in Fig. 5.

3.7 Finecare™ Showed Excellent Performance and Reproducibility in Another Laboratory

To ensure the reproducibility of the results, the performance of Finecare™ was evaluated in another laboratory. Finecare™ showed 80% sensitivity and 100% specificity compared to the reference method (Table 3). Nevertheless, similar to our results, a very strong correlation was observed ($r = 0.97$, $p < 0.0001$) between Finecare™ and Cobas Pro c503 as shown in Fig. 6.

4. Discussion

To our knowledge, this is the first study conducted to evaluate the performance of fluorescence-LFIA-based HbA1c test, which marks the novelty of this research work. In this study, we demonstrated that Finecare™ results are comparable to the reference method Cobas Pro c503.

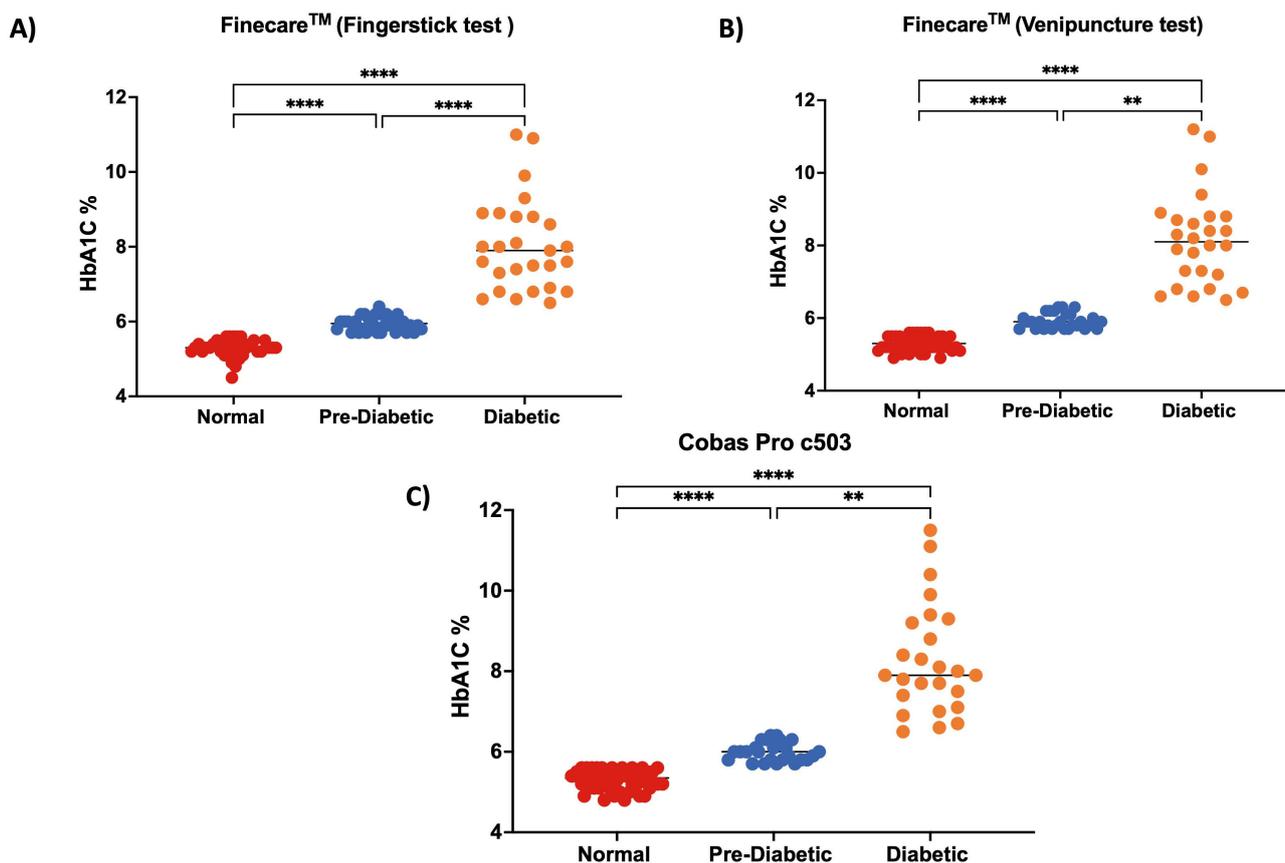


Fig. 5. Distribution of numerical results. (A) fingerstick whole blood using Finecare™ machine; (B) venous blood using Finecare™ machine; and (C) venous blood using the reference method; Cobas Pro c503. Results are represented as dot plots. Data are presented for 100 patients (normal <5.7%, prediabetic 5.7–6.4%, diabetic ≥6.5%) from each assay. Nonparametric Kruskal-Wallis test was used to compare the differences between groups. ** $p < 0.01$, **** $p < 0.0001$.

