

## ORIGINAL ARTICLE

# Comparison of HbA1c analysers: Agilent 1100 HPLC using kits produced by Gordion Diagnostic (Turkey) with Premier Hb9210 using kits produced by Trinity Biotech (USA) in different patient groups

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### Abstract

**Background:** The aim of this study was to compare the performance of Agilent 1100 HPLC analyser using HbA1c kits manufactured by Gordion Diagnostic (Turkey) with that of Premier Hb9210 using the original kits for the measurement of HbA1c in different patient groups. **Methods:** Subjects were divided into four groups: Group 1 included 140 diabetic and non-diabetic subjects with normal urea and haemoglobin levels; Group 2 included 84 diabetic and non-diabetic subjects with high urea levels; Group 3 included 44 diabetic and non-diabetic subjects with iron deficiency anaemia; and Group 4 included 52 diabetic and non-diabetic subjects with high haemoglobin levels. EP Evaluator Release 8 program was used to evaluate the resultant data. **Results:** According to the comparison results of the two methods in all groups, there was an excellent correlation between the two methods ( $R > 0.98$ ). Moderate–low correlation was found between increased urea concentration and the difference of the two methods ( $R = -0.374$ ,  $p = 0.0005$ ). The difference between the methods was found to be increased with increased urea concentrations. This difference, although statistically significant, was within the permitted limits. The observed correlation between the difference of the two methods and the low and high haemoglobin concentrations was statistically non-significant ( $R = 0.149$ ,  $p = 0.3343$ ;  $R = 0.263$ ,  $p = 0.0594$ ). **Conclusions:** We found that Agilent 1100 HbA1c analyser and Gordions' HbA1c kit comply with the clinical requirements and are suitable for HbA1c analysis at high levels of urea and Hb and low levels of Hb in diabetic and non-diabetic patients.

**Key words:** HbA1c, boronate affinity, ion exchange chromatography, iron deficiency anaemia

## INTRODUCTION

Diabetes management needs efficient and reliable measurement techniques applicable for measuring glycated haemoglobin-A1c (HbA1c) levels. It is important to have compatibility of repeated measurements in the same laboratories as well as between different laboratories. Therefore, the performances of different methods carried out in different laboratories have been evaluated.<sup>1,2</sup>

There are more than 30 methods for HbA1c measurement. These methods depend on two principles: charges (ion-exchange chromatography and electrophoresis) and structural difference (boronate affinity chro-

matography and immunoassays).<sup>3</sup> HbA1c measurement performed using high-performance liquid chromatography (HPLC) has been accepted as the reference method by the National Glycohemoglobin Standardization Program (NGSP) in the USA. Reference methods such as HPLC–mass spectrometry (HPLC–MS) and capillary electrophoresis HPLC (HPLC–CE) were developed by the *International Federation of Clinical Chemistry (IFCC)*.<sup>4</sup>

HbA1c levels are affected not only by blood glucose levels but also by situations affecting the survival of red blood cells (RBC), the presence of haemoglobin variants, anaemia, uraemia, pregnancy and acute blood loss.<sup>5</sup> It has been

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proved that haemoglobinopathies and chemically modified derivatives of haemoglobin (such as carbamyl haemoglobin in uraemic patients (CarbHb)) positively and negatively interfere with some HbA1c measurement methods.<sup>6</sup> CarbHb is formed as a result of the non-enzymatic reaction between haemoglobin and isocyanate that is derived from urea in uraemic patients.<sup>7</sup> It has been proved that carbamylation and glycation compete for the amino groups.<sup>8</sup> Previous studies have shown that both CarbHb and other haemoglobin components can interfere with HbA1c measurements.<sup>7</sup> There are studies that indicate that there is a positive correlation between increased HbA1c levels and iron deficiency anaemia (IDA).<sup>9,10</sup> IDA is one of the reasons for the potential interference that can limit the use of HbA1c use in both diagnosis and treatment.<sup>11</sup>

Depending on the method used, different methods give different HbA1c measurement results, since these techniques measure the different fractions of glycosylated haemoglobin via different ways.<sup>12-17</sup> Therefore, there are concerns in the selection of the measurement methods for HbA1c. Thus, it is important to compare the different measurement methods used for HbA1c with the international standardisation of HbA1c measurement.<sup>2</sup>

In this study, we aimed to compare the performances of Premier Hb9210 HbA1c analyser (boronate affinity) and Agilent 1100 analyser (ion-exchange chromatography–HPLC, IEX–HPLC) in HbA1c measurements by using original kits and Gordion kits produced in Turkey, respectively. The performances of these two techniques were compared in different patient groups: non-diabetic and diabetic patient group with normal haemoglobin and urea, non-diabetic and diabetic patient group with high urea, non-diabetic and diabetic patient group with IDA and non-diabetic and diabetic patient group with high haemoglobin.

To our knowledge, this is the first study that has been performed by using the Gordion HbA1c measurement kit. Furthermore, it is also the first study in which HbA1c levels have been measured and compared between patient groups with IDA and high haemoglobin.

