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Multicentre evaluation of the Premier Hb9210 HbA_{1c} analyser

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Abstract

Background: The accurate and precise quantification of HbA_{1c} is essential for the diagnosis and routine monitoring of patients with diabetes. We report an evaluation of the Trinity Biotech Premier Hb9210 analyser (Bray, Ireland/Kansas City, MO, USA), a boronate affinity chromatography-based high performance liquid chromatography (HPLC) system for the measurement of glycated haemoglobin.

Methods: We evaluated the analytical performance of the Hb9210 as part of a multicentre evaluation. The effect of haemoglobin variants, other potential interferences and the performance in comparison to both the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and National Glycohemoglobin Standardization Program (NGSP) reference systems, was assessed. Most of the centres participating also act as reference laboratories for both the IFCC standardisation network for HbA_{1c} and the NGSP.

Results: The combined data from all centres showed total coefficients of variation (CV) of 2.71%, 2.32% and 2.14% at low, medium and high values, respectively, for mmol/mol (SI units) and 1.62%, 1.59% and 1.68% for % (NGSP units), which are well below the recommended upper limits of 3% CV for mmol/mol (SI units) and 2% CV for % (NGSP). The analyser showed a good correlation to HbA_{1c} methods currently used in clinical practice and the IFCC reference method procedure. Haemoglobin variants AC, AS, AE and AD do not affect the measurement of HbA_{1c}. Overall the Hb9210 performs well across the whole analytical range.

Conclusions: The Hb9210 performs well and is suitable for clinical application in the analysis of HbA_{1c}.

Keywords: affinity chromatography; diabetes; glycated haemoglobin; HbA_{1c}; multicentre evaluation.

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Introduction

With the publication of the World Health Organization (WHO) and American Diabetes Association (ADA) guidelines advocating the use of HbA_{1c} for the diagnosis of diabetes there has been a significant shift in the role of HbA_{1c} testing [1, 2]. However, it still remains the cornerstone for monitoring and managing long-term glycaemic control in patients with type 1 and type 2 diabetes [3, 4]. The move towards using HbA_{1c} for diagnosis has been supported by improvements in the analytical performance of HbA_{1c} methods through advances in technology and the implementation of the IFCC standardisation system for HbA_{1c} measurement and the NGSP [5]. There is also an increasing body of evidence that HbA_{1c} testing may have uses outside of traditional diabetes roles and as such the need for rapid, accurate, precise and cost effective testing is set to increase [6, 7].

The main analytical principles for HbA_{1c} or glycated haemoglobin quantification are based on charge separation, including ion exchange (HPLC) and capillary electrophoresis (CE), or the structural differences between glycated and non-glycated forms of haemoglobin (such as immunoassay and boronate affinity HPLC). Here we present data from a new generation of boronate affinity HPLC analysers, the Premier Hb9210 from Trinity Biotech (Bray, Ireland/Kansas City, USA) in a multicentre trial. The study was co-ordinated by reference laboratories of the

IFCC standardisation network and the National Glycohemoglobin Standardization Program (NGSP).

Affinity separation relies on the specific and reversible binding of cis diol groups on the glucose molecule of glycated haemoglobin to m-aminophenylboronic acid, in gel form, in the column. The haemoglobin with glucose bound binds to the column, while the non-glycated haemoglobin passes straight through. Once all of the non-glycated haemoglobin has been eluted, a counter-ligand is used to compete with the binding sites and the glycated haemoglobin subsequently elutes off the column. The absorbance at 413±2 nm of both fractions can then be measured. Unlike separation relying on ion exchange chromatography, the affinity separation produces only two discrete peaks; a non-glycated fraction and a glycated fraction, from which the ratio of the fractions can be calculated. Following calibration using IFCC traceable manufacturer's calibrators results are reported as HbA_{1c}, calculated as a fraction of total haemoglobin.

This evaluation used Clinical and Laboratory Standards Institute (CLSI) based protocols to determine the suitability of the Hb9210 analyser for routine determination of HbA_{1c} values in the Clinical Laboratory. We paid particular attention to the overall performance of multiple analysers, located in a range of evaluating laboratories, which is not only important in clinical practice but also for comparing results between clinical and research centres.

