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HbA1c ENZYMATIC ASSAY EVALUATION AT AN ACADEMIC LABORATORY

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ABSTRACT

Background: HbA1c is a well-known and widely accepted marker for monitoring glycaemic control in patients with Diabetes Mellitus. It has also been shown to predict long term microvascular complications associated with Diabetes Mellitus. As a result the International Expert Committee has endorsed HbA1c as a diagnostic marker for Diabetes Mellitus. There are several assays available for the measurement of HbA1c and the recommended assay performance is an intralaboratory coefficient of variation of <2%. The aim of the study was to evaluate the analytical performance of the HbA1c enzymatic assay on the Abbott Architect ci8200.

Method: The precision, reportable range and bias were determined by following The Clinical and Laboratory Standards Institute (CLSI) EP05, EP06 and EP15 guidelines. Bias was determined by comparison between the Abbott Architect and the Biorad Variant II analyser.

Results: The total impresicion for low and high controls was 1.06% and 1.8% respectively. The assay demonstrated excellent correlation with the Biorad Variant II analyser with a slope of 0.923, intercept of 0.569 and R2 of 0.978. The Bland-Altman mean difference was 0.044 between the Abbott Architect and Biorad Variant II analysers. At the diagnostic and monitoring cut-off, the bias was found to be 1.05% and 0.43% respectively.

Conclusion: The new enzymatic HbA1c assay demonstrated good precision and had the advantage of rapid analysis thus prompt results reporting. The study also demonstrated acceptable agreement of the Abbott Architect and Bio-Rad Variant II methods across the measured range. Notably, the assay met analytical goals for the diagnosis and monitoring of Diabetes Mellitus using, as the total allowable error was 1.13% and 1.03% respectively, which was well below the 3% total allowable recommended error from biological data.

KEYWORDS

HbA1c, Diabetes Mellitus, Coefficient of variation, Analytical performance, The Clinical and Laboratory Standards Institute (CLSI)

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of carbohydrate metabolism where there is hyperglycaemia as a result of overproduction or underutilisation of glucose.[1] Chronic hyperglycaemia is associated with long-term microvascular complications thus reducing quality of life for many people.[2,3] DM is a common worldwide non-communicable disease. In 2016 the WHO in their global account on diabetes, reported that an estimated 422 million adults were living with DM in 2014, compared to 108 million in 1980. In 2010 the cost of managing an individual with DM in South Africa was approximately R5000. In 2015 this figure had increased alarmingly, to R26, 743.69.[4]

Traditionally, the following criteria have been used to diagnose DM: a fasting plasma glucose (FPG) of ≥7 mmol/l; a 2 hour plasma glucose concentration of ≥11.1 mmol/l during an oral glucose tolerance test (OGTT), and a random plasma glucose concentration of ≥11.1 mmol/l in a patient with symptoms of DM. However, these only provide a snapshot of the patient's glycaemic status.[5,6] Plasma glucose is readily available and inexpensive however, there are pre-analytical challenges in obtaining accurate plasma glucose results. Patients have to fast overnight for measurement of FPG and an OGTT requires administration of a solution with high glucose concentrations. In addition, there is loss of glucose post sample collection due to *ex vivo* glycolysis; this is quite common with the widely used sodium fluoride containing collection tubes. Sodium fluoride is unable to prevent short term glycolysis, as the anti-glycolytic action of fluoride is delayed for up to four hours. During the first 1-2 hour after blood collection sodium flouride has little effect on glycolysis and the glucose level can fall by 0.5 mmol/l. Other measures, to prevent glycolysis, like the use of ice-water slurry are not practical.[5,7]

HbA1c has been used for many years to monitor glycaemic status, but has in recent years been endorsed by the International Expert committee to diagnose DM. HbA1c is formed from non-enzymatic binding of glucose on the N-terminal valine residues of the haemoglobin beta chain.[1,8] HbA1c is preferred over plasma glucose as it provides a measure of glycaemic control during the preceding 2-3 months. In addition there is no need for fasting or timed sampling and it is unaffected by acute stress or illness.[2,9]

An HbA1C concentration of ≥6.5% is used for the diagnosis of DM and a concentration between 5.7%-6.4% indicates impaired glucose tolerance.[5,10] In patients on anti-diabetic

therapy, the American Diabetes Association (ADA) recommends that HbA1c concentrations should be <7%. However, in those patients with a history of severe hypoglycaemia or with advanced microvascular or macrovascular complications HbA1c concentrations of <8% are acceptable.[11] There are several methods available for the determination of HbA1c. These methods are based on different analytical principles and performance. To ensure comparability of HbA1c, results from all these different methods have to be traceable to the internationally acceptable reference method and should meet the recommended analytical goals i.e., an intra-laboratory coefficient of variation (CV) <2% and an inter-laboratory CV <3.5%.[5,12] With global harmonisation of HbA1c testing there is also uniformity in the reporting of results. The National Glycohemoglobin Standardisation Programme (NGSP) and International Federation of Clinical Chemistry (IFCC) result units, are reported in the form of percentage (%) and millimoles per mole (mmol/mol) respectively. Some authors believe results should be reported as an estimated average glucose (eAG) as the eAG is in the same units as patients self-monitoring.[5]

Abbott has recently developed an HbA1c enzymatic assay for routine use, which is based on the quantification of fructosyl dipeptides.[13] The aim of this study was to assess the analytical performance of the enzymatic HbA1c assay on the Abbott Architect ci8200 analyser and its correlation to that of the Biorad Variant II analyser.

MATERIALS AND METHODS

Study design and instrumentation

We assessed the performance of the HbA1c on the Abbott Architect ci8200, using an enzymatic assay against that of the Boirad Variant II analyser. Abbott Architect calibrators and Biorad Variant II controls were used, according to the manufacturer's instructions. Calibration of the Abbott Architect ci8200 was performed using Abbott calibrators (REF 4P52-02) as they are traceable to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method.

The Abbott HbA1c enzymatic assay principle is based on the quantification of fructosyl dipeptides. Whole blood is first haemolysed on-board using a specific dilution buffer and haemoglobin is oxidised with sodium nitrite to obtain methaemoglobin. The methaemoglobin is then quantified photometrically at 476nm. Following protease digestion, fructosyl dipeptide oxidase generates hydrogen peroxide, which reacts with chromogens in the presence of peroxidase and is measured at 660nmn (i.e., the HbA1c fraction). HbA1c (%/mmol/mol) concentration is then calculated automatically by the system, from the ratio of the HbA1c fraction to that of the total haemoglobin (THb).[13,14] The manufacturers claim performance is imprecision with a Standard deviation (SD) of less than 0.13 for a HbA1c of less than 5.7%. They also claim a within-laboratory CV of less that 2% for samples with a HbA1c targeted to 6.5% (5.7-7.0% HbA1c) and a within-laboratory CV of less than 3.5% for samples with a HbA1c level greater than 7%.

The Biorad Variant II principle is based on ion-exchange high-perfomance liquid chromatography (HPLC). Samples are automatically diluted on the sampling station and injected into analytical cartridge. The dual pumps of the variant chromatographic station will then deliver a programmed buffer gradient of increasing ionic interaction to the cartridge. This will then separate haemoglobins (Hbs) based on their ionic interactions with the cartridge material. The separated Hbs will then pass through the flow cell of the filter photometer, where changes in the absorbance at 415nm are measured, notably there is an additional filter at 690nm that corrects for the background absorbance.[15]

Precision

The assay imprecision was evaluated according to the CLSI EP05-A3 guideline, using Biorad Lyphocheck Diabetes control materials (REF740). Five replicates of a low (level 1) and a high level (level 2) controls were run daily for 5 days with a total number of 25 results; thus establishing the within-run and between-run precision. The target mean for the HbA1c low and high control materials was 4.9% and 9.44% respectively. Imprecision was then expressed as the CV. The precision performance of the instrument was a CV = 0.99% and a CV = 0.69% for levels 1 and 2 respectively. The Biorad Variant II is NGSP certified.

Method comparison

The study was completed following the CLSI EP09 guideline, where 40 EDTA blood samples are recommended for comparison studies. These were then sent to the laboratory for routine HbA1c analysis. Samples were collected for a period of five days. All samples were immediately analysed on the Abbott Architect analyser using the enzymatic assay method. They were then sent daily in a cooler-box with an ambient temperature of 8°C, to the referral laboratory for analysis on the Bio-Rad Variant II analyser.

Linearity

Linearity was assessed using the CLSI EP6-A guideline. Five concentrations spanning the analytical range were determined using 2 patient samples with low (4.6%) and high (14.8%) HbA1c concentrations. The sample with high concentration was mixed with the low concentration samples, generating 5 levels, including one close to the medical decision limit and the samples were then analysed in duplicate. The acceptability was assessed using the recovery between 95% and 105%.

Statistical analysis

Statistical evaluation for method comparison was performed using commercial IT software. The relationship between the methods was determined using the Passing-Bablok non-parametric regression analysis. Bias and agreement intervals were assessed using the Bland-Altman plot. For precision studies, data was analysed using one way analysis of variance (ANOVA). For linearity acceptance criteria was assessed using recovery studies.

Analytical goals

To assess if the enzymatic assay methodology met the analytical goals for routine use in diagnosing and monitoring DM, Total Allowable Error (TAE) was calculated at a HbA1c of 6.5% and 7% respectively and then compared with TAE from biological data.

RESULTS

Precision study

The within run imprecision was 0.9% and 0.7% for levels 1

and 2, whereas the between-day imprecision was 1.6% and 0.8% for levels 1 and 2. The total imprecision was 1.06% and 1.8% for levels 1 and 2.

Method comparison

There was excellent correlation between the Abbott Architect enzymatic method and the Biorad Variant II HPLC method. The Pearson CV of the two methods was 0.978 and the Passing-Bablok non parametric regression analysis showed a slope of 0.923 with a 95% confidence interval (CI) of 0.8750-1.00 and an intercept of 0.569 with 95% CI of 0.100-0.950 (see Figure 1). The Bland-Altman (BA) plot showed a mean difference of 0.04 with a CI of 0.51 to 0.68. The percentage bias at the diagnostic cut-off (6.5%) was 1.05%, whereas for the monitoring target level (7%) the percentage bias was 0.43% (see Figure 2).

Linearity

Dilution of the high concentrated sample revealed recovery rates between 100-113%. 40% of the measured samples did not dilute linearly at the concentration of 9% and 12%.

DISCUSSION

The aim of this study was to evaluate the performance of enzymatic HbA1c and its correlation to that of the Biorad Variant II analyser. Currently, HPLC is the robust reference method as it is able to report HbA1c accurately in the presence of haemoglobin variants (HbS, C, D, E, traits; HbF as high as 25% and HbC and LA1c traits). The HPLC method is known for its excellent precision, high throughput rate, NGSP verifiability and is traceable to the Diabetes Control and Complications Trial (DCCT) and also to the IFCC reference method.[15] Immunoassays, have a quick turnaround time and are well automated, but are known to have poor precision and unstable calibration curves.[14] The advantage of the enzymatic method however, is that, manual sample haemolysate is not required for whole blood samples with volumes above 200µlitres. It also has a short turn-around time, high throughput, is fully automated and has excellent precision.[14]

Since HbA1c has been endorsed for diagnosing DM the repeatability and reproducibility of the assay should meet the expected performance.[2] Precision studies demonstrated an acceptable performance of the assay as demonstrated by within-run CV's of 0.9% and 0.7% for levels 1 and 2 respectively. The between-run CV's were 1.6% and 0.8% for levels 1 and 2 respectively. The imprecision performance is comparable to the 0.9% desirable analytical CV from biological variation data and the 1.3% analytical goal derived by Braga et al. The exception was that

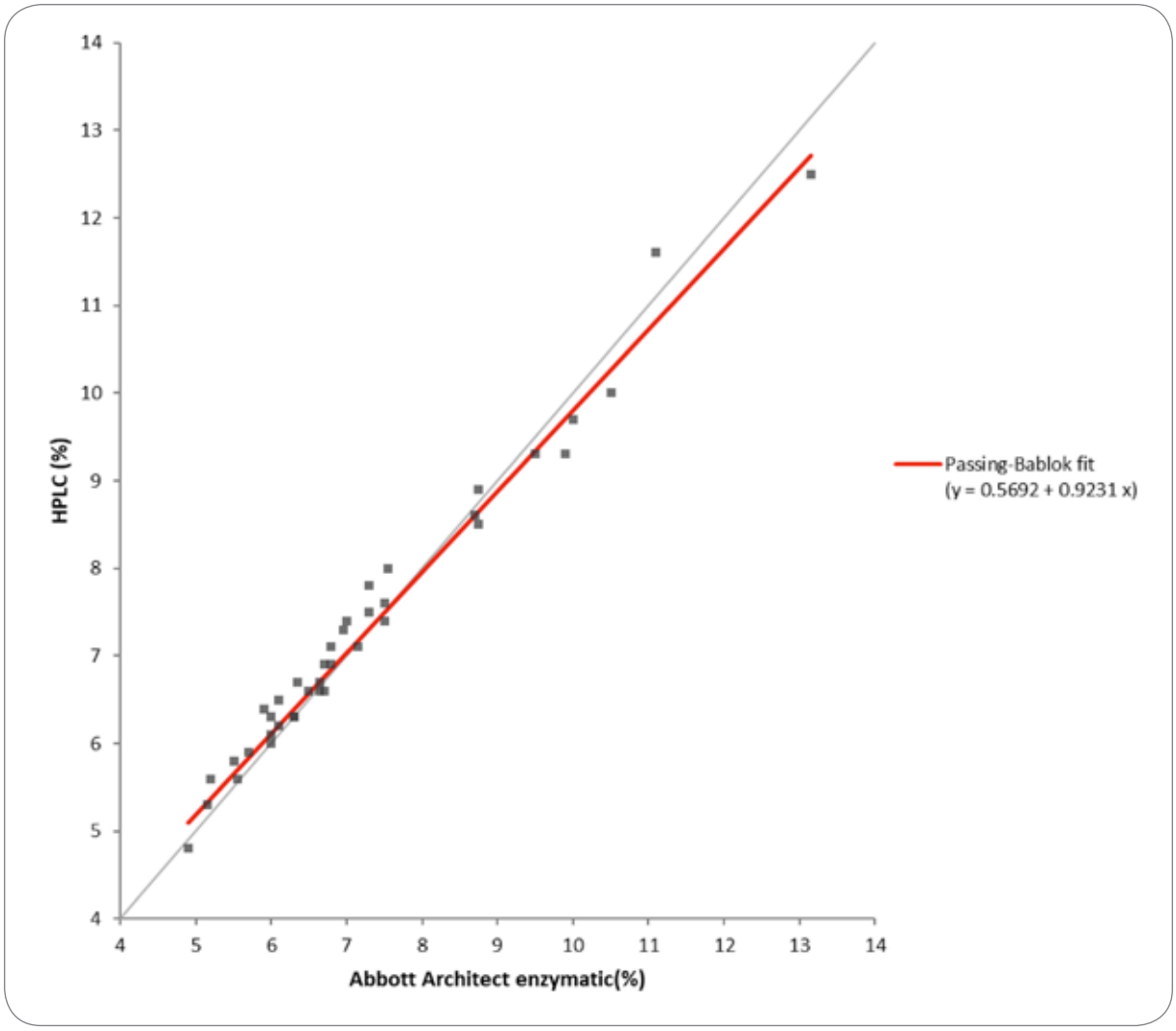

Figure 1: Method comparison of enzymatic HbA1c with HPLC

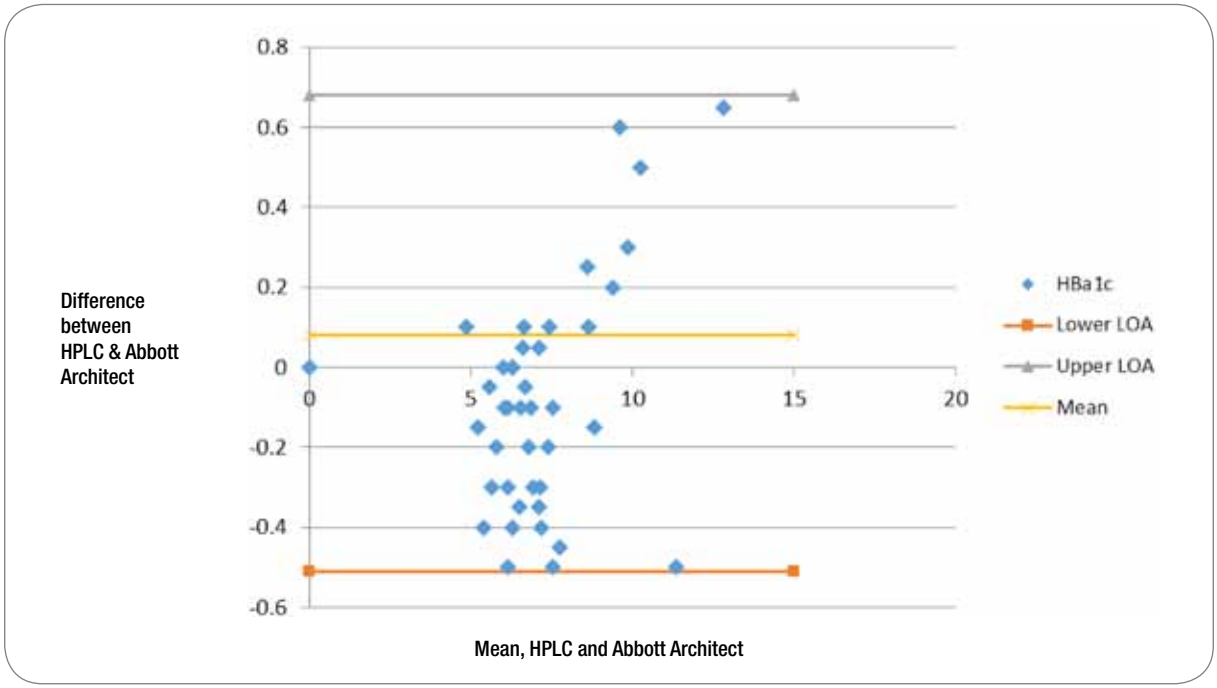

Figure 2: Bland-Altman plot of the comparison between HPLC and Abbott Architect (enzymatic assay)

of level 1 between day precision and this could be attributed to different temperatures between days.[16] Both within-run and between run CVs were comparable to CVs obtained by other studies, where Lee et al., reported a within-run imprecision of 0.6% and 0.4% for levels 1 and 2 respectively. Jaisson et al., reported an imprecision of 1.42% and 0.42% for within day and 0.95% and 0.47% for between-day for levels 1 and 2 respectively.[13,17] The total imprecision for both low (1.06%) and high (1.8%) level controls was within the recommended intra-laboratory CV of <2%.[9]

There was excellent correlation between the results obtained from the enzymatic assay and those from the Variant II with a Pearson correlation coefficient of 0.978. This is comparable to values obtained from other studies, where they compared the Tosoh G8 and Capillarys 2 Flex Piercing with the Bio-Rad Variant II.analyser.[13,17]

The regression analysis demonstrated both a constant and proportional bias between the two methods. The constant bias is noted at low HbA1c concentration, whereas the proportional bias was noted at the higher HbA1c concentration. However, these were within the allowable recommended bias ranges. These findings were similar to those seen in a study by Jaisson S et al. A study by Maesa et al., demonstrated a small proportional bias at high HbA1c concentration. The observed bias might be due to the calibration procedures of the analyser.

The enzymatic method showed acceptable linearity in the range between 4.6% and 14.8%, this covers both the HbA1c diagnostic and monitoring cut-offs. Notably, at concentrations of 9% and 12% we noted an over-recovery and this might be due to operator/pipetting errors. The assay fulfilled the analytical goals for the diagnosis and monitoring of DM. The TAE was 1.13% and 1.03% for diagnosis and monitoring, which was well below the 3% TAE as recommended from biological data.

STUDY LIMITATIONS

Interference studies due to Haemoglobin (Hb) variants were not carried out on the Abbott Architect ci8200. Although patient samples did not demonstrate Hb Variants on HPLC, interference from these variants has not been ruled out. There was an over-recovery at 9% and 12% concentrations, when the linearity experiment was done, despite the levels being above the diagnostic and monitoring levels. This was probably due to poor manual skills regarding the techniques involved.

CONCLUSION

The new enzymatic HbA1c assay demonstrated good precision and had the advantage of rapid analysis, hence prompt results reporting. The study also demonstrated acceptable agreement of the Abbott Architect and Bio-Rad Variant methods across the measured range. Notably, the assay fulfilled the analytical goals for the diagnosis and monitoring of DM, as the TAE was 1.13% and 1.03% respectively, which was well below the 3% TAE, as recommended from biological data.

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