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Evaluation of a New Point-of-Care HbA1c Assay: The Pixotest

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Abstract:

Introduction: *The PixoTest (iXensor) has been introduced for point of care testing (POCT) of glycated hemoglobin (HbA1c), utilizing a colorimetric assay to assess HbA1c on a handheld device with a short turn-around time of around 3 minutes. Such POCT would be very useful in the assessment and diagnosis of diabetes in the outpatient setting. We evaluated the PixoTest's analytical performance for HbA1c against our automated laboratory Cobas (Roche Diagnostics) analyser.*

Methods: *The performance evaluation included assay linearity, analytical precision, Bland-Altman analysis and regression analysis against the Roche Cobas c513 turbidimetric HbA1c assay. Statistical analyses were performed using MedCalc software v18.11.3 (MedCalc, Ostend, Belgium). Our laboratory is a CAP accredited laboratory and both HbA1c assays are NGSP certified.*

Results: *The PixoTest assays were linear for HbA1c from 4.8-15.0%. Inter-assay precision (n=20) of Roche controls was satisfactory with a %CV of of 3.7 and 3.1 respectively. Bland-Altman analysis showed no significant difference between the Cobas and Pixos HbA1c (-0.07%, p = 0.27). Deming Regression analyses showed a close correlation coefficient of r = 0.96 between the 2 methods.*

Conclusion: *The performance of HbA1c assay on the PixoTest is good, within the manufacturer's claims, comparable to the Roche assays and fit for operational use.*

Keywords: *Diabetes, HbA1c, Evaluation, Point-of-care, PixoTest*

1. INTRODUCTION

Glycated hemoglobin (HbA1c) is recommended by the American Diabetes Association (ADA) for the diagnosis, monitoring, and screening of diabetes/prediabetes [1]. HbA1c correlates with microvascular and macrovascular complications of diabetes [2-6]. The within-subject biological variation of HbA1c is smaller (3.6%) when compared to fasting plasma glucose (FPG – 5.7%) and the oral glucose tolerance test (oGTT – 16.7%) [7]. HbA1c assays are internationally harmonized due to the National Glycohemoglobin Standardization Program (NGSP), with all-method precision (coefficient of variation - CV) consistently <3.0% over their past 8 surveys [8].

Modern assessments of HbA1c use either separation techniques (ion exchange chromatography, capillary electrophoresis and affinity chromatography) or chemical methods (immunoassays and enzymatic assays) [9]. HbA1c point of care testing (POCT) is growing in demand due to the convenience and speed of testing allowing same session decision-making in the clinic. The PixoTest (iXensor, Taiwan) is a recently introduced NGSP-certified handheld POCT device for HbA1c with a short turn-around time of 3 minutes. We evaluated the analytical performance of the PixoTest HbA1c against our laboratory automated HbA1c analyser – the Cobas c513 (Roche Diagnostics, Asia-Pacific, Singapore).

2. METHODS

The PixoTest Hba1c assay is an immunocolorimetric assay. Ethylenediaminetetraacetic acid (EDTA) whole blood (5uL) is acquired by capillary action into a proprietary calibrated transfer pipette containing blue-dyed latex microparticles conjugated to specific antibodies directed against the N-terminus of the hemoglobin A0 beta chain. Thereafter, the admixture is added into a buffer solution containing a precise amount of hemolyzing reagent followed by 6-8 agitations to lyse the RBCs. This entire buffer mixture is then loaded onto a test strip coated with immobilized anti-HbA1c antibody. The intensity of resultant blue conjugate on the strip is measured optically and the HbA1c result is available after 3 minutes. The PixoTest has a reported HbA1c measuring range of 4.0-15.0%. The Cobas c513 is an NGSP-certified immuno-turbidimetric inhibition assay using the Roche Tina-quant reagents [10, 11]. The PixoTest HbA1c assay attained NGSP certification in 2017.

Linearity was studied following the Clinical and Laboratory Standards Institute (CLSI) EP-6 protocol [12]. Patient samples collected in EDTA tubes (Becton-Dickinson, Singapore) with low and high HbA1c values were mixed to generate samples across a wide range of HbA1c concentrations. Precision was assessed according to the CLSI EP05-A3 protocol [13]. For method comparison, we used the CLSI EP-9 method [14]. Anonymised leftover EDTA whole blood samples that had been previously tested on the Cobas c513 for HbA1c across a broad clinically relevant range (n=100) were recruited and stored at 4ºC. These samples were mixed thoroughly and examined on the PixoTest within 16 hours. As this work was part of our routine evaluation of new diagnostic assays it was exempt from institutional review board approval. Our laboratory is certified by the College of American Pathologists (CAP) and our performance for the Cobas c513 HbA1c assay on the CAP external quality assurance program has been satisfactory. Statistical analyses including Bland-Altman analysis and correlation/regression were performed using MedCalc software v18.11.3 (MedCalc, Ostend, Belgium).