Materials and methods

Participating centres

This multicentre evaluation was performed across five clinical laboratories located in Europe and USA; Norfolk and Norwich University Hospital, UK (Centre 1), Diabetes Diagnostic Laboratory, University of Missouri, USA (Centre 2), Department of Laboratory Medicine, National Institutes of Health, USA (Centre 3), European Reference Laboratory, located in Queen Beatrix Hospital, The Netherlands (Centre 4) and European Reference Laboratory, located in Isala, The Netherlands (Centre 5).

Characteristics of the analyser

The Premier Hb9210 is an automated benchtop HbA_{1c} analyser using boronate affinity HPLC for measurement of HbA_{1c}. The Premier Hb9210 system was calibrated using a two-point calibration with calibrators provided by the manufacturer and at time intervals prescribed by the manufacturer's instructions. Calibrator values were assigned by the manufacturer using secondary calibration material supplied by the IFCC Reference Laboratory Network as recommended in the consensus statement [8].

The quoted working range of the system is 20–173 mmol/mol [4%–18% (NGSP units)]. The instrument can be preloaded with up to 210 samples. Specimen sampling can be either from primary tubes with barcode ID scanning and cap piercing or haemolysates analysed using offline dilution tubes. Specific racks for whole blood and haemolysates have to be used which are recognised by the instrument, using a barcode on the rack. A sample volume of 10 µL whole blood is used to make a 1:150 haemolysate by automated dilution. If offline pre-diluted samples are to be used, a volume of 0.5 mL is required to make a direct injection. If the total peak area is too small, which is the case when the haematocrit (Hct) level is too low, a result will not be reported and an error flagged. The blood volume should then be manually increased. Sample types accepted include fresh anticoagulated whole blood (EDTA, heparin or potassium oxalate/sodium fluoride are recommended by the manufacturers) or thawed from frozen state (maximum one freeze-thaw cycle).

The column used is a patented boronate affinity column, maintained at 55 °C on the analyser. Haemoglobin fractions are identified with a LED photodiode single wavelength detector at 413±2 nm. Columns for either 500 patient samples or 1000 patient samples are available depending on the throughput required. The 500 and 1000 injection columns have the same length and diameter and are filled with the same boronate affinity resin. The 1000 injection columns undergo an extended conditioning process as part of the production QA process, to ensure a guaranteed number of patient injections can be achieved. This allows what is essentially the same column to support low, medium and high workloads. For this evaluation only columns with a 500 patient sample capacity were used.

The instrument has a single operation mode for the measurement of glycated haemoglobin. The reported result is derived from the ratio of glycated haemoglobin to total haemoglobin and expressed in SI units of mmol/mol, NGSP % units, derived using the master equation [9] or both.

Study design

We used CLSI-based protocols EP-5 and EP-9 to assess reproducibility and trueness, respectively. Linearity, bias and carryover as well as interferences from variant haemoglobins were also assessed.

Imprecision

Assessment of precision was based on CLSI-EP5, and performed in four of the evaluation laboratories (Centres 1–3, 5) using pooled samples with high and low HbA_{1c} values. In addition three of the evaluation laboratories (Centres 1–3) also assessed imprecision at a medium HbA_{1c} value. Sample pools were prepared at the required levels then were divided into several aliquots and frozen at –70 °C. Aliquots were then thawed daily and analysed twice per day in duplicate over a period of 20 working days. Centre 2 performed a repeat precision study after re-calibration of their system using a new batch of calibrators provided by the manufacturer and the CVs at low, medium and high HbA_{1c} levels all improved. There were no extenuating reasons for exclusion of the first set of data; therefore they have been included in the evaluation data.

Trueness

An EP-9 based protocol was used to evaluate trueness and to compare the Hb9210 with other analysers used in routine practice in the participating laboratories (Centres 2 and 5). Fifty samples with HbA_{1c} values ranging from 20 to 115 mmol/mol (4.0%–12.7% NGSP), were used at Centre 2 and 40 samples ranging from 27 to 83 mmol/mol (4.6%–9.7% NGSP) at Centre 5. Samples were analysed in duplicate at each Centre and compared to the values assigned using the comparison methods, detailed below. At Centre 2 the Hb9210 was calibrated with the manufacturer's calibrators and compared to two NGSP network methods which were calibrated to the NGSP. At Centre 5 the Hb9210 was calibrated with the manufacturer's calibrators and compared to three comparison methods each of which were calibrated to IFCC secondary reference material. All data was analysed using Deming regression analysis.