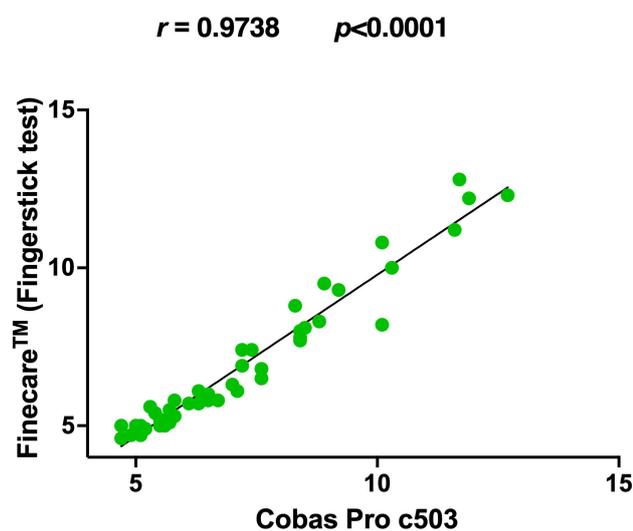


Fig. 6. Pairwise correlation and linear regression analysis of Finecare™ in comparison to Cobas Pro c503. Spearman correlation coefficient (r) and p -value were indicated. The coefficient of determination (R^2) was calculated to be 0.95. $n = 47$.

In addition, there was no significant difference between Finecare™ and Roche Cobas Pro c503 analytical analyser using the fingerstick or venous blood samples. Furthermore, a strong significant positive correlation (spearman correlation) and excellent agreement (Cohen Kappa) were observed between Finecare™ and Cobas Pro c503 with both fingerstick and venous blood samples ($r > 0.9$, $p < 0.001$). The excellent concordance between the Finecare™ and Roche Cobas Pro c503 makes it a very attractive alternative for the reference laboratory technique in POC settings.

One of the key advantages of Finecare™ is obtaining quantitative results within 5 minutes using fingerstick blood samples. Even though anti-HbA1c antibodies are more stable and persistent in venous whole blood samples, the fingerstick whole blood samples are more convenient and easier to use. Further, a small sample size is used in Finecare™ (10 μL) compared to Cobas Pro c503, which needs 300 μL as a dead volume for most of the assays. Thus, we evaluated the efficacy of Finecare™ utilizing whole blood samples obtained through fingerstick to blood samples obtained via venipuncture. Using Finecare™, a very good correlation (r

= 0.905, $p < 0.0001$) was detected between fingerstick and venous samples. Our findings validated the feasibility of utilizing whole blood samples from fingerstick for the detection of HbA1c and demonstrated excellent concordance with venous blood samples using the Finecare™ test. The collection of fingerstick samples is simple, easy, and eliminates the need for a phlebotomist.

In this study, we showed that Finecare™ assay could be used efficiently for the long-term monitoring of HbA1c in diabetic patients. To ensure the reproducibility of the results, the performance of Finecare™ was evaluated in another laboratory. The assay performs with high reproducibility (80%) with the two runs repeats done on two different samples taken from two different laboratories. In addition, Finecare™ showed reproducible very high specificity (100%) compared to the reference method. Furthermore, a very strong correlation was also observed ($r = 0.97$, $p < 0.0001$) between Finecare™ and Cobas Pro c503. Nevertheless, we noticed that there were differences in the calculated sensitivity between the two labs (public and referral labs) despite the fact that both labs used whole venous blood for analysis. This difference could be due (i) personal error that is related to the differences in technical expertise between the public and the referral lab, and (ii) differences in sample size used in both labs ($n = 100$ vs. $n = 47$); the larger sample size used, the more accurate analysis. In other words, with a small sample size, the probability of false negative results is high, which is the type II error. The main determinant of type II error is the sample size.

There were a few limitations to our study. We did not show that the Finecare™ assay was free of artifact interference that could affect the test results, including samples from patients with hemoglobinopathies (examples: hemoglobin C disease, hemoglobin S-C disease, sickle cell anemia, and thalassemia's). In addition, the size of Finecare™ analyser could be considered large in size ($270 \times 238 \times 146$ mm) for self-testing. Finally, we could improve our validation study by performing each using two different Finecare™ analysers or by using different kit lot numbers.

5. Conclusions

The Finecare™ HbA1c Rapid Quantitative Test showed very good performance, including excellent sensitivity, specificity, correlation, agreement, and concordance with the Roche Cobas Pro c503 (reference method). After further analysis to address the limitations listed above, Finecare™ might be a reliable assay that can be easily implemented for long-term monitoring of HbA1c in diabetic patients.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

Methodology—MMA. Data analysis—GKN and NY. First draft writing—GKN and NY. Review and editing—SID, EAZ, MMA, AFM, and NAD. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Ethical approval exemption (QU-IRB 1766-E/22) was granted by Qatar University.

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Conflict of Interest

The authors declare no conflict of interest. All kits and Finecare™ readers were provided as free of charge as an in-kind support from Wndfo to support this study. GKN is a member of the editorial board of this journal. We declare that GKN had no involvement in the peer review of this article and has no access to information regarding its peer review.

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