3. RESULTS

The PixoTest assay was linear for HbA1c from 4.8-15.0% against a claimed measuring range of 4-15%. Inter-assay precision (CV%) was 3.7% at HbA1c level of 4.8% and 3.1% at HbA1c level of 8.0% (package insert claimed CV was 3.2% and 3.5% for HbA1c levels of 5.73% and 8.35% respectively). Bland-Altman analysis showed no significant difference between the Cobas and PixoTest assays, with a mean difference in HbA1c of -0.07% (95% CI -0.1909% to 0.055%) (See Figure1). Deming regression analysis (Figure 2) showed close agreement (r = 0.96; 95% CI 0.9436-0.9742) between PixoTest and Roche HbA1c (stated r = 0.99 against the Tosoh G7 analyzer).

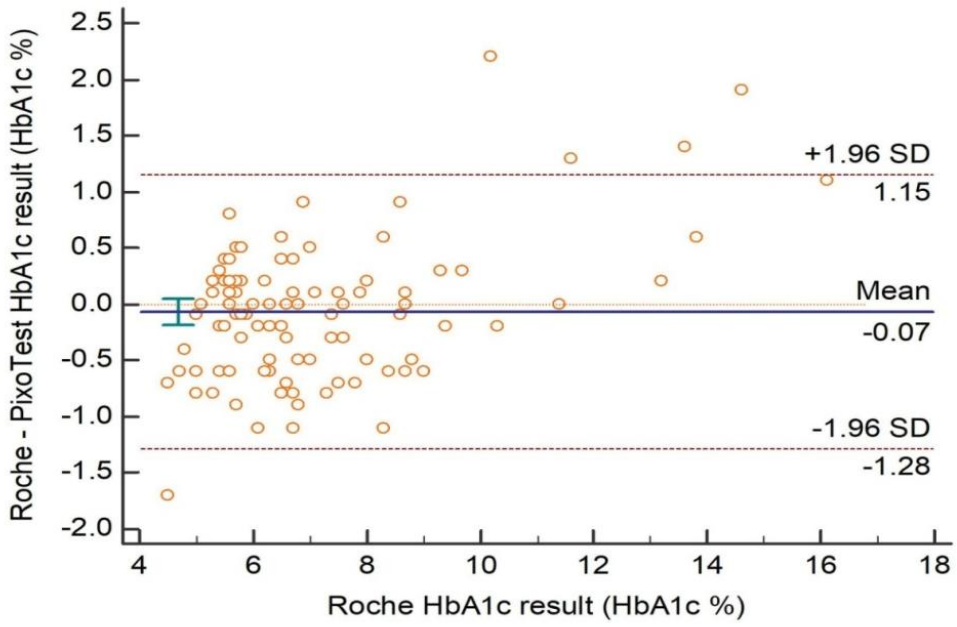

Figure1. *Bland-Altman analysis of PixoTest versus Cobas c513 HbA1c results*

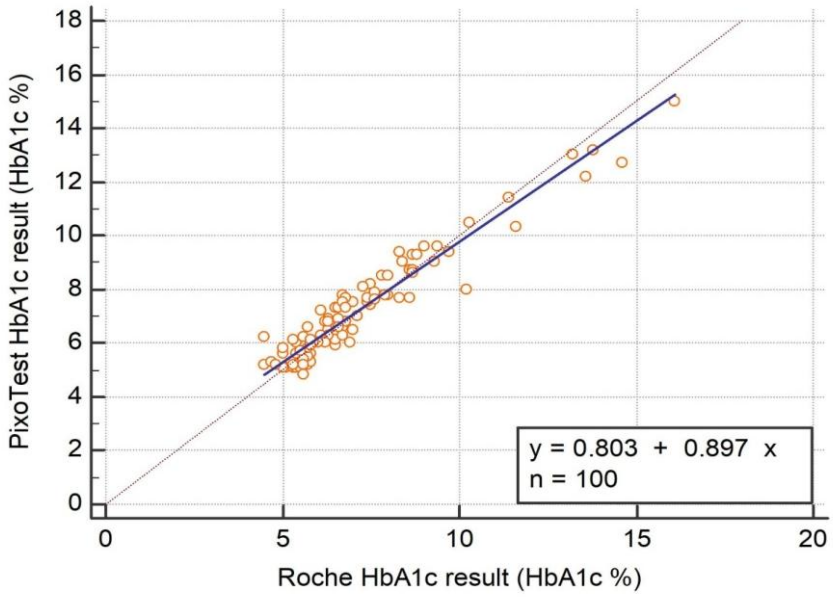

Figure2. *Deming regression analysis of PixoTest versus Cobas c513 HbA1c*

4. DISCUSSION

Our evaluation of the PixoTest HbA1c assay confirms that its performance is good, within the manufacturer's claims and is comparable to the Cobas c513. The PixoTest assay precision was quite acceptable - 3.1% for the high HbA1c control (8.0%) and 3.7% for the low control (4.8%). To simulate real-world conditions, precision testing was performed by 4 operators on 4 different instruments. The PixoTest is fit for operational use. However, several other NGSP-certified HbA1c POCT devices are available [15-17] (see Table 1). They all have good precision (CVs <4%) in the clinically relevant range. The newer devices [17] and the PixoTest have improved assay time (<4 min).

Table 1: Comparison of HbA1c POCT devices

Name	Assay type	Assay time (min)	HbA1c Precision Concentration (%)	CV (%)
DCA Vantage (Siemens)	Latex agglutination inhibition immunoassay	6	5.1/11.2 [15]	1.8/3.7
			5.3/6.1/8.1 [16]	2.3/2.5/2.7
Afinion (Axis-Shield)	Affinity separation	10	4.7/6.3/8.2 [15]	2.4/1.4/1.8
			5.3/6.1/8.1 [16]	2.8/3.1/2.4
Afinion2 (Abbott)	Affinity separation	3	6.2/9.0 [17]	1.2/0.9
Quo-Lab (EKF Diagnostics)	Affinity separation and fluorescence quenching	4	6.4/8.6 [17]	1.6/1.8
PixoTest (iXensor)	Immunocolorimetric	3	4.8/8.0	3.7/3.1

This report is novel in that the PixoTest HbA1c is a new assay that has not been reported before. It adds to the body of literature and provides additional information for consideration when selecting a POCT device for HbA1c [16]. The utility of HbA1c is growing. Studies [18] show that POCT HbA1c is superior to regular diabetes screening, diagnosing 63% of the population to be dysglycemic (prediabetic or diabetic) compared to 41%. HbA1c also has a greater concordance with pre-diabetes and diabetes [19] than oGTT. Besides, HbA1c has a stronger association with cardiovascular disease than oGTT or fasting plasma glucose (FPG) [20]. An Australian study performed on an Aboriginal