## MATERIALS AND METHODS

This study was performed in the biochemistry laboratory of the Diskapi Yildirim Beyazit Training and Research Hospital. The study was approved by the Clinical Research Ethics

Committee on 17 March 2014, with the code 14/43, and informed consent was obtained from all participants.

### Characteristics of the analysers

#### *Premier Hb9210 boronate affinity chromatography method*

Haemoglobin samples were injected into the HPLC of Premier Hb9210 with aminophenylboronic acid column, which was previously balanced in terms of pH and ionic power. When the haemoglobin solution (haemolysate) passes through the column, glycosylated component was captured with the help of the diol groups that were complexed with boronate. When the uncaptured unglycosylated component left the column, glycosylated component left the column with a reactive that distinguished this component from boronate.

The principles of Premier Hb9210 boronate affinity and HPLC were followed. The pump transferred the reagents through the analytical column, which contained the aminophenylboronic acid that was embedded in the porous polymer support (gel). Haemolysed samples used in the HbA1c analysis were injected automatically into the column during the flow of the Premier Hb9210 Buffer Reagent A (elution reagent, 1). Glycosylated component adhered to the boronate, whereas unglycosylated component passed through the column and reached the spectrophotometric detector. Detection was performed at  $413 \pm 2$  nm. Premier Hb9210 pumped the Premier Hb9210 Buffer Reagent B (elution reagent, 2) after the solution of unglycosylated component passed through the column, and it separated the glycosylated component from the column. Buffer Reagent A and Buffer Reagent B were designed as to virtually offer the same absorption in order to obtain a stable baseline in the range of  $413 \pm 2$  nm. Detector signal acted through the dual-beam technique.<sup>18</sup>

#### *Agilent 1100 HPLC ion-exchange chromatography method*

Ion-exchange chromatography separates the Hb types according to their charges. Negatively charged cation exchange column was used for the measurement of positively charged Hb in the ion-exchange chromatography. HbA1c analysis was performed by using Agilent 1100 HPLC containing gradient pump system, automatic sampling equipment, automatic injector and UV detector. Gordion Hemoglobin A1c (Lot:

01-0213 Ref: 60400) HbA1c reagents and ClinRep (Lot: 1110913 Ref: 11130) HbA1c analytical columns were used for the analysis. Column temperature was kept constant at 28°C by the help of column oven. During the sample preparation step, Gordion haemolysis reagent was used to prepare the lysates, primarily from erythrocytes. A volume of 5 µl of whole blood sample containing EDTA was thoroughly mixed with 1000 µl of haemolysis reagent. This mixture was incubated at 37°C for 20 min and immediately cooled, and 10 µl of each sample was injected through the system. Gordion Buffer A and Gordion Buffer B, as two different eluents, were used for the mobile phase. The flow rate was adjusted at 2 ml/min. It was adjusted such that 88% of the mobile phase passed through Buffer A in the first 30 s and then the remaining 12% passed through Buffer B. After the 30<sup>th</sup> second till the 45<sup>th</sup> second, 78.4% passed through Buffer A and the remaining 21.6% passed through from Buffer B. The concentration of Buffer B was increased to 100% in the 46<sup>th</sup> second, and this concentration was kept stable till the 75<sup>th</sup> second. Then, after the 76<sup>th</sup> second till 1.25 min, it was kept constant as 100% passed through Buffer A and 12% passed through Buffer B. After 1.25 min, it was adjusted such that 88% of the mobile phase passed through Buffer A and the remaining 12% passed through Buffer B. The total time taken for each sample was 2 min. The retention time at a flow rate of 2 ml/min for HbA1c was determined as  $0.79 \pm 0.03$  min and for HbA0 it was determined as  $1.3 \pm 0.05$  min. The absorbances of the eluent that reached the UV detector were measured at 415 nm wave length, and then the glycosylated Hb was expressed as the percentage of total Hb.

### Study design

The precision study was performed by using CLSI EP 5A protocol, and the EP6-A Protocol Linearity and recovery studies, Protocol EP9-A Systematic Errors and Method Comparison studies were also performed.

### Imprecision

For the precision study performed by CLSI EP 5A protocol, Bio-Rad Diabetes Control samples were used (Normal control (Level 1 Lot: 33 841) and pathological control (Level 2 Lot: 33 842)). These control samples were divided into 21 portions, and the first portion was used for the pre-precision study and the remaining

portions were stored at -20°C for the analyses. The control samples, which were prepared for the simple precision study, were repeated successively 10 times for the same study. Other samples were analysed after thawing of samples at room temperature for 30 min, and they were run for certain times (each day, two times in the morning and two times in the afternoon). This was repeated for 20 days at the same times.

Minimum allowable total errors according to biological variation data were (TEa) 4.5%, CV 1.4% and bias 2.2% in all steps of the process.<sup>19</sup>

### Linearity and recovery study

Bio-Rad and BIO-RAD HbA1c Linearity Set (Level 1 Lot: 34651, Level 2 Lot: 34652, Level 3 Lot: 34653, Level 4 Lot: 34654), which had four different levels of HbA1c, were used for the CLSI EP6-A Linearity study. Each sample was run four times, and the mean of the results was used.