In addition Centres 2–5 each analysed 40 samples distributed by Centre 2 for comparison across the sites. Each centre had aliquots of the same samples in order to allow direct comparison of performance. The values obtained at each site using manufacturers calibrators were then compared to the offline IFCC calibrated values obtained from the Premier at Centre 4. It was apparent that the values for Centre 2 had a significant positive bias compared to the other centres. In line with the repeat precision analyses performed at Centre 2, with new calibrators from the manufacturer, the trueness study was also repeated and the 9210 was compared with the Trinity Ultra[2] (Kansas City, MO, US).

Comparison methods

The Hb9210 was compared to the following methods at Centre 2: NGSP SRL3 – Trinity Ultra[2] (boronate affinity separation) (Kansas City, MO, USA) and NGSP SRL9 – Tosoh G8 (ion exchange chromatography) (Japan). Centre 5: Tosoh G8 (Japan), Trinity Ultra[2] (Bray, Ireland) and Roche Tina-quant Gen2 HbA_{1c} on the Integra 800 (immunoassay) (Rotkreuz, Switzerland).

Linearity and carryover

Linearity of the method was assessed at two centres (Centres 1 and 2). Samples with high and low levels of HbA_{1c} were mixed in incremental amounts to generate a series of samples over a broad concentration range. The theoretical HbA_{1c} value (calculated from the ratios of the mixed packed cells with high and low HbA_{1c} values) and the measured values were compared. It is not possible to dilute samples in buffer and obtain a linear dilution profile due to the fact that HbA_{1c} is expressed as a ratio to total Hb, therefore a dilution would serve only to dilute the hematocrit levels.

To examine the potential effect of specimen to specimen carryover a standard protocol was used (Centre 1). Samples with very high HbA_{1c} were followed by specimens with low values in a prescribed order (L, L, L, H, H, L, H, H, L, L, L, H, H, L, H, H, L, H, H, L). If the results for the high-low sequences are statistically the same as those for the low-low sequences, then there is deemed to be no carryover. The mean of the high-low values should be within three times the SD of the low-low sequence samples.

Interference from haemoglobin variants

Samples with the common haemoglobin variants AC, AS, AD and AE were analysed at two of the centers (Centres 2 and 4) and the values obtained compared with those from a method that is known to not be affected by the individual variants [Comparison method at Centre 2, Trinity Ultra2, Centre 4, Sebia Capillarys 2 Flex Piercing (Lisse, France)] [10, 11]. A difference >±7% was considered clinically significant [12].

Other interferences

To investigate the effects of a range of potential interferences (Centres 1 and 4), samples with increased levels of labile HbA_{1c}, icteric samples (red cells resuspended in plasma containing 356 µmol/L bilirubin) and samples with increased levels of carbamylated haemoglobin were used (in vitro carbamylated red cells). In addition, specimens with a broad range of haemoglobin concentrations (33–221 g/L) were assessed to investigate the effects of haematocrit on HbA_{1c} measurement.

Labile HbA_{1c} samples were prepared as follows; whole blood was incubated with 15, 30, 60 and 120 mmol/L glucose at 37 °C for 4 h. Labile HbA_{1c} was estimated using HPLC (Menarini 8180V, Italy), the Labile HbA_{1c} is separated from HbA_{1c} and other peaks and the percentage value is the percentage of total area as reported by the instrument. Then HbA_{1c} (Premier 9210) and glucose (Roche Cobas 6000) were measured. Samples were stored in the refrigerator and HbA_{1c}/glucose measured again after 1, 2 and 5 days of storage. At a glucose concentration of 30 mmol/L labile HbA_{1c} is around 3.5% [13].

Carbamylated haemoglobin samples were prepared as previously published method [14], briefly; a sample with an HbA_{1c} of 39 mmol/mol (5.7%) and 12% carbamylated haemoglobin (measured with ion exchange chromatography, Menarinin 8180V, carbamylated Hb is separated from HbA_{1c} and other peaks and the percentage value is the percentage of total area as reported by the instrument) was mixed with a sample with an HbA_{1c} of 39 mmol/mol and 0.2% carbamylated haemoglobin, in incremental ratios. The mixtures, which ranged from 0.25% to 12% carbamylated haemoglobin, were measured with the Hb9210.

Samples with a range of haematocrit levels were prepared by separating red cells from the plasma and then mixing increasing amounts with plasma to generate a range of values that may be encountered in clinical practice.

Sample stability

Sample stability was assessed, at Centre 2, and compared with the Trinity Ultra[2] (Kansas City, MO, USA) at a range of temperatures and HbA_{1c} concentrations. Aliquots of five samples with values between 33 and 75 mmol/mol, (5.2% and 9.0%) were stored at temperatures of –70 °C, –20 °C, +4 °C, room temperature (17–23 °C), 30 °C and 37 °C. Samples were analysed in duplicate at designated time points results were considered acceptable if within ±3 SD for all samples stored at –70 °C.

Calibration and controls

Offline calibrations to align results to the IFCC reference system were performed by running calibrators of known values and then

using the assigned values to calibrate the instruments. To perform an offline calibration to IFCC values reference standards code; Berlin 2011.108 were used, values were assigned by all laboratories of the IFCC network. To perform an online calibration to NGSP values in-house pooled whole blood calibrators generated by the Central Primary Reference Laboratory of the NGSP were used.

Evaluation samples

Specimens used in this study for the determination of trueness and imprecision were fresh frozen or fresh whole blood, anticoagulated with EDTA, from either a single donor (trueness) or pooled whole blood (imprecision).

Statistical analyses

Statistical analyses were performed using either EP-Evaluator, Release 9, Data Innovations LLC, or Microsoft Excel.

Results

Imprecision

Table 1 shows the results obtained at four centres (3 out of 4 centres also included a mid-range value for the precision study). As each centre used their own patient samples to perform the analysis, the data have not been pooled. Nevertheless, it is possible to present the mean of the total CVs and also the standard error of the mean (SEM) across all levels.

The values presented show a good imprecision with the majority of total CVs below the recommended values of <3% for SI units and <2% for NGSP units [15–17]. The mean CVs were 2.71%, 2.32% and 2.14% at low, medium and high values, respectively, for mmol/mol (SI units) and 1.62%, 1.59% and 1.68% for NGSP units. The CVs and the SEM values are also detailed within Table 1.

Trueness

The EP-9 based protocol compared 40 samples in duplicate using both the Hb9210 and a comparator method. The methods used for the comparison were either NGSP SRLs or methods which had been offline calibrated with IFCC secondary reference material. In most cases there was a good correlation between the Hb9210 and the comparator methods as demonstrated by the slopes not being significantly different to 1.00 (the comparison with the Tosoh G8 at Centre 5 was borderline) but the results showed a

Table 1 Overview of total CVs from all centres in both SI and NGSP units[a].

Centre number	SI (IFCC) units, mmol/mol							NGSP units, %						
	1	2 – 1st study	2 – 2nd study	3	5	Mean CV for all centres	SEM for all centres	1	2 – 1st study	2 – 2nd study	3	5	Mean CV for all centres	SEM for all centres
Mean low HbA_{1c} value	37.5	34.0	34.0	35.0	30.8			5.56	5.26	5.27	5.36	4.97		
Total CV (low value)	3.3%	3.4%	2.6%	1.8%	2.4%	**2.71**	0.30	2.0%	2.03%	1.55%	1.1%	1.4%	**1.62**	0.18
Mean mid HbA_{1c} value	49.8	48.5	49.2	46.5	–			6.69	6.59	6.65	6.41	–		
Total CV (mid value)	2.8%	2.6%	2.1%	2.0%	–	**2.37**	0.19	1.9%	1.75%	1.41%	1.3%	–	**1.59**	0.14
Mean high HbA_{1c} value	74.0	77.9	64.4	77.7	86.9			8.89	9.28	8.05	9.26	10.09		
Total CV (high value)	2.2%	2.8%	2.0%	2.1%	1.6%	**2.14**	0.19	1.9%	2.15%	1.45%	1.6%	1.3%	**1.68**	0.15

[a]The data are derived from analysis of samples in duplicate, twice per day over a period of 20 working days at each centre.

significant positive bias (intercept does not overlap zero) when compared to the NGSP SRL3. This difference was not significant when compared to methods which were offline calibrated to IFCC targets and to the NGSP SRL9, when using Deming regression analysis. The method comparison was repeated after recalibration of the Premier instrument in Centre 2 and using the Ultra² (NGSP SRL3) as the comparator method, it can be seen that the correlation was improved and the bias seen in the first set of results was diminished. All results from Centre 2 are detailed in Table 2A and from Centre 5 in Table 2B.

Linearity and carryover

Linearity was determined by two centres and was shown to be good throughout and beyond the quoted working range of the analyser. Table 3 shows the data from Centre 1 and details the deviation in measured values from the theoretical HbA_{1c} values (calculated from the ratios of the mixed packed cells with high and low HbA_{1c} values), which is below the maximum recommended difference of 5% of the expected value.

There was no effect of carryover evident from the analysis of alternate high and low samples of HbA_{1c}. The difference between the means of the high-low and the low-low sequence samples was 0.6 mmol/mol (0.05% NGSP), which is less than three times the SD of the low-low values [1.64 mmol/mol, (0.15% NGSP)].

Interference from haemoglobin variants

Four of the most common heterozygous haemoglobin variants were investigated: AC, AD, AE and AS at two centres. Approximately 10 of each type of heterozygous variant were analysed and the results compared with methods that have been previously shown to have no significant interferences from these variants such as the Trinity Ultra² [10, 18]. The results for each variant are detailed in Table 4; none of the samples showed a clinically significant (>±7%) interference.

Table 2A Results of trueness study[a].

			Deming
	Trinity Ultra² at Centre 2[b]	**Tosoh G8 at Centre 2[b]**	**Trinity Ultra² at Centre 2[c]**
Parameters derived from EP-9 based accuracy/trueness study			
Slope[a]	1.009 (0.993–1.025)	0.995 (0.986–1.022)	0.978 (0.944–1.011)
Intercept[a]	0.284 (0.157–0.412)	0.219 (0.000–0.439)	0.247 (0.0006–0.488)
	Measured	**Measured**	**Measured**
HbA_{1c} level (assigned)			
Low (4.9%)	5.2%	5.1%	5.0%
Medium (7.6%)	8.0%	7.8%	7.7%
High (10.4%)	10.4%	10.6%	10.4%

[a]Based on data from Centre 2 in NGSP units; [b]1st calibration; [c]repeat study using new calibration.

Table 2B Results of Trueness study[a].

			Deming
	Trinity Ultra² at Centre 5	**Tosoh G8 at Centre 5**	**Tinaquant at Centre 5**
Parameters derived from EP-9 based accuracy/trueness study			
Slope[b]	0.998 (0.972–1.024)	0.963 (0.934–0.993)	1.006 (0.972–1.040)
Intercept[b]	−0.98 (−2.38 to 0.42)	−0.33 (−1.96 to 1.31)	−1.30 (−3.14 to 0.54)
	Measured	**Measured**	**Measured**
HbA_{1c} level (assigned)			
Low 30.0 mmol/mol	29.0 mmol/mol	28.6 mmol/mol	28.9 mmol/mol
Medium 60.0 mmol/mol	58.9 mmol/mol	57.5 mmol/mol	59.1 mmol/mol
High 90.0 mmol/mol	88.8 mmol/mol	86.3 mmol/mol	89.2 mmol/mol

[a]Based on data from Centre 5 in SI units; [b]results of trueness study.

Table 3 Results of linearity study[a].

Sample	SI units, mmol/mol			NGSP, % units		
	Observed value	Theoretical value	% difference	Observed value	Theoretical value	% difference
1	32.0	32.0	0.0	5.08	5.08	0.0
2	64.3	63.3	1.58	8.03	7.94	1.13
3	79.7	79.0	0.89	9.44	9.38	0.64
4	95.7	94.7	1.06	10.91	10.82	0.83
5	125.7	126.0	−0.24	13.65	13.68	−0.22

[a]Based on data from a single centre (Centre 1) as a representative example.

Other interferences

Interference study results are shown in Table 4. The results of the incubation of whole blood with increasing concentrations of glucose indicates the production of labile HbA_{1c} (Schiff base) in the samples, which causes some degree of interference at higher concentrations of glucose. At very high glucose levels (60 and 120 mmol/L) measured HbA_{1c} was 2–5 mmol/mol higher (0.2%–0.5% NGSP) than the basic level suggesting a small interference in the measurement. At a glucose concentration of 30 mmol/L the HbA_{1c} would be approximately 1 mmol/mol higher. At 15 mmol/L glucose the effect is <0.5 mmol/mol (<0.1% NGSP). Increasing concentrations of synthetically prepared carbamylated haemoglobin [14] from 0.2% to 12%, as verified using ion exchange chromatography, had no significant effect on the measured value of HbA_{1c}, when using the Hb9210.

Decreased haematocrit levels below 0.306 (Hb 102 g/L) result in the HbA_{1c} value being unreliable, the value will not be reported and an error message will be flagged. At this stage the user should refer to the manufacturer's instructions and perform a manual sample dilution for analysis (this will be a lesser dilution than the automated one) in order to obtain an accurate assessment of HbA_{1c} in the sample. Incubation of red cells samples over a range of HbA_{1c} concentrations with icteric plasma (total bilirubin 356 µmol/L) did not significantly affect the measured HbA_{1c} (<6% difference in value).

Sample stability

Samples are stable at room temperature for 7 days and for ≥56 days at temperatures of 4 °C, –20 °C and –70 °C. This is in accordance with the manufacturer's recommendations which, along with the sample stability study results, are detailed in Table 5. The sample stability for the Hb9210 was comparable with that of the Trinity Ultra[2]. It

Table 4 Table of interferences and effect of haemoglobin variants on HbA_{1c} measurement using the Hb9210.

Potential interferent	Level of interfering substance used	Levels of interference observed
Labile HbA_{1c}	0–120 mmol/L glucose	When glucose is 60–120mmol/L HbA_{1c} is 2–5 mmol/mol (0.2%–0.5% NGSP) higher When glucose is 30 mmol/L HbA_{1c} is approx. 1 mmol/mol (<0.1% NGSP) high
Carbamylated haemoglobin	0.2%–12% carbamylated Hb	No significant effect
Low haematocrit	0.091–0.387 (Hb levels 69–140 g/L)	HCT values below 0.306 are not reliable and manual dilution is required to obtain HbA_{1c} values
Icteric plasma	Total bilirubin 356 µmol/L	−1.7 mmol/mol HbA_{1c} (NS)
Haemoglobin variant	**Level at which interference is deemed significant[a]**	**Level of interference observed (compared to Trinity Ultra[2], affinity method)**
Haemoglobin AC	±7% bias	0%–0.3% (NS)
Haemoglobin AD	±7% bias	−0.2% (NS)
Haemoglobin AE	±7% bias	−1.6% to −1.7% (NS)
Haemoglobin AS	±7% bias	2.6%–2.8% (NS)

NS, not significant. [a]Based on NGSP criteria, current at the time of the evaluation, this has now been reduced to ±6% but all values remain not significant.

Table 5 Table of sample stability.

	Duration of acceptable storage, days		
	Ultra[2]	Premier 9210	Manufacturer recommendations (9210)
37 °C	6 h	1	1
30 °C	2	2	–
Room temperature	9	7	6
4 °C	≥56	≥56	45
−20 °C	≥56	≥56	360

Data from Centre 2.

should be noted, however, that in routine practice not all patient samples are stable over this time period at temperatures above –70 °C. Therefore, if long-term sample storage is anticipated this should be at –70 °C.

Discussion

With the rapidly increasing global burden of diabetes and the move towards HbA_{1c} for its diagnosis, the need for precise HbA_{1c} measurement, free from bias, is essential. The concerted efforts of the IFCC HbA_{1c} standardisation programme in partnership with the NGSP and the continual improvements made by manufacturers has resulted in the performance characteristics of new analysers being continually improved. The data presented here are the evaluation of a new generation of dedicated affinity chromatography HbA_{1c} analyser. The evaluation was performed across several geographically distant sites, each using a separate instrument. The majority of sites were members of the IFCC and/or NGSP reference system networks. The use of several participating centres enables the simulation of the performance of the analysers across a network of laboratories rather than in isolated institutes. With the growing move towards integrated laboratory networks providing health services for groups of hospitals this evaluation demonstrates the suitability of the Hb9210 for use across multisite networks providing stringent quality assurance procedures are in place.

The Hb9210 shows good imprecision across all sites and generally the CVs are below the upper recommended levels of <3% CV (SI units) or <2% CV (NGSP % units). The combined data from all centres showed mean total CVs of 2.71%, 2.32% and 2.14% at low, medium and high values, respectively, for mmol/mol (SI units) and 1.62%, 1.59% and 1.68% for % (NGSP units) [15, 17].

During the course of the evaluation it was noted that there was an apparent small drift in the QC values over time, when there was a low throughput of patient samples and a long calibration interval. On discussion with the manufacturer calibration intervals that are appropriate for both the sample number and the intervals between sample analyses were detailed. It is important to adhere to these calibration intervals, that are derived based on the column size and the anticipated throughput of the column, otherwise may occur. If low volumes of samples are anticipated then more frequent calibration is required, however, additional calibration measurements are not included in the column total and do not count as patient samples in column usage so will not reduce the patient sample numbers that can be processed. The manufacturer provides calibration intervals for each of the 500 sample and 1000 sample columns, based on anticipated usage, these times need to be adhered to in order to prevent the apparent drift in QC values over time. Some drift is an acknowledged historical property of affinity methods however this can be negated by using the advised calibration intervals.

The trueness study demonstrated excellent correlation with IFCC/NGSP Secondary Reference Methods; however, one Centre had to recalibrate the Premier Hb9210 with a new calibration lot before adequate performance was seen. This demonstrates that the Hb9210 is comparable with common routine methods currently available. It is important to note that the instruments were supplied with manufacturer's calibrators with different lot numbers which will lead to some variation in comparison results; but this reflects routine practice. It is imperative that manufacturers use stringent criteria for their calibrator value assignments; manufacturers must assign calibrator values using secondary calibrators available from the IFCC Laboratory Network. This manufacturer complies with this requirement. Nonetheless, whenever possible, laboratories should independently verify their calibration whenever instituting/changing calibrator lots to ensure consistency of results.

The method showed excellent linearity over the full quoted working range of the assay [20–173 mmol/mol (4%–18%)] with no evidence of carryover of high HbA_{1c} samples.

The presence of haemoglobin variants is an important issue with up to 7% of the world's population being heterozygous carriers of haemoglobin disorders [19]. The common heterozygous haemoglobin variants AC, AD, AE and AS showed no clinically significant interference (>±7%) with the Premier Hb9210 method. However, even though the measured HbA_{1c} is not affected by the presence of these variants, affinity chromatography methods

such as the Premier Hb9210 do not indicate the presence of haemoglobin variants on the chromatograph and thus a variant will go unnoticed when using this method for the determination of HbA_{1c} levels. The same is true for immunoassay and enzymatic methods.

Glycation can occur at some 15 different locations on the haemoglobin chains. The most prevalent position is the N-terminal valine of the β-chain (forming HbA_{1c}, approx. 60% of total glycated haemoglobin).

Haemoglobin F is missing the β-chain and thus the prevalent position for glycation. Due to this total glycated haemoglobin in haemoglobin F is expected to be (much) lower than in haemoglobin A. The authors have conducted experiments to estimate the degree of glycation of HbF and found that the glycation rate is approximately one third that of HbA. Work is in progress to confirm this finding and publish it. The impact of this lower glycation rate is that in samples with HbF, the measured analyte (total glycohaemoglobin) and the recalculated HbA_{1c} will be lower than when measured with methods measuring true HbA_{1c}.

The presence of renal disease in patients with diabetes is not uncommon and high levels of uraemia have previously been shown to generate carbamylated haemoglobin, which caused significant interference in older generations of assays [20]. There was no evidence of interference in the Hb9210 from carbamylated haemoglobin. However there was some evidence of interference with high levels of labile HbA_{1c}, formed from high glucose concentrations above 30 mmol/L. Note that glucose concentrations >30 mmol/L are uncommon in patients and will rarely be present when HbA_{1c} is measured.

The data presented are unique in that the evaluation was performed by laboratories of the IFCC and NGSP reference systems and also across multiple sites. Overall this multicentre evaluation has shown that the Hb9210 performs at a high standard with respect to analytical precision as demonstrated in the mean CV values <3% and 2% using IFCC (mmol/mol) or NGSP (%) HbA_{1c} units, respectively. Common interferences, including those from common Hb variants, do not affect this method.

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