community [21] at high risk of developing diabetes showed good concordance between a POCT HbA1c (Siemens DCA 2000+) and the Roche Cobas HbA1c.

A slight limitation of this PixoTest device is the need for adequate mixing of the test sample with the hemolyzing reagent and the completeness of transferring the entire reaction contents onto the measuring cup of the test strip. Moreover, we did not test the effect of Hb variants on this system. While hemoglobin (Hb) variants are the bane of HbA1c measurements, the effect of Hb variants on modern HbA1c immunoassays is becoming less of a concern. Except for homozygous hemoglobinopathy, heterozygous variant states have minimal effects on HbA1c results. A recent study [22] comparing 100 normoglycemic beta-thalassemia carriers and 100 controls without diabetes showed that the mean HbA1c concentration on the Cobas Integra 800 analyzer was almost identical in both groups - 5.23% versus 5.22%. Besides, homozygous hemoglobinopathies are extremely rare in our practice. In one national referral center near our hospital, Hb variants were encountered in 2.3% (129/5628) of the HbA1c tests done over a 5 month period [23]; of these only 5 (0.09%) were homozygous hemoglobinopathies.

5. Conclusion

The PixoTest HbA1c POCT device has excellent correlation with the Cobas c513 auto-analyzer results with no significant differences. As HbA1c is recommended for the screening, diagnosis and monitoring of diabetes, rapid HbA1c testing using accurate POCT devices is desirable. With a rapid assay time of 3 minutes, PixoTest can aid the clinician in quick decision-making.

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Author Contributions

All authors have made substantial contributions (design of study, data acquisition, drafting article and approval of final version for submission).

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ABBREVIATIONS:

ADA – American Diabetes Association

CAP – College of American Pathologists

CLSI –Clinical and Laboratory Standards Institute

CV – coefficient of variation

EDTA – Ethylenediaminetetraacetic acid

FPG – Fasting plasma glucose

Hb – Hemoglobin

HbA1c – Glycated hemoglobin

HPLC – high performance liquid chromatography

NGSP – National Glycohemoglobin Standardization Program

oGTT – oral glucose tolerance test

POCT – Point-of-care testing

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