Recovery study was performed by calculation of the results obtained from the linearity study via running the results in the EP Evaluator Release 8 Program.

### Method comparison study

For HbA1c measurement, Premier Hb9210 that used boronate affinity chromatography and Agilent 1100 HPLC that used ion-exchange chromatography were compared with each other. The Gordion kits (Gordion Diagnostic Ltd. Turkey) were used in Agilent 1100 HPLC whereas the original kits (Premier Hb9210, Trinity Biotech USA) were used in Premier Hb9210.

All samples were run on the same day and in both instruments. The samples were selected from the newly arrived ones as they did not exceed an average of eight patients in each group, and they were run every day. The samples were run twice using the two techniques. In order to perform HbA1c measurements, venous blood samples were collected in EDTA tubes from individuals in each group. The two methods were compared with each other between the patient groups.

### Inclusion criteria of patient groups

Clinical & Laboratory Standards Institute (CLSI) Guideline recommendations were implemented for the present study. Approved guideline CLSI EP9 for method comparison studies recommends using at least 40 patient samples.<sup>20</sup> In the present study, after defining the inclusion criteria for each

group, patients were randomly selected such that at least 40 patients were included in each group from our study population.

*Group 1:* A total of 140 patients were included in the study, who had haemoglobin (Hb) >13 g/dl in males, and >12 g/dl in females, HbF <5%, urea <48 mg/dl in males and females, creatinine <1.3 mg/dl in males and <1.1 mg/dl in females, without haemoglobin variant and chronic disease except diabetes.<sup>21</sup>

*Group 2:* A total of 84 patients were included in the study who were without anaemia, without haemoglobin variant, had HbF <5%, with urea >48 mg/dl in males and females, creatinine >1.3 mg/dl in males and >1.1 mg/dl females and who had kidney disease according to the test results performed at least 1 month later.

*Group 3:* A total of 44 patients were included in the study who had normal urea and creatinine, without haemoglobin variant, HbF <5%, haemolytic anaemia, anaemia of chronic disease and with haemoglobin (Hb) <13 g/dl in males and <12 g/dl in females, with MCV <80 fL, MCH <26 pg/cell, with ferritin <29 ng/ml in males and <10 ng/ml in females.<sup>5,22</sup>

*Group 4:* A total of 52 patients with normal urea and creatinine, without haemoglobin variant, HbF < 5% and with haemoglobin (Hb) >16.5 g/dl, were included in the study.

While creating the patient groups, tests for glucose, urea and creatinine were performed in the ADVIA 2400 Chemistry Analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA); ferritin levels were measured in ADVIA Centaur XP (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA); whole blood test was performed in Coulter LH 780 Hematology Analyzer (Beckman Coulter, Fullerton, CA, USA) and haemoglobin variants (HbF, HbS, HbC, HbE) were measured in Tosoh Automated

Glycohemoglobin Analyzer at G8 (Tosoh Corporation, Tokyo, Japan).

The findings were evaluated by using EP Evaluator Release 8 CLSI EP9 program.

*Statistical analyses*

Statistical analyses were performed by EP-Evaluator Release 8, Data Innovations LLC, and Microsoft Excel programs.

**RESULTS**

*Imprecision*

In this study, the values of within run, between run, between day and total CV% values were calculated with high- and low-level control samples in both Premier Hb9210 HbA1c analyser and Agilent 1100 (IEX-HPLC) Table 1. Target total CV% values accepted for the IFCC (SI unit mmol/mol) and the NGSP (%) imprecision are recommended as <3% and <2.0%, respectively.<sup>23</sup> In our study, the total CV% values were <2.0%, as recommended.

*Linearity*

Four different HbA1c standards were used (varying between 4.4% and 14.6%) in the linearity experiment performed by using Premier Hb9210 HbA1c analyser, and it was found that the system was linear between 4.30% and 14.27%. Besides, four different HbA1c standards were used (varying between 4.6% and 15.1%) in the linearity experiment performed by using Agilent 1100 (IEX-HPLC), and it was found that the system was linear between 4.50% and 14.55% Table 2.

*Recovery*

The recovery rates of HbA1c measurements performed by using Premier Hb9210 (boronate

**TABLE 1: Precision study results**

	Within Run CV%	Between Run CV%	Between Day CV%	Total CV%
<b>Premier Hb9210</b>				
Low % 5.1	1.1	0.0	1.1	1.5
High % 9.50	0.5	0.8	0.6	1.2
<b>Agilent 1100</b>				
Low % 5.28	0.8	0.9	1.0	1.6
High % 9.55	0.6	0.6	0.8	1.1

TABLE 2: Linearity and recovery study results

Sample	Premier Hb9210			Agilent 1100		
	Assigned	Mean	% Recovery	Assigned	Mean	% Recovery
SET 1	4.6	4.30	97.7	4.6	4.50	97.8
SET 2	6.1	5.98	98.0	6.4	6.28	98.0
SET 3	9.7	9.50	97.9	10.0	9.80	98.0
SET 4	14.6	14.27	97.8	15.1	14.55	96.4

affinity) at 14.6% concentrations were between 97.7% and 98%. The recovery rates of HbA1c measurements performed by using Agilent 1100-Gordian Kit (IEX-HPLC) at 4.6%–10% concentrations were between 97.7% and 98%, whereas the recovery rate at 15.6% concentration was found 96.4% and it exceeded the acceptable limit Table 2.

