UNIVERSITY OF BIRMINGHAM

Research at Birmingham

Comparison of IFCC-calibrated HbA1c from laboratory and point of care testing systems

Manley, Susan; Hikin, Laura J.; Round, Rachel A.; Manning, Peter W.; Luzio, Stephen D.; Dunseath, Gareth J.; Nightingale, Peter; Stratton, Irene M.; Cramb, Robert; Sikaris, Kenneth A.; Gough, Stephen; Webber, Jonathan

Document Version
Peer reviewed version

Link to publication on Research at Birmingham portal

Accepted Manuscript

Title: Comparison of IFCC-calibrated HbA1c from Laboratory and Point of Care Testing Sy<!–<query id="Q1">Please provide a reduced form of the main title that doesn't exceed 80 characters.</query>–><!–<RunningTitle>Comparison of IFCC-calibrated HbA$_{1c}$ from Laboratory and Point of Care Testing Sy</RunningTitle>–>stems
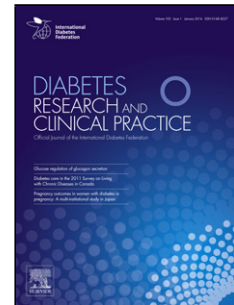
Author: Susan E. Manley Laura J. Hikin Rachel A. Round Peter W. Manning Stephen D. Luzio Gareth J. Dunseath Peter G. Nightingale Irene M. Stratton Robert Cramb Kenneth A. Sikaris Stephen C.L. Gough Jonathan Webber

Please cite this article as: S.E. Manley, L.J. Hikin, R.A. Round, P.W. Manning, S.D. Luzio, G.J. Dunseath, P.G. Nightingale, I.M. Stratton, R. Cramb, K.A. Sikaris, S.C.L. Gough, J. Webber, Comparison of IFCC-calibrated HbA$_{1c}$ from Laboratory and Point of Care Testing Systems, *Diabetes Research and Clinical Practice* (2014), http://dx.doi.org/10.1016/j.diabres.2014.05.003

Comparison of IFCC-calibrated HbA$_{1c}$ from Laboratory and Point of Care Testing Systems

Running Title: Comparing Laboratory and Point of Care HbA$_{1c}$

Susan E. Manley PhD, FRCPath,[1,2] Laura J. Hikin MSc,[1] Rachel A. Round MSc,[1] Peter W. Manning MSc,[1] Stephen D. Luzio PhD, FRCPath,[3] Gareth J Dunseath M Phil,[3] Peter G. Nightingale PhD,[4] Irene M. Stratton MSc,[5] Robert Cramb MSc, FRCPath,[1,2] Kenneth A. Sikaris MBBS, FRCPathA,[6] Stephen C.L. Gough MD, FRCP,[7] Jonathan Webber DM, FRCP,[8]

[1]Clinical Biochemistry, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK,

[2]Divisional of Medical Sciences, University of Birmingham, Birmingham, UK,

[3]Diabetes Research Group, Swansea University, Swansea, UK,

[4]Wellcome Trust Clinical Research Facility, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK,

[5]Gloucester Diabetic Retinopathy Research Group, Gloucestershire Hospitals NHS Foundation Trust, Cheltenham, UK,

[6]Melbourne Pathology, Melbourne, Australia,

[7]Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK,

[8]Diabetes Centre, University Hospitals Birmingham NHS Foundation Trust, Birmingham. UK.

Corresponding author:

Dr Sue Manley,

Office 2, Level -1,

Clinical Laboratory Services,

Clinical Biochemistry - University Hospitals Birmingham NHS Foundation Trust,

Queen Elizabeth Hospital Birmingham,

Mindelsohn Way,

Edgbaston,

Birmingham,

B15 2WB

United Kingdom

Tel: #44(0) 121 371 5991

Fax: #44(0) 121 371 5974

E-mail: susan.manley@uhb.nhs.uk

ABSTRACT

OBJECTIVE— WHO, IDF and ADA recommend HbA_{1c} ≥6.5% (48 mmol/mol) for diagnosis of diabetes with pre-diabetes 6.0% (42 mmol/mol) [WHO] or 5.7% (39 mmol/mol) [ADA] to 6.4% (47 mmol/mol). We have compared HbA_{1c} from several methods for research relating glycaemic markers.

RESEARCH DESIGN AND METHODS—HbA_{1c} was measured in EDTA blood from 128 patients with diabetes on IE HPLC analysers (Bio-Rad Variant II NU, Menarini HA8160 and Tosoh G8), point of care systems, POCT, (A1cNow+™ disposable cartridges and DCA $2000^{®}$+ analyser), affinity chromatography (Primus Ultra2) and the IFCC secondary reference method (Menarini HA8160 calibrated using IFCC SRM protocol).

RESULTS— Median (IQ range) on IFCC SRM was 7.5%(6.8 to 8.4) (58(51 to 68) mmol/mol) HbA_{1c} with minimum 5.3%(34 mmol/mol)/maximum 11.9%(107 mmol/mol). There were - positive offsets between IFCC SRM and Bio-Rad Variant II NU, mean difference (1SD), +0.33%(0.17) (+3.6(1.9) mmol/mol), r^2=0.984, p<0.001 and Tosoh G8, +0.22%(0.20) (2.4(2.2) mmol/mol), r^2=0.976, p<0.001 with a very small negative difference -0.04%(0.11) (-0.4(1.2) mmol/mol), r^2=0.992, p<0.001 for Menarini HA8160. POCT methods were less precise with negative offsets for DCA $2000^{®}$+ analyser -0.13%(0.28) (-1.4(3.1) mmol/mol), r^2=0.955, p<0.001 and A1cNow+™ cartridges -0.70%(0.67) (-7.7(7.3) mmol/mol), r^2=0.699, p<0.001 (n=113). Positive biases for Tosoh and Bio-Rad (compared with IFCC SRM) have been eliminated by subsequent revision of calibration.

CONCLUSIONS— Small differences observed between IFCC-calibrated and NGSP certified methods across a wide HbA$_{1c}$ range were confirmed by quality control and external quality assurance. As these offsets affect estimates of diabetes prevalence, the analyser (and calibrator) employed should be considered when evaluating diagnostic data.

250 words

HbA_{1c} is important for the management of diabetes [1,2] with its relationship to complications described by the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS). In both clinical trials ion exchange high performance liquid chromatography (IE HPLC) was employed for reporting HbA_{1c} using Bio-Rad analysers [3]. More recently, HbA_{1c} has been recommended by the ADA [4], WHO [5] and IDF [6] for the diagnosis of diabetes with a level of $\geq 6.5\%$ (48 mmol/mol) selected as the cut-point because of its relationship to diabetic retinopathy in epidemiological studies [7,8].

HbA_{1c} is an attractive alternative to glucose being more stable after collection of blood, not as readily affected by short-term variations in glycaemia and not requiring fasting or time consuming procedures e.g. oral glucose tolerance testing (OGTT) [9]. It is easily measured in laboratories and at point of care (POCT) using a variety of techniques including IE HPLC, immunochemistry or boronate affinity chromatography [10]. Precise International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) calibrated methods are recommended in current guidelines [4-6] following the introduction of the IFCC reference method for HbA_{1c} [11] involving mass spectrometry and capillary electrophoresis which has been used to anchor calibration of routine methods since 2003.

Rather than a single HbA_{1c} cut-point for diagnosis some guidance from Germany [12] and the US [13] recommends using ranges e.g. $\geq 7.5\%$ (58 mmol/mol) for ruling in diabetes and $\leq 5.5\%$ (37 mmol/mol) for ruling diabetes out with subsequent glucose testing for individuals who fall between 5.5% (37 mmol/mol) and 7.5% (58 mmol/mol) [14,15]. Knowledge of any off-sets between field methods and IFCC HbA_{1c} values is important when cut-points are being used for the diagnosis of diabetes and narrow ranges for pre-diabetes.

5 | P a g e

In this study, we have measured IFCC-calibrated HbA_{1c} in 128 patients with diabetes and normal haemoglobin using six systems certified by the National Glycohemoglobin Standardization Program (NGSP) and widely used in laboratories and at POCT, Table 1. We have compared HbA_{1c} results with an IFCC secondary reference method (IFCC SRM) based in a laboratory in the Netherlands comprising an IE HPLC analyser calibrated using special IFCC-calibrators. This IFCC SRM is used for the assignment of quality assurance and other materials. We have translated the differences observed in HbA_{1c} into effects on estimates of the prevalence of diabetes using two data sets from patients undergoing OGTT with concurrent HbA_{1c} measurement already published by the authors [15]. In addition, we have compared the calibrator used for Tosoh equipment during this study with the revised calibrator issued in Europe in September/October 2013 in 45 patient samples stratified across the range.

RESEARCH DESIGN AND METHODS

Ethical approval for the study was obtained from the West Midlands Local Research Ethics Committee and complies with the current revision of the Declaration of Helsinki.

Study Population

Adult patients attending the diabetes centre for routine care of Type 1 or Type 2 diabetes (n=128) with normal haemoglobin were recruited for a research study, Glucose, Fructosamine & HbA_{1c} Study (GFH). HbA_{1c} was measured in EDTA blood samples in the clinical biochemistry laboratory and in capillary heparinised blood samples in the diabetes centre between June 2007 and June 2009 using laboratory and POCT analysers, Table 1. After recruitment ended, HbA_{1c} was also measured in EDTA blood samples in March and April 2010 on two additional ion exchange HPLC analysers and an IFCC SRM in blood samples stored at $-70^{o}C$, Table 2.

HbA$_{1c}$ Methods

Three IE HPLC analysers, one affinity chromatography analyser and two systems used for POCT were compared with an IFCC secondary reference method as described below, Table 1.

Ion Exchange HPLC

One Tosoh G8 IE HPLC analyser was located in the diabetes centre and another in the clinical biochemistry laboratory at University Hospitals Birmingham NHS Foundation Trust. The Bio-Rad Variant II NU and Menarini HA8160 IE HPLC analysers were located in other laboratories described in the acknowledgements. The IFCC SRM Menarini HA8160 ion exchange HPLC analyser was located in an IFCC secondary reference laboratory in the Netherlands.

Point of Care Testing: Immunochemistry

The A1cNow^{+TM} disposable, hand-held cartridges were calibrated and programmed to perform ten analyses with the reagents provided. They contained onboard internal quality control (IQC) but additional IQC (Bio-Rad Lyphochek$^{®}$ Diabetes Control Levels 1 and 2) were analysed with each batch of samples. Due to limited access to consumables, HbA$_{1c}$ could not be measured in all samples, (n=113). Since the study was performed the company have specified in their kit leaflet that there is interference from EDTA as evidenced in the latest College of American Pathologists (CAP) data [16]. The DCA 2000$^{®}$+ analyser was calibrated using cards specific for each reagent lot.

Boronate Affinity Chromatography

IQC samples provided by the manufacturer were analysed on the Primus Ultra2 analyser at the beginning and end of each batch with two-point calibration.

7 | P a g e

Calibration

Assays were calibrated in the individual laboratories according to their routine standard operating procedures. All manufacturers confirmed the provision of IFCC-calibrators. The IFCC SRM Menarini HA8160 IE HPLC analyser was calibrated using the IFCC-network three-level calibrator panel (Lot 2009.1021; 2009.1022; 2009.1023).

Recalibration of Tosoh G8 IE HPLC analyser by manufacturer post GFH Study

In September 2013, Tosoh alerted laboratory staff in Europe to a revision of their calibrator with immediate effect via a Product Information leaflet (Release of the new "Hemoglobin HbA$_{1c}$ Calibrator Set" Lot ZS3001). The leaflet stated that ' *For samples with HbA$_{1c}$ values of 6 to 7% (NGSP) or 42 to 53 mmol/mol (IFCC), variations of 0.1 to 0.2% (NGSP) or 1.4 to 2.2 mmol/mol (IFCC) can be seen with the new Lot (as compared to the current Lot), depending on the specific Lot being used. These variations were deemed acceptable based on 0.3% (NGSP) criteria. For samples with HbA$_{1c}$ values <6% and for samples with HbA$_{1c}$ values between 7 to 10% (NGSP), a decrease in HbA$_{1c}$ values of ≤0.2% and ≤ 0.3% (NGSP), respectively, can be seen with the new Lot (as compared to the current Lot).'*

As a result of this notification, 45 EDTA blood samples and IQC samples were measured on the laboratory Tosoh G8 IE HPLC analyser with the calibrator in use at the time i.e. Lot ZS2002 and also after the introduction of the revised calibrator Lot ZS3001.

QC

IQCs recommended by manufacturers were used with additional materials provided for samples processed in batch-mode.

Sample Collection and Storage

HbA$_{1c}$ results were available from the diabetes centre on heparinised capillary blood using a Tosoh G8 IE HPLC analyser for 112 patients. For the remaining 16 patients, HbA$_{1c}$ was measured in venous EDTA blood on a similar analyser in the laboratory, Table 2.

In addition, HbA$_{1c}$ was measured in the laboratory in EDTA blood using a Primus Ultra2 affinity chromatography analyser (n=128) and by 2 POCT methods, A1CNow^{+TM} cartridges (n=113) and DCA 2000$^{®}$+ analyser (n=128). Measurements were performed within 5 days of collection of blood where possible but if not, samples were stored at -70°C. Note that collection into EDTA is not usually performed when blood samples are tested on these POCT devices but testing in situ was not possible due to the requirements for obtaining consent for the research study from patients.

According to study protocol, 75µl aliquots of 128 EDTA venous whole blood samples were prepared in triplicate in 2ml microtubes and stored at -70°C. They were sent by courier on dry ice to the outside laboratories for measurement on IFCC SRM, Bio-Rad Variant II NU and Menarini HA8160 analysers in March 2010. The samples were stored at -70°C on receipt and not thawed until required. Samples were analyzed in 6 batches on different days following the laboratory's standard operating procedures along with IQC material (lyophilized Lyphochek® levels I and II from Bio-Rad Laboratories Ltd) provided by the study. This IQC material was reconstituted following manufacturer's instructions, divided into aliquots and stored at -20°C.

Additional Studies

Duplicate HbA$_{1c}$ measurements were performed on a subsequent assay/day on a sub-set of aliquots stratified across the range (n=23) on the IFCC SRM, Menarini HA8160 and laboratory Tosoh G8 analysers.

9 | P a g e

Statistical Analysis

Data were entered into Excel and checked after entry by another person. Statistical analysis was performed using Excel and Analyse-it Ver 2.22 (Analyse-it Software Ltd, Leeds, UK). HbA_{1c} was reported by the study centre in DCCT aligned units and converted to IFCC using the following equation IFCC = (NGSP – 2.15) x 10.929 before analysis. The other laboratories reported HbA_{1c} in both IFCC and DCCT aligned units. CVs quoted in the paper were calculated from DCCT aligned units (%) but it should be noted that they are higher when calculated using IFCC units (mmol/mol) due to the nature of the conversion equation [17].

The different methods for HbA_{1c} measurement were compared to the IFCC SRM with Bland-Altman difference plots presented for each method [18-20]. Each data point, zero (nil difference) lines, mean differences, and 1.96 SD lines are shown. Pearson correlation coefficients (r^2) were calculated for each assay versus the IFCC SRM. Student's t-test was used to provide p values for the various other comparisons. During the study, the diabetes centre Tosoh G7 analyser was updated to a G8 analyser and results re-aligned using a linear regression equation derived from samples analysed on both systems (G8 HbA_{1c} = 0.9532*G7 HbA_{1c}+ 0.1138 (13), n=49). A further re-alignment was required following the release of the consensus statement on the worldwide standardisation of HbA_{1c} measurement [21], when Tosoh implemented a new 'anchor' for their calibrator value. The regression equation provided by the manufacturer was as follows; re-aligned HbA_{1c} = 0.917*original HbA_{1c} + 0.407, n=729. The laboratory Tosoh G8 analyser did not require any re-alignment as all analyses on this system took place after this changeover. A national quality assurance scheme (NEQAS, UK) classifies the Bio-Rad Variant II NU with other Variant II analysers but Bio-Rad confirmed that there are no appreciable differences between the various Variant II systems.

10 | P a g e

Data sets for patients undergoing OGTT for diagnostic purposes with concurrent HbA$_{1c}$ [15] were examined to assess the effect of any offset between routine HbA$_{1c}$ assays and the IFCC SRM using probability density function graphs produced for these data sets and also for those obtained in this paper for patients referred to a university hospital for treatment of diabetes, Figures 2 & 3.

RESULTS

HbA$_{1c}$ ranged from 5.3% to 11.9% (34 to 107 mmol/mol) for 128 blood samples on the IFCC SRM, Table 2. The inter-assay CVs achieved compared well with those quoted by manufacturers with the increased scatter for immunoassay POCT methods reflecting imprecision, Table 1 & Figure 1. In a stratified subset of 23 samples, there were minimal differences between duplicates on IE HPLC analysers with mean difference (1SD) in HbA$_{1c}$ on IFCC SRM -0.06%(0.09), p=0.004 (-0.7(0.9) mmol/mol), Menarini HA8160 analyser (n=19 only) 0.05%(0.10), p=0.046 (0.5(1.1) mmol/mol) and laboratory Tosoh G8 analyser -0.02%(0.07), p=0.171 (-0.2(0.8) mmol/mol).

Significant differences in HbA$_{1c}$ were apparent between laboratory/POCT methods and the IFCC SRM, all at p<0.001, Table 2, Figure 1. Small positive offsets were observed for the Bio-Rad Variant II NU analyser +0.33% HbA$_{1c}$ (3.6 mmol/mol) and Tosoh G8 analyser located in the diabetes centre +0.22% HbA$_{1c}$ (2.4 mmol/mol). For the Menarini HA8160 analyser situated in a routine hospital laboratory, there was a very small offset -0.04% HbA$_{1c}$ (-0.4 mmol/mol) similar to the differences observed between duplicates. There were negative biases for Primus Ultra2 affinity chromatography analyser, -0.23% HbA$_{1c}$ (-2.5 mmol/mol) and   DCA 2000$^{®}$+ immunoassay analyser, -0.13% HbA$_{1c}$ (-1.4 mmol/mol). These differences in bias between methods were confirmed by internal quality control results obtained for the

11 | P a g e

study and in national external quality assessment schemes. The difference in A1cNow^{+TM} disposable, immunoassay cartridges of -0.7% HbA$_{1c}$ (-7.7 mmol/mol) in EDTA blood is similar to that observed in College of American Pathologists (CAP) data for 2013 [16] and may be attributed to the presence of EDTA. When 23 EDTA blood samples were re-measured at the end of the study on the laboratory Tosoh G8 IE HPLC analyser after being frozen at -70°C, the higher bias for Tosoh G8 IE HPLC analyser versus IFCC SRM was confirmed, +0.30%(0.12) (+3.3(1.3) mmol/mol) HbA$_{1c}$, p<0.001.

In Birmingham, UK, 1457 patients with impaired fasting glucose (6.1 to 6.9 mmol/L) were referred by their family doctors for OGTT for diagnosis of diabetes with HbA$_{1c}$ measured on a Tosoh G8 analyser at the diabetes centre [15]. In Melbourne, Australia, 4083 patients at risk of diabetes for various reasons were referred for OGTT with HbA$_{1c}$ measured on a Bio-Rad Variant II Turbo analyser. Patients with abnormal haemoglobins were excluded from both datasets [15]. Figure 2 shows the effect of the small offsets identified in this study on the prevalence of diabetes when HbA$_{1c}$ was measured on different IE HPLC analysers in these two populations. Probability density plots for both the method comparison reported in this paper and OGTT data sets, Figure 3, highlight the differences in the distributions of HbA$_{1c}$ for patients with diabetes and those being diagnosed with diabetes and also the bias of the different methods used for HbA$_{1c}$ measurement.

Introduction of the revised Tosoh calibrator in October 2013 resulted in a decrease in HbA$_{1c}$ values as outlined in the product information sheet i.e. an offset across the range of HbA$_{1c}$ as identified in the GFH Study versus the IFCC SRM. On calibrator lot (ZS2002) comparable to that used for this paper, HbA$_{1c}$ was 7.5 (6.3 to 9.7)%, (58 (46 to 83) mmol/mol), median IQ range, in 45 blood samples with minimum 5.4% (36 mmol/mol) and maximum 12.3% (111

mmol/mol) compared to 7.4 (6.2 to 9.6)%, (57 (44 to 81) mmol/mol, 5.4% (35) mmol/mol and 12.0% (108 mmol/mol), respectively, for the revised calibrator (ZS3001), r^2 =0.999, p<0.0001. The difference (mean 1SD) between the calibrators (revised [RC] minus GFH study equivalent [PC]) was -0.23(0.06)% or -2.5(0.6) mmol/mol, Figure 1c, with linear regression equation , $HbA_{1c}[RC]$ = -0.044 + 0.9866 $HbA_{1c}[PC]$ in % or $HbA_{1c}[RC]$ = -0.797 + 0.9866 $HbA_{1c}[PC]$ in mmol/mol. The change in assay bias on revision of the calibrator was also reflected in IQC as indicated by the additional leaflet *Calibrator and Control Values when using Calibrator lot ZS3001* that introduced new ranges for IQC issued in September 2013 by Tosoh.

CONCLUSIONS

Changes in HbA_{1c} of 0.5% (6 mmol/mol) to 1.0% (11 mmol/mol) or greater within a patient are considered clinically significant for the management of diabetes [22] with targets of 6.5% (48 mmol/mol) or 7.5% (58 mmol/mol) depending on circumstances. However, now that a single HbA_{1c} cut-point ≥6.5% (48 mmol/mol) is recommended for diagnosis of diabetes with pre-diabetes defined as 5.7% (39) or 6.0% (42 mmol/mol) to 6.4% (47 mmol/mol) HbA_{1c} [5], the criteria for assessing the performance of HbA_{1c} assays have changed as small variations in assay performance will result in the movement of people from one category to another.

Although routine methods for measuring HbA_{1c} are calibrated using the IFCC reference method, it is important to know whether there are any differences in the accuracy and imprecision of IFCC-calibrated and NGSP certified methods that will affect diagnostic procedures. In this study, the values obtained for HbA_{1c} from two IE HPLC laboratory analysers were significantly higher than the IFCC SRM across the range of blood samples as a constant unrelated to glycation. A small positive HbA_{1c} offset of 0.22% (2.4 mmol/mol) IFCC

13 | P a g e

(mean difference) was identified for the Tosoh G8 IE HPLC analyser, $p<0.001$, and 0.33% (3.6 mmol/mol) for Bio-Rad Variant II NU IE HPLC analyser, $p<0.001$, compared with the IFCC SRM. The difference between the Menarini HA8160 IE HPLC analyser located in a routine hospital laboratory was significant, $p<0.001$, but much smaller at -0.04% (-0.4 mmol/mol) although this is not surprising as it is the same IE HPLC analyser as that used for the IFCC SRM but calibrated using the manufacturer's rather than IFCC SRM calibrators.

The offsets observed for particular HbA_{1c} assays studied would result in different proportions of people being diagnosed with diabetes depending on the assay used. Translation of the positive biases observed in this study performed between 2007 and 2010 down to IFCC SRM HbA_{1c} values in two populations presenting for OGTT would result in a lowering of the prevalence of diabetes by approximately one third i.e. from 36% to 22% for HbA_{1c} results obtained using a Tosoh G8 analyser in Birmingham, UK and from 24% to 15% for the Bio-Rad analyser used in Melbourne, Australia, Figure 2. Since this study was performed, we have been informed that Bio-Rad adjusted their calibration to account for this positive bias as evidenced by the CAP review for 2013 [16].

For another laboratory analyser involving affinity chromatography, there was a negative difference of -0.23% (-2.5 mmol/mol) HbA_{1c} compared with IFCC SRM, $p<0.001$, giving possible differences in HbA_{1c} between routine laboratory analysers of up to 0.6% (6 mmol/mol). Use of this analyser would lead to an underestimation of the prevalence of diabetes compared with the IFCC SRM. Bias can be introduced during the calibration process despite acceptable imprecision due to matrix effects in artificial calibration materials rather than blood, Figure 3. These small differences observed between laboratory/POCT methods are within the limits of accuracy for NGSP certification.

This study is limited because only a few analysers were involved with particular batches of assay consumables and calibrators. In addition, some samples were measured at the end of the study after storage at -70°C. However, the results are in line with manufacturers' expectations and confirmed in internal quality control and national external quality assurance schemes e.g. United Kingdom National External Quality Assessment Service (UK NEQAS) and CAP. The performance of the A1cNow^{+TM} disposable cartridges may have been be affected by EDTA as the company have since reported interference from EDTA in kit inserts published after the study was completed. This paper does not include widely used immunoassays available on routine automated clinical chemistry analysers that do not detect abnormal haemoglobins. In a recent publication comparing an immunoassay method with a Bio-Rad IE HPLC analyser, the correlation quoted was r^2 =0.996 but readings of 9.5% (80 mmol/mol) for Bio-Rad and 8.6% (70 mmol/mol) on the Cobas c502 [23] were apparent on the scattergram.

These differences between IFCC-calibrated methods are in line with some attributed to populations in research studies without due consideration of the analyser employed for HbA$_{1c}$ measurement [24-28]. In our previously published paper relating OGTT data to concurrent HbA$_{1c}$ in the UK and Australia, it was unclear whether the differences in HbA$_{1c}$ ranges quoted were related to the population, sampling or analysers [15,25]. In Birmingham, HbA$_{1c}$ was measured using the Tosoh G8 situated in the diabetes centre (as described in this paper) and in Australia using a Bio-Rad Variant II analyser – analysers with similarly high biases versus the IFCC SRM as observed in this study. Inspection of the HbA$_{1c}$ distribution in these two populations in Figure 2 shows how the differences between results from routine IFCC-calibrated methods would impact on the number of patients being diagnosed with diabetes [29]. Figure 3 demonstrates the requirements for the performance of HbA$_{1c}$ assays now based on the differing populations presenting for diagnosis or treatment. It is worth noting that since

15 | P a g e

the introduction of HbA_{1c} for diagnosis of diabetes in Birmingham in June 2012, the HbA_{1c} workload in our hospital laboratory has increased by 75% during the year since then with only a few OGTTs being performed.

In September/October 2013, Tosoh introduced a new calibrator that revised their HbA_{1c} values downwards, yielding results similar to the Menarini analysers in this study and the Bio-Rad IE HPLC analyser after introduction of a revised calibrator. The Tosoh IE HPLC data presented in this paper has not been realigned to the new revised calibrator but the comparison data from October 2013 shows that the bias detected in this study compared with the IFCC secondary reference method has been eliminated, Figure 1c.

In conclusion: currently there is some debate about the particular requirements for the performance of IFCC-calibrated and NGSP certified HbA_{1c} assays for diagnostic purposes in addition to the treatment of patients with diabetes. Although using HbA_{1c} for diagnosis has been subject to systematic review [30] following much debate about its relationship to OGTT [31,32], the methods used for HbA_{1c} measurement are rarely discussed. Small differences i.e. offsets between IFCC-calibrated methods identified in this study across a wide range of HbA_{1c} values would affect estimates of the prevalence of diabetes markedly. When evaluating HbA_{1c} in individuals/populations, it is important to consider the method used for HbA_{1c} measurement (and any revisions to calibration by the manufacturers) especially when HbA_{1c} is being used for diagnostic purposes [33].

16 | P a g e

17 | P a g e

References

1. DCCT Study Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications of insulin-dependent diabetes mellitus; the Diabetes Control and Complications Trial. N Engl J Med 1993;329:977–986.

2. UK Prospective Diabetes Study Group. UK Prospective Diabetes Study (UKPDS) 33 Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. Lancet 1998;352:837–853.

3. Manley S. Haemoglobin A_{1c} – a marker for complications of type 2 diabetes: the experience from the UK Prospective Diabetes Study (UKPDS). Clin Chem Lab Med 2003;41:1182–1190.

4. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33 Suppl 1:S62–S69.

5. Report of a World Health Organization Consultation: Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. Diabetes Research and Clinical Practice. 2011; 93: 299-309.

6. IDF Clinical Guidelines Task Force. 2012 Global guideline for type 2 diabetes. Diabetes Research and Clinical Practice. DOI: 10.1016/j.diabres.2012.10.001

7. Buell C, Kermah D, Davidson MB. Utility of A_{1c} for diabetes screening in the 1999–2004 NHANES population. Diabetes Care 2007;30:2233–2235.

8. 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;93:2447–2453.

9. International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009;32:1327–1334.

10. Manley SE, Round RA, Smith JM. Calibration of HbA1c and its measurement in the

presence of variant haemoglobins: report on questionnaire to manufacturers. Ann Clin Biochem 2006;43:135–145.

11. Hoelzel W, Weykamp C, Jeppson J-O, Miedema K, Barr JR, Goodall I et al. IFCC reference system for measurement of haemoglobin A_{1c} in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem 2004;50:166–174.

12. Brückel J, Joost HG, Kerner W, Müller UA, Schleicher E, Schulze M et al. Stellungnahme der Deutschen Diabetes Gesellschaft, diabetes DE und des Kompetenznetzes Diabetes mellitus zur Verwendung des HbA1c-Wertes als Biomarker zur Diabetesdiagnose. German Diabetes Society 2010.

13. Pogach L, Conlin PR, Hobbs C, Vigersky RA, Aron D. Updates in Specialty Care VADoD Update of Diabetes Guidelines: What Clinicians Need to Know About Absolute Risk of Benefits and Harms and A1c Laboratory Accuracy. Fed Pract 2011;28(4):39-44.

14. Lu ZX, Walker KZ, O'Dea K, Sikaris KA, Shaw JE. A1C for screening and diagnosis of type 2 diabetes in routine clinical practice. Diabetes Care 2010;33:817–819.

15. Manley S, Nightingale P, Stratton I, Sikaris K, Smith J, Cramb R et al. Diagnosis of diabetes using HbA_{1c} levels compared with WHO criteria. Diabetes & Primary Care 2010;12:87–96.

16. College of American Pathologists (CAP) Survey Data. CAP GH2 Proficiency Survey. CAP GH-2a 2013 Summary.

17. 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;57:1204–1206.

18. Cull CA, Manley SE, Stratton IM, Andrew H, Neil W, Ross IS, et al. Approach to maintaining comparability of biochemical data during long-term clinical trials. Clin Chem 1997;43:1913–1918.

19. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1(8476):307–310.

20. Twomey PJ: How to use difference plots in quantitative method comparison studies. Ann Clin Biochem 2006;43:124–129.

21. ADA, EASD, IFCC and IDF. Consensus statement on the worldwide standardisation of the HbA_{1c} measurement. Diabetologia 2007;50:2042 – 2043.

22. UK Prospective Diabetes Study Group. UK Prospective Diabetes Study (UKPDS) 35. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes: prospective observational study. BMJ 2000: 321:405-412.

23. Pease R. Routine automated HbA_{1c} immunoassay. The Biomedical Scientist 2012;56: 664–665.

24. Bennett CM, Guo M, Dharmage SC. HbA(1c) as a screening tool for detection of type 2 diabetes: a systematic review. Diabet Med 2007;24:333–343.

25. Manley SE, Sikaris KA, Lu ZX, Nightingale PG, Stratton IM, Round RA, et al. Validation of an algorithm combining haemoglobin A_{1c} and fasting plasma glucose for diagnosis of diabetes mellitus in UK and Australian populations. Diabet Med 2009;26:115–121.

26. Carson AP, Reynolds K, Fonseca VA, Muntner P. Comparison of A1C and fasting glucose criteria to diagnose diabetes among U.S. adults. Diabetes Care 2010;33:95–97.

27. Kramer CK, Araneta MR, Barrett-Connor E. A1C and diabetes diagnosis: The Rancho Bernardo Study. Diabetes Care 2010;33:101–103.

28. van't Riet E, Alssema M, Rijkelijkhuizen JM, Kostense PJ, Nijpels G, Dekker JM. Relationship between A1C and glucose levels in the general Dutch population: the new Hoorn study. Diabetes Care 2010;33:61–66.

29. Manley SE, Nightingale PG, Sikaris KA, Stratton IM, Cramb R, S.C.L. Gough SCL. How should HbA_{1c} be incorporated into the diagnostic pathway for diabetes mellitus?

Diabetologia 2010;53(Suppl.1):S398.

30. Zhang X, Gregg EW, Williamson DF, Barker LE, Thomas W, Bullard KM et al.A1c level and future risk of diabetes: a systematic review. Diabetes Care 2010;33:1665–1673.

31. Olson DE, Rhee MK, Herrick K, Ziemer DC, Twombly JG, Phillips LS. Screening for diabetes and pre-diabetes with proposed A1c-based diagnostic criteria. Diabetes Care 2010;33:2184–2189.

32. Buse JB. Screening for diabetes and pre-diabetes with proposed A1C-based diagnostic criteria. Diabetes Care 2010;33:e174.

33 American Diabetes Association Standards of Medical Care in Diabetes—2014. Diabetes Care 2014;37 Suppl 1:S14–S80.

Table 1—*Information on HbA₁c Methods from Manufacturers and GFH study*

Analyser	Principle	Detects variant hemoglobin	Conversion equation	Assay range	Manufacturer inter-assay CV* low/high	Study inter-assay CV*
Bayer A1cNow+™	Immuno-chemistry	No	$NGSP = 0.0915*IFCC + 2.15$	4% to13% 20 to 119 mmol/mol	3.0% to 4.0%	4.8%/6.3%
Bio-Rad VARIANT II NU	IE HPLC	Yes	$NGSP = 0.09148*IFCC + 2.152$	3.1% to 18.5% 10 to 179 mmol/mol	<2.0%	1.5%/1.2%
IFCC SRM	IE HPLC	Yes	$NGSP = 0.0915*IFCC + 2.15$	all physiological values	1.3%/1.4%	1.0%/0.6%
Menarini HA8160	IE HPLC	Yes	$IFCC = (NGSP - 2.15)*10.929$	all physiological values	1.3%/1.6%	1.0% /1.3%
Primus Ultra2	Affinity chromato-graphy	No	$IFCC = (NGSP - 2.15)/0.0915$	2% to 25% -2 to 250 mmol/mol	<2.0%	1.7%/1.5%
Siemens DCA 2000®+	Immuno-chemistry	No but HbA₁c reported for Hbs S & C	$IFCC = (10.93*NGSP - 23.50)$	2.5% to 14.0% 4 to 130 mmol/mol	2.2% to 3.2%	1.6%/4.6%
Tosoh G8	IE HPLC	Yes	$NGSP = (0.09148*IFCC) + 2.152$	2.4% to 22.3% 3 to 220 mmol/mol	<2.0%	1.2%/1.0%

*obtained from DCCT values

23 | P a g e

Table 2—*Comparison of HbA$_{1c}$ from IFCC-calibrated Field Methods with an IFCC Reference Method, n=128*

Method	Median (IQ range) % mmol/mol	Minimum to maximum % mmol/mol	Mean difference (SD) % mmol/mol	r^2	p value
Bayer A1cNow+™*	6.7 (6.1 to 7.4)	4.9 to 11.1	-0.70 (0.67)	0.70	<0.001
	50 (43 to 57)	30 to 98	-7.7 (7.3)		
Bio-Rad VARIANT II NU**	7.8 (7.1 to 8.8)	5.7 to 12.3	0.33 (0.17)	0.98	<0.001
	62 (54 to 73)	39 to 111	3.6 (1.9)		
IFCC SRM**	7.5 (6.8 to 8.4)	5.3 to 11.9	-	-	-
	58 (51 to 68)	34 to 107			
Menarini HA8160**	7.5 (6.7 to 8.4)	5.3 to 12.2	-0.04 (0.11)	0.99	<0.001
	58 (50 to 68)	34 to 110	-0.4 (1.2)		
Primus Ultra2***	7.3 (6.6 to 8.1)	5.4 to 11.3	-0.23 (0.28)	0.95	<0.001
	56 (49 to 65)	36 to 100	-2.5 (3.1)		
Siemens DCA 2000®+***	7.3 (6.7 to 8.1)	5.2 to 13.2	-0.13 (0.28)	0.95	<0.001
	56 (50 to 65)	33 to 121	-1.4 (3.1)		
Tosoh G8†	7.8 (7.0 to 8.6)	5.3 to 12.2	0.22 (0.20)	0.98	<0.001
	62 (53 to 70)	34 to 110	2.4 (2.2)		

*n=113 **Stored at -70°C ***Samples measured fresh or after storage at -70°C †112 samples capillary heparinised blood measured in diabetes centre and 16 samples venous EDTA blood measured in laboratory

FIGURE LEGENDS

Figure 1a

Scatterplot with linear regression lines for HbA$_{1c}$ measured by different IFCC-calibrated field methods and IFCC SRM, n=128.

— IFCC SRM   ● Tosoh G8   ● Menarini HA8160   ● Bio-Rad VARIANT II NU
                            ● Primus Ultra   ● Siemens DCA 2000®+ ● Bayer A1cNow+™

Figure 1b

Difference plots for HbA$_{1c}$ measured by IFCC-calibrated field methods versus IFCC SRM, n=128.

Colours of symbols as per Figure 1a, — zero line, --- mean difference & …. 1.96 SD

Figure 1c

Difference plot as above for Tosoh G8 IE HPLC analyser post recalibration (ZS3001) by manufacturer in September/October 2013 versus calibrator (ZS2002) with a bias equivalent to those calibrators used in the GFH Study, n=45.

26 | P a g e

Figure 2

Effects of bias of IE HPLC assays on prevalence of diabetes in patients presenting for OGTT with concurrent HbA_{1c} in Birmingham and Australia

━━   4083 Australian patients with risk factors for diabetes (Bio-Rad Variant II Turbo IE HPLC analyser)
━━   1457 UK patients with impaired fasting glucose, 6.1 – