Comparison of HbA$_{1c}$ measurement methods between automatic boronate affinity point of care testing and high-performance liquid chromatography

Ricky Tjahjadi, 1* Astuti Giantini 2

ABSTRACT

Background: This study aimed to assess the comparison between two HbA$_{1c}$ analyzers, Alere Afinion AS100, a boronate affinity based point of care testing (POCT), and High-Performance Liquid Chromatography (HPLC) based analyzer Bio-Rad Variant II Turbo HbA1c kit-2.0 as reference method.

Methods: This study involved 120 samples of peripheral K$_3$EDTA whole blood sent to Clinical Pathology Laboratory of Cipto Mangunkusumo National Hospital for HbA$_{1c}$ measurement. Based on reference method, 40 samples with HbA$_{1c}$ ≤ 6.4%, 40 samples with HbA$_{1c}$ > 6.4%, and 40 samples with variant hemoglobin or hemoglobinopathy were included. Precision and accuracy of both analyzers were assessed using control materials. The goodness of fit between these methods were assessed by Bland-Altman plot and Passing-Bablok regression test.

Results: Reference method had total error (TE) ranging from 3.15% to 4.9%, while Afinion ranged from 2.16% to 3.24%. Both methods correlated well with Passing-Bablok regression showing no proportional or systematic differences. Linearity between tests was proven by Cusum test value of p < 0.05. Bland-Altman plot yielded 91.74% goodness of fit. No significant differences were observed in hemoglobinopathy and variant hemoglobin analysis of HbA$_{1c}$.

Conclusion: Afinion was precise, accurate, and linear to HPLC reference method. Both methods exhibited no systematic or proportional differences. Despite Bland-Altman plot of less than 95% goodness of fit, no clinically significant result was found based on NGSP criteria.

Keywords: Afinion, boronate affinity, HbA$_{1c}$, HPLC, POCT, variant II Turbo.

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INTRODUCTION

Diabetes is a chronic disease with increasing incidence around the globe. In 2014, it was estimated that 380 million of world population suffered from diabetes with projected incidence of 592 million in 2035. Diabetes usually diagnosed by utilizing fasting blood glucose, oral glucose tolerance test and HbA$_{1c}$. The HbA$_{1c}$ has the added value of therapy monitoring. 1

Diabetes management is proposed to keep blood glucose in normal range with minimal fluctuation. Blood glucose measurement could be done in laboratory using serum and plasma, or personally done using point of care testing (POCT) device on capillary blood sample. Since blood glucose naturally fluctuates, glycated hemoglobin is a better substitute for long term monitoring. Of all glycated hemoglobin, HbA$_{1c}$ is the most studied and checked parameter. 2

Accurate measurement of HbA$_{1c}$ guides clinicians to manage patient’s glycemic status and to aid in the setting of clinical decision making. The American Diabetes Association (ADA) recommended at least two measurements a year on diabetic patient whose HbA$_{1c}$ is well-controlled, or even four measurements for those with uncontrolled glycemia. 3 World Health Organization (WHO) emphasizes that HbA$_{1c}$ could be utilized as a diagnostic tool only if the assay is well-controlled, interference free, and standardized to an international reference. 4,5 Harmonization of variable assays is achieved by standardization and certification by National Glycohemoglobin Standardization Program (NGSP) and Diabetes Control and Complications Trial (DCCT). 6

NGSP certified methods have to be periodically monitored since not all assays perform well in certain condition. 7 Erythrocyte age and conditions have to be taken into account when interpreting HbA$_{1c}$. Patients with hemolysis or other conditions resulting in shortened erythrocyte age tends to have lower value of HbA$_{1c}$, while other conditions such as iron deficiency anemia will result in higher HbA$_{1c}$. Variant haemoglobins such as Hbf, Hbs and Hbc also interferes with certain HbA$_{1c}$ assays. Manufacturers modify assays to eliminate variant hemoglobin interferences, but studies find that
variations exist between NGSP certified methods which could change HbA1c diagnostic value.1,4

To date, there are more than 150 HbA1c assays with only two main principles, which are separation of hemoglobin fractions and chemical reaction.3,4 Each method has different interferences, but all methods have to be traceable to NGSP.4 POC devices offer convenience in reagents preparation, sampling and analysis, but need to be reliable enough to hold clinically relevant information.7 An ideal HbA1c assay needs to consistently have CV under 2% and minimal bias, also considering each method’s interfering factors.9 Quality of HbA1c assays is a major concern, since many comparative studies showed high CV and bias, even between NGSP certified assays.9 According to NGSP, both Afinion and Variant II are not interfered by the presence of HbC, HbS, HbE and HbD. Variant II is not interfered when HbF concentration is less than 25%, while no specific data is available on Afinion although it is assumed that boronate affinity is free from interference with HbF concentration 10–15%.9

This research was conducted to assess the comparability between two HbA1c assays, HPLC in Bio-Rad Variant™ II TURBO HbA1c kit-2.0 and boronate affinity POCT Alere Afinion HbA1c on varying HbA1c concentration and haemoglobinopathy samples. Both methods used in this study have been certified and can be traced back to NGSP at www.ngsp.org.

MATERIALS AND METHODS

The study was a cross-sectional study and taken from April 2017 to June 2017. Sample analysis was done in Clinical Pathology Laboratory, Cipto Mangunkusumo General Hospital. A sum of 120 samples were obtained from patients’ remaining K_EDTA venous blood samples whose HbA1c were measured in RSCM Clinical Pathology Laboratory from April to May 2017. Samples were simultaneously analyzed on Variant II and Afinion. Based on Variant II results, 40 samples had HbA1c concentration equal to or less than 6.4%, 40 samples with HbA1c concentration of more than 6.4%, and the rest consisted of hemoglobinopathy samples. These 120 samples fulfilled minimum requirement for comparison test.13 Hemolytic samples were excluded from study population.15 Ethical approval was obtained from Ethics Committee, Faculty of Medicine, University of Indonesia according to approval letter 430/UN2.F1/ETIK/2017.

This study used Bio-Rad’s Variant™ II TURBO HbA1c kit-2.0 as reference method which utilizes ion-exchange principle. Total retention time is 90 seconds per sample.13,14 Afinion AS100 Analyzer (serial number AS0043636) as this study’s POCT method examined HbA1c by boronate affinity principle. The HbA1c analysis on Afinion has a detection limit of 4-15% (NGSP) or 20-140 mmol/mol (IFCC).9 Accuracy and precision for both methods were assessed using two levels of control materials in which were optimized for each device before sample analyses. Statistical analysis was performed with IBM Statistical Package for the Social Sciences (SPSS) v20.0 and MedCalc Statistical Software v15.2. Accuracy and precision were described by mean, standard deviation (SD), coefficient of variant (CV) and bias (d). Total error (TE) was calculated by formula of: TE = d + 1.65 CV. Desirable TE was less than 3% according to Ricos’ allowable total error recommendation (TEa) or less than 5% according to Royal College of Pathologists of Australasia (RCPA)’s recommendation.15,16

Samples were analyzed separately according to HbA1c ≤ 6.4%, HbA1c > 6.4%, haemoglobinopathy samples, and as a whole comparison. Distribution of each data group was analyzed using Shapiro Wilk or Kolmogorov Smirnov when analyzed as a whole sample. Passing-Bablok regression was used to determine systematic or proportional differences between methods used in the sturry. Linearity was assessed by cumulative sum (cusum) test. Cusum test’s p value of more than 0.05 meant methods compared were linear and Passing-Bablok formula could be applied.17,18 Bland-Altman plots were generated to analyze goodness of fit through mean difference line, limit of agreement (LoA) lines, and 95% confidence interval lines.19,20 Even if there is yet no consensus on bias limitations which held clinical significance, NGSP determined two methods should only have 95% confidence interval difference value of ±0.75% to be in accordance.21,22

RESULTS

Performance summary of Variant II and Afinion between within-run and Afinion groups were depicted in Table 1. In Table 1, Afinion’s level 1 within-run test which was slightly lower than factory recommendation. HbA1c analysis on Afinion had a mean of 5.70% ± 0.35%, while Variant II yielded 5.45% ± 0.40% (Figure 1). Cusum test’s p value was 0.10, while Passing-Bablok regression had intercept of 1.36 (95% CI, 0.2 – 2.07), slope of 0.8 (95% CI, 0.67 – 1). Systematic difference as shown by Passing-Bablok intercept meant that Afinion always yield higher HbA1c than Variant II (Figure 1).

Shapiro Wilk analysis on sample group with HbA1c > 6.4% showed that data was not normally distributed. HbA1c analysis on Varian II Turbo had
Table 1  Performance summary of Variant II and Afinion

<table>
<thead>
<tr>
<th>Sample Grouping</th>
<th>Within-run</th>
<th>Between-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV (%)</td>
<td>d (%)</td>
</tr>
<tr>
<td>Variant II level 1</td>
<td>1.54</td>
<td>-3.9  -0</td>
</tr>
<tr>
<td>Variant II level 2</td>
<td>2.08</td>
<td>-5.15 -2.06</td>
</tr>
<tr>
<td>Afinion level 1</td>
<td>0.83</td>
<td>0 - 1.58</td>
</tr>
<tr>
<td>Afinion level 2</td>
<td>1.25</td>
<td>-3.53 - (-1.18)</td>
</tr>
</tbody>
</table>

Table 2  HbA1c analysis summary on Variant II and Afinion

<table>
<thead>
<tr>
<th>Sample Grouping</th>
<th>HbA1c ≤ 6.4%</th>
<th>HbA1c &gt; 6.4%</th>
<th>Haemoglobino-pathy samples</th>
<th>All samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afinion</td>
<td>5.7 ± 0.7%</td>
<td>8.2%</td>
<td>5.7% (5 – 12.2%)</td>
<td>7% (5 – 13.5%)</td>
</tr>
<tr>
<td></td>
<td>(6.6 – 13.5%)</td>
<td></td>
<td>(4.8 – 11.3%)</td>
<td></td>
</tr>
<tr>
<td>Variant II</td>
<td>5.5 ± 0.8%</td>
<td>8.1%</td>
<td>5.7% (4.7 – 13.7%)</td>
<td>5.9%</td>
</tr>
<tr>
<td></td>
<td>(6.4 – 13.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean difference (95% CI)</td>
<td>-0.25</td>
<td>-0.32</td>
<td>-0.08</td>
<td>-0.21</td>
</tr>
<tr>
<td></td>
<td>(-0.31 – (-0.20))</td>
<td>(-0.43 – (-0.20))</td>
<td>(-0.16 – (0.01))</td>
<td>(-0.27 – (-0.16))</td>
</tr>
<tr>
<td>Slope (95% CI)</td>
<td>0.80</td>
<td>1.10</td>
<td>1.00</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>(0.67 – 1.00)</td>
<td>(1.04 – 1.16)</td>
<td>(0.97 – 1.08)</td>
<td>(1 – 1.07)</td>
</tr>
<tr>
<td>Intercept (95% CI)</td>
<td>1.36</td>
<td>-0.44</td>
<td>0.10</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
<td>(0.20 – 2.07)</td>
<td>(-0.96 – 0.07)</td>
<td>(-0.43 – 0.28)</td>
<td>(-0.26 – 0.2)</td>
</tr>
<tr>
<td>Cusum test</td>
<td>0.10</td>
<td>0.97</td>
<td>0.35</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Figure 1  Passing-Bablok regression analysis on three HbA1c categories

A median of 8.1%, ranging from 6.4% to 13.7%, while Afinion’s median was 8.2% and ranged from 6.6% to 13.5%.

Linearity was proven by Cusum test (p=0.97) and no systematic difference existed (intercept value was -0.44 and 95% CI ranging from -0.96 to 0.07), while proportional difference was observed on this higher value group (1.10 slope and 95% CI ranged from 1.04 – 1.16).

Haemoglobinopathy group data was not normally distributed according to Shapiro Wilk analysis. HbA1c analysis on Varian II resulted in a median of 5.7% (4.8% – 11.3%), while Afinion’s median was 5.7% (5% – 12.2%). Passing-Bablok regression analysis showed that there was no systematic (0.10 intercept, 95% CI, -0.43 – 0.28) and proportional differences (1.00 slope, 95% CI, 0.97 – 1.08) (Table 2).

Bland-Altman analysis on HbA1c ≤ 6.4% group showed mean difference of -0.25 between methods and LoA between -0.59 to 0.09 (Figure 2). The goodness of fit was 92.5% as 3 out of 40 samples exceeded LoA. In previous study comparing Afinion to HPLC, Wood JR, et al, found that analysis on samples with HbA1c < 8%, LoA ranged from -0.2 to 0.6, wider than this study.

Samples were ranging from 5% to 13.5% with a median of 6% according to Afinion, while Varian II results ranged from 4.7% to 13.7% and median was 5.9%. Passing-Bablok regression showed linearity between methods with Cusum test p value of 0.07. Intercept was -0.03 (95% CI, -0.26 – 0.2) and slope was 1.04 (95% CI, 1 – 1.07), so Afinion result could be formulated as 1.04 x – 0.03, given x is Varian II measurement (Figure 3). This result pointed that no systematic or proportional differences existed between methods. Bland-Altman Plot gave -0.21% mean difference, with lower LoA of -0.78 (95% CI, -0.88 – (-0.69)) and upper LoA of 0.36 (95% CI, 0.27 – 0.45). The goodness of fit was 91.67% as ten out of 120 samples were not in accordance with the suitability test (Figure 3).
DISCUSSION

Both methods’ CV got beyond each respective factory recommendations, except for Afinion’s level 1 within-run test which was slightly lower than factory recommendation (Table 1). This result was still acceptable since most publications recommended CV ≤ 4% for HbA1c assays, even if it still could cause HbA1c deviation of 0.3% in 7 out of 100 examinations. NGSP consistently encourage manufacturers to achieve CV of less than 2% as desirable value,7,22 and this was achieved by Afinion in current study. Accuracy was analyzed and compared to Ricos’ recommendation which took imprecision, bias, and biological variation into account. Variant II had TE < 5% on all test levels which surpassed Ricos’ recommendation of less than 3%, but it still fulfilled RCPA’s criteria. Afinion averaged lower TE than Variant II with level 2 within-run test as the only test surpassing 3%.15,16 This result was in accordance to previous Afinion performance study done by Wood JR, et al (2012) which yielded CV value of 2%.23

No systematic difference in linearity existed in this study while proportional difference was observed on this higher value group. Similar result was achieved by Wood, et al who compared Afinion to HPLC, resulting in differences of -0.5% to 0.7% in HbA1c range from 8 to 10%, and differences of -0.7% to 1.1% for those whose HbA1c were more than 10%.23,24

Based on the haemoglobinopathy assessment, our study found that the mean relative difference between methods was higher than other population, in accordance to this study.23 On HbA1c > 6.4% group, mean difference was -0.32 with LoA between -1.03 to 0.4, which resulted in 97.5% agreement. Compared to NGSP’s criteria which is 95% confidence interval of difference within 0.75% HbA1c, then both methods still meet the criteria (agree).25 Bland–Altman analysis on variant Hb and haemoglobinopathy samples showed that 95% of data compatible (fit) with -0.08% mean difference, upper LoA was 0.42 and -0.57 for lower LoA.

CONCLUSION

Afinion was precise with a CV value of less than 2%, but still had bias when analyzing pathological control and yielded TE of 3.24%. Analysis by boronate affinity on Afinion was linear with HPLC method on Bio-Rad’s Variant II. Neither systematic nor proportional differences were found between methods when all samples were analyzed altogether. No significant bias was found on haemoglobinopathy samples analysis. Bland-Altman plot showed less than 95% goodness of fit, but the differences were still acceptable according to NGSP’s criteria.

Afinion had decent performance in accuracy and precision, so it could be utilized as HbA1c monitoring device. Should analysis turned in doubtful result, clinical judgment and patient’s condition should be considered. Reference method should be consulted if needed.

ETHICS APPROVAL

This study has been approved by the Ethics of Committee, University of Indonesia, Jakarta, Indonesia

COMPETING INTEREST

The authors declare that they have no competing interests.
AUTHORS’ CONTRIBUTION

Ricky Tjahjadi carried out data collection, laboratory analysis, statistical analysis, and drafted manuscript. Ricky Tjahjadi and Astuti Giantini designed the study. Astuti Giantini supervised the study and revised manuscript. All authors read and approved the final manuscript.

REFERENCES