#### Comparison of methods

Based on the experiment in which the techniques were compared in Group 1, Group 2, Group 3 and Group 4, there was an excellent correlation between the two methods ( $R > 0.98$ ) Table 3. (Fig. 1).

Moderate–low correlation was found between increased urea concentration and the difference of the two methods ( $R = -0.374$ ,  $p = 0.0005$ ). It was observed that as the urea concentration increased, the difference between the two techniques also increased. The difference was statistically significant and was within the acceptable limits. There was a non-significant correlation between high and low Hb concentrations in the two methods (Fig. 2).

#### DISCUSSION

HbA1c measurement method should have adequate repeatability and accuracy since it is used in the long-term follow-up of HbA1c in diabetes management.<sup>9</sup> HbA1c analyser has a critical importance in order to obtain accurate results. Any new method that is commercially available should at least give the same or intermediate performance compared to currently available methods.<sup>24</sup>

This is the first study in which Gordian HbA1c kit is used. Furthermore, HbA1c measurement is also performed for the first time by two different methods in patients with IDA and high haemoglobin. In this study, the performance of Agilent 1100 (Gordian Diagnostic) IEX-HPLC was compared with Premier Hb9210 HbA1c boronate affinity method. We have shown that Agilent 1100 (IEX-HPLC) HbA1c technique meets the clinical requirements and it is a suitable method for HbA1c measurements in normal diabetic and non-diabetic, uremic diabetic and non-diabetic, anaemic diabetic and non-diabetic and diabetic and non-diabetic patients with high Hb levels.

TABLE 3: EP-9. Comparison of methods

	Number of Patients	Slope	Intercept	Deming	
				Std. Error Estimation	R
Group 1	140	1.012 (1.000 to 1.024)	-0.137 (-0.227 to -0.047)	0.217	0.9950
Group 2	84	0.982 (0.955 to 1.008)	0.44 (0.25 to 0.62)	0.30	0.9849
Group 3	44	0.982 (0.946 to 1.017)	0.33 (0.10 to 0.55)	0.25	0.9861
Group 4	52	1.001 (0.979 to 1.022)	-0.17 (-0.34 to 0.00)	0.26	0.9941

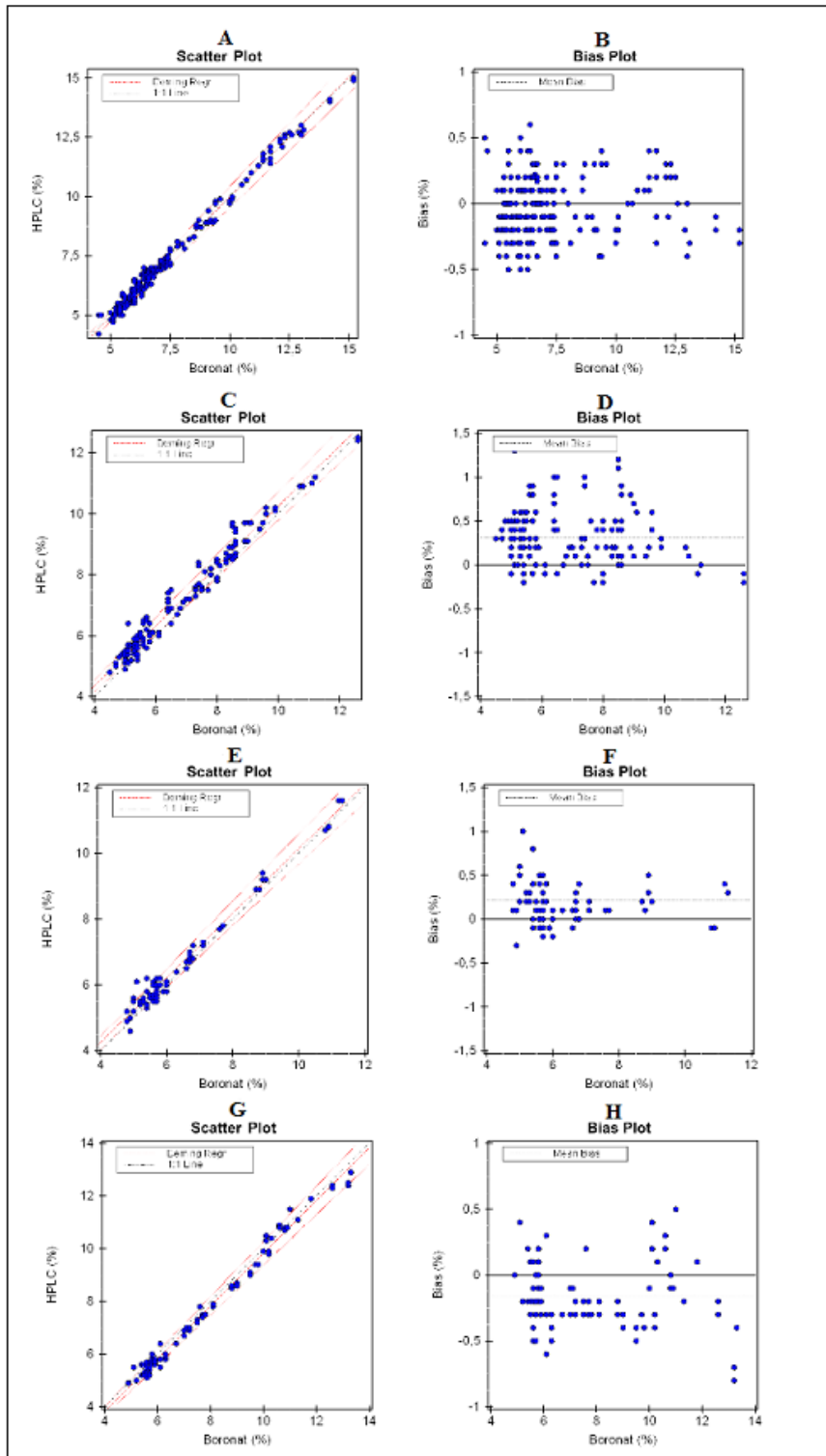


FIG. 1: Distribution of HbA1c results between Premier Hb9210 and Agilent 1100 devices in all groups. (A) Group 1 Distribution of HbA1c results between boronate affinity method and IEX–HPLC technique. (B) % bias according to boronate affinity method. (C) Group 2 Distribution of HbA1c results between boronate affinity method and IEX–HPLC technique, (D) % bias according to boronate affinity method. (E) Group 3 Distribution of HbA1c results between boronate affinity method and IEX–HPLC technique. (F) % bias according to boronate affinity method. (G) Group 4 Distribution of HbA1c results between boronate affinity method and IEX–HPLC technique, (H) % bias according to boronate affinity method.

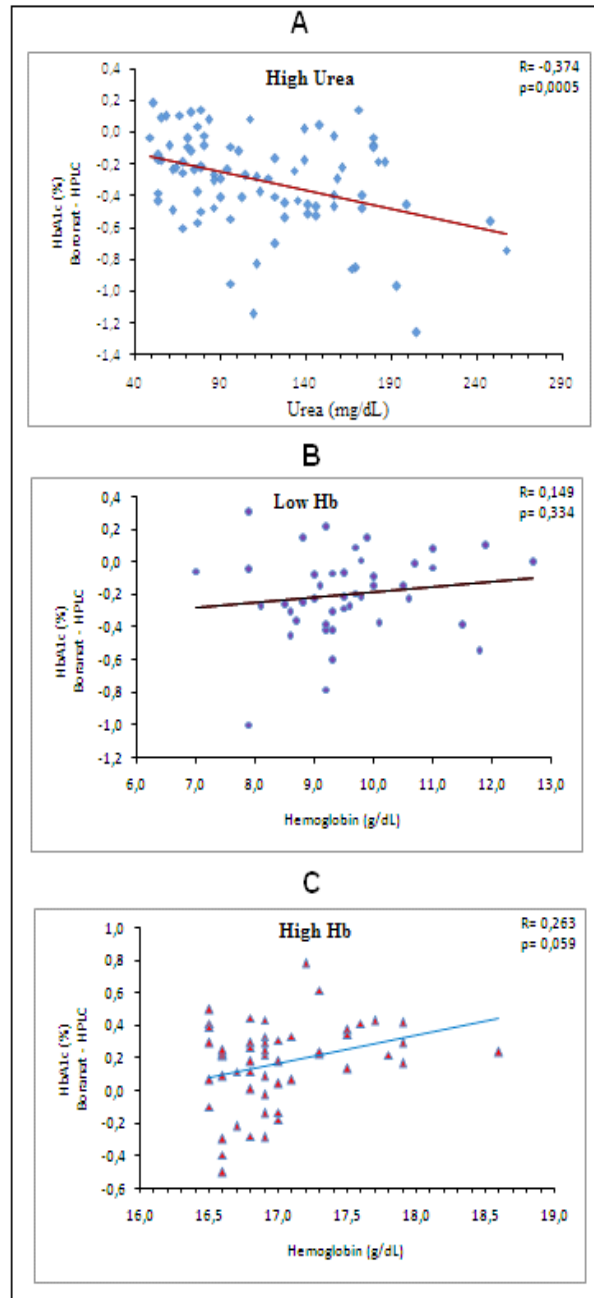


FIG. 2: Distribution graphs of increased urea as well as increased and decreased haemoglobin concentrations between boronate affinity method and IEX-HPLC technique. (A) Distribution graph of increased urea concentrations between boronate affinity method and IEX-HPLC technique. (B) Distribution graph of decreased haemoglobin concentrations between boronate affinity method and IEX-HPLC technique. (C) Distribution graph of increased haemoglobin concentrations between boronate affinity method and IEX-HPLC technique.

The maximum total CV% value was found to be 1.6 for the two instruments, according to the reproducibility studies. John *et al.* performed validation studies by using Hb9210 HbA1c analyser in different countries in five different

laboratories (two of them were reference laboratories). They found that the total CV% value was 1.68 in all laboratories.<sup>25</sup> Even though the CV% value of Hb9210 HbA1c analyser used in our study was slightly lower compared to

the CV% value obtained by John *et al*, it was similar to the CV% value that was reported by John *et al*. In our study, the total CV% values for both instruments were found to be <2%, as recommended by the NGSP.

Premier Hb9210 HbA1c analyser was linear between 4.30% and 14.27% and Agilent 1100 was linear between 4.50% and 14.55%. Similar to our study, John *et al* indicated that the system was linear between 5.08% and 13.68% by using Premier Hb9210 HbA1 analyser.<sup>25</sup>

The recovery did not exceed the allowable limits in Premier Hb9210 HbA1c analyser, and it was valid. However, only one value exceeded the acceptable limit when the recovery study was performed using Agilent 1100 (IEX-HPLC). The recovery results of validity studies performed by John *et al* by using Premier Hb9210 HbA1c analyser were similar to our results that were obtained by our Premier Hb9210 HbA1c analyser.<sup>25</sup>

There was a high correlation between the two methods according to the method comparison study performed for the non-diabetic and diabetic patient group with normal urea and haemoglobin levels. There are limited number of studies in which Premier Hb9210 HbA1c analyser was compared with other methods.<sup>2,25</sup> One of these studies is the one performed by John *et al* in which they compared Premier Hb9210 HbA1c analyser with Trinity Ultra 2 (boronate affinity), Tosoh G8 (IEX-HPLC) and Roche Tina-quant Gen2 HbA1c Integra 800 (immunoassay) in five laboratories (two of them were reference laboratories). They showed that Premier Hb9210 has a good correlation with routine methods and the IFCC reference method procedure.<sup>25</sup> Our comparison results were similar to the results of John *et al*.

It has been accepted that high urea levels interfere with ionexchange-HPLC method and HbA1c measurement, whereas they do not affect boronate affinity and HbA1c measurement. This interference occurs due to high urea concentrations and CarbHb present in the blood circulation.<sup>26-28</sup> There was a high correlation between the two methods in a study performed with non-diabetic and diabetic patient group with high urea. It has been shown that the difference between the boronate affinity method and IEX-HPLC method increased as the urea concentrations increased. This difference was statistically significant. However, this difference was within the acceptable limits and clinically non-significant.

It was reported that CarbHb interfered with IEX-HPLC methods, whereas it did not affect the affinity chromatography method.<sup>26,28-30</sup>

Studies conducted in 2013 stated that CarbHb did not have a clinical effect on HbA1c measurement. Little *et al* compared HbA1c measurements of chronic renal failure patients using a total of 11 instruments. Eight of these were based on the IEX-HPLC method, two were based on the immunoassay method and one was based on the enzymatic method. The comparison results showed that there was a statistically significant but small difference between five of them. The authors specified that this difference was not clinically significant and chronic renal failure and CarbHb levels did not lead to interference with these methods in HbA1c measurement.<sup>31</sup> Li *et al* compared between three different IEX-HPLC methods and one boronate affinity method. They did not show a statistically significant difference between the four instruments in the non-diabetic and diabetic groups.<sup>32</sup> John *et al* used Premier Hb9210 HbA1c analyser and they stated that 0.2%–12% CarbHb concentrations did not affect HbA1c measurement.<sup>25</sup> Our results are similar to the findings of other studies and reveal that CarbHb does not affect HbA1c measurement clinically.

IDA is the most frequently observed anaemia and one of the interference reasons that can limit its use in diagnosis and treatment.<sup>33</sup> In this study, whether IDA leads to a difference between boronate affinity method and IEX-HPLC method was examined. The method comparison study was performed in non-diabetic and diabetic patient groups with IDA using the two methods. According to our results, there was a high correlation between the two methods. The difference between the two methods was not statistically significant as the haemoglobin concentrations decreased. It was observed that IDA did not lead to any difference between the two methods. There are studies performed in which the relationship between IDA and HbA1c was evaluated. HbA1c values of patients with/without IDA were compared with the HbA1c values of patients with IDA before and after therapy. Various HbA1c measurement methods were used in these studies. However, there is no such study as ours that has been performed to examine whether IDA leads to significant difference between the two HbA1c measurement methods. In some of these studies, it has been reported that IDA interfered



with HbA1c measurements performed using different methods.<sup>5,10,34-38</sup> However, it has also been specified that IDA did not affect HbA1c measurement.<sup>11,39-43</sup>

Such contradiction between studies can be due to the severity and duration of IDA, the type and duration of diabetes and other different features. Furthermore, the HbA1c levels before and after treatment can be different due to the laboratory conditions. Results can also be affected by different methods used for HbA1c measurement.

There is only one study in which whether high Hb concentrations influence HbA1c measurement was assessed.<sup>43</sup> We concluded that high concentrations of Hb did not lead to a significant difference between the results of the two methods. Thus, we compared the two methods in the non-diabetic and diabetic patient group. There was a high correlation but non-significant difference between the two methods. Even though different from our study, the only study that examined the effect of high Hb concentrations on HbA1c measurement was performed by Ford *et al.* They showed that there was a positive and significant correlation between Hb and HbA1c. When Hb was >17 g/dl, there was a significant increment in HbA1c measurements.<sup>43</sup> There is no other study that shows that high Hb concentrations lead to different HbA1c measurements. According to our results, high Hb levels do not cause any difference in results among the two different methods.

The performance of the kit of Agilent 1100 IEX-HPLC produced by Gordion Diagnostics in Turkey was compared to the kits of Premier Hb9210 HbA1c analyser (boronate affinity) method. These kits can be valid according to the NGSP and IFCC guidelines, are well correlated with frequently used and routine methods, are not influenced with haemoglobin variants and CarbHb and have a good performance. The Gordion HbA1c kit complies with the clinical needs and it is convenient to be used in patients with CarbHb, IDA and high hemoglobin that are believed to have negative effects on HbA1c measurements.

#### Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

#### REFERENCES

- Aslan D, Can Yilmazturk G, Akalin N, Akif Buyukbese M, Sermez Y, Fenkci S. [Global and national harmonization and standardization of clinical laboratory testings: The example of HbA1c]. *Turk J Biochem.* 2011; 36: 374-83.
- Genc S, Omer B, Aycan-Ustyol E, Ince N, Bal F, Gurdol F. Evaluation of turbidimetric inhibition immunoassay (TINIA) and HPLC methods for glycated haemoglobin determination. *J Clin Lab Anal.* 2012; 26: 481-5.
- John WG, Mosca A, Weykamp C, Goodall I. HbA1c standardisation: history, science and politics. *Clin Biochem Rev.* 2007; 28: 163-8.
- Gillery P. A history of HbA1c through Clinical Chemistry and Laboratory Medicine. *Clin Chem Lab Med.* 2013; 51: 65-74.
- Sinha N, Mishra TK, Singh T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. *Ann Lab Med.* 2012; 32: 17-22.
- Thevarajah TM, Nani N, Chew YY. Performance evaluation of the Arkray Adams HA-8160 HbA1c analyser. *Malays J Pathol.* 2008; 30: 81-6.
- Lamb E, Dawnay A. Glycated haemoglobin measurement in uraemic patients. *Ann Clin Biochem.* 1992; 29: 118-20.
- Kwan JT, Carr EC, Bending MR, Barron JL. Determination of carbamylated hemoglobin by high-performance liquid chromatography. *Clin Chem.* 1990; 36: 607-10.
- Christy AL, Manjrekar PA, Babu RP, Hegde A, Rukmini MS. Influence of iron deficiency anemia on hemoglobin A1c levels in diabetic individuals with controlled plasma glucose levels. *Iran Biomed J.* 2014; 18: 88-92.
- Tarim O, Küçükerdo an A, Günay U, Eralp O, Ercan I. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. *Pediatr Int.* 1999; 41: 357-62.
- Rafat D, Rabbani TK, Ahmad J, Ansari MA. Influence of iron metabolism indices on HbA1c in non-diabetic pregnant women with and without iron-deficiency anemia: effect of iron supplementation. *Diabetes Metab Syndr.* 2012; 6: 102-5.
- Bodor GS, Little RR, Garrett N, Brown W, Goldstein DE, Nahm MH. Standardization of glycohemoglobin determinations in the clinical laboratory: three years of experience. *Clin Chem.* 1992; 38: 2414-8.
- Wettre S, von Schenck H. Batch to batch imprecision in the affinity chromatography assay of glycated hemoglobin. *Clin Biochem.* 1986; 19: 364-6.
- Bruns DE. Standardization, calibration, and the care of diabetic patients. *Clin Chem.* 1992; 38: 2363-4.
- Little RR, Wiedmeyer HM, England JD, *et al.* Interlaboratory standardization of measurements of glycohemoglobins. *Clin Chem.* 1992; 38: 2472-8.
- Goodall I. HbA1c standardisation destination--global IFCC Standardisation. How, why, where and when--a tortuous pathway from kit manufacturers, via inter-laboratory lyophilized and whole blood comparisons to designated national comparison schemes. *Clin Biochem Rev.* 2005; 26: 5-19.

17. Holmes EW, Er ahin C, Augustine GJ, *et al.* Analytic bias among certified methods for the measurement of hemoglobin A1c: a cause for concern? *Am J Clin Pathol.* 2008; 129: 540-7.
18. Premier Hb9210™ with Affinity Software™ Analytical System For Glycated Hemoglobin Operator's Manual (v3). Ireland: Trinity Biotech; 2011. p. 3-4 – 3-6
19. Westgard J. Tools, Technologies and Training for Healthcare Laboratories [Internet]. 2009. Available from: <https://www.westgard.com/minimum-biodatabase1.htm>.
20. CLSI. Method Comparison and Bias Estimation using Patient Samples; Approved Guideline. CLSI document EP9-A. Wayne, PA: Clinical and Laboratory Standards Institute; 1995.
21. Lee KF, Szeto YT, Benzie IF. Glycohaemoglobin measurement: methodological differences in relation to interference by urea. *Acta Diabetol.* 2002; 39: 35-9.
22. Rastogi T, Mathers C. Global burden of iron deficiency anaemia in the year 2000 [Internet]. 2002 [updated 2007 April]. Available from: [http://www.who.int/entity/healthinfo/statistics/bod\\_irondeficiencyanaemia.pdf](http://www.who.int/entity/healthinfo/statistics/bod_irondeficiencyanaemia.pdf).
23. Weykamp CW, Mosca A, Gillery P, Panteghini M. The analytical goals for hemoglobin A(1c) measurement in IFCC units and National Glycohemoglobin Standardization Program Units are different. *Clin Chem.* 2011; 57: 1204-6.
24. Chapelle JP, Teixeira J, Maisin D, *et al.* Multicentre evaluation of the Tosoh HbA1c G8 analyser. *Clin Chem Lab Med.* 2010; 48: 365-71.
25. John WG, Little R, Sacks DB, *et al.* Multicentre evaluation of the Premier Hb9210 HbA1c analyser. *Clin Chem Lab Med.* 2015; 53: 319-27.
26. Weykamp CW, Penders TJ, Siebelder CW, Muskiet FA, van der Slik W. Interference of carbamylated and acetylated hemoglobins in assays of glycohemoglobin by HPLC, electrophoresis, affinity chromatography, and enzyme immunoassay. *Clin Chem.* 1993; 39: 138-42.
27. Weykamp CW, Penders TJ, Muskiet FA, van der Slik W. Effect of calibration on dispersion of glycohemoglobin values determined by 111 laboratories using 21 methods. *Clin Chem.* 1994; 40: 138-44.
28. Weykamp CW, Miedema K, de Haan T, Doelman CJ. Carbamylated hemoglobin interference in glycohemoglobin assays. *Clin Chem.* 1999; 45: 438-40.
29. Weykamp CW, Penders TJ, Muskiet FA, van der Slik W. Influence of hemoglobin variants and derivatives on glycohemoglobin determinations, as investigated by 102 laboratories using 16 methods. *Clin Chem.* 1993; 39: 1717-23.
30. Lee KF, Szeto YT, Benzie IF. Glycohaemoglobin measurement: methodological differences in relation to interference by urea. *Acta Diabetol.* 2002; 39: 35-9.
31. Little RR, Rohlfing CL, Tennill AL, *et al.* Measurement of Hba(1C) in patients with chronic renal failure. *Clin Chim Acta.* 2013; 418: 73-6.
32. Li Q, Ju Y, Jin T, *et al.* Haemoglobin A(1)c measurement in patients with chronic kidney disease. *Clin Biochem.* 2014; 47: 481-4.
33. Rafat D, Rabbani TK, Ahmad J, Ansari MA. Influence of iron metabolism indices on HbA1c in non-diabetic pregnant women with and without iron-deficiency anemia: effect of iron supplementation. *Diabetes Metab Syndr.* 2012; 6: 102-5.
34. Brooks AP, Metcalfe J, Day JL, Edwards MS. Iron deficiency and glycosylated haemoglobin A. *Lancet.* 1980; 2: 141.
35. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. *Acta Haematol.* 2004; 112: 126-8.
36. Davis RE, McCann VJ, Nicol DJ. Influence of iron-deficiency anaemia on the glycosylated haemoglobin level in a patient with diabetes mellitus. *Med J Aust.* 1983; 1: 40-1.
37. Shanthy B, Revathy C, Manjula Devi AJ, Subhashree. Effect of iron deficiency on glycation of haemoglobin in nondiabetics. *J Clin Diagn Res.* 2013; 7: 15-7.
38. Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB. A new look at screening and diagnosing diabetes mellitus. *J Clin Endocrinol Metab.* 2008; 93: 2447-53.
39. Gram-Hansen P, Eriksen J, Mourits-Andersen T, Olesen L. Glycosylated haemoglobin (HbA1c) in iron- and vitamin B12 deficiency. *J Intern Med.* 1990; 227: 133-6.
40. van Heyningen C, Dalton RG. Glycosylated haemoglobin in iron-deficiency anaemia. *Lancet.* 1985; 1: 874.
41. Rai KB, Pattabiraman TN. Glycosylated haemoglobin levels in iron deficiency anaemia. *Indian J Med Res.* 1986; 83: 234-6.
42. Kim C, Bullard KM, Herman WH, Beckles GL. Association between iron deficiency and A1C Levels among adults without diabetes in the National Health and Nutrition Examination Survey, 1999–2006. *Diabetes Care.* 2010; 33: 780-5.
43. Ford ES, Cowie CC, Li C, Handelsman Y, Bloomgarden ZT. Iron-deficiency anemia, non-iron-deficiency anemia and HbA1c among adults in the US. *J Diabetes.* 2011; 3: 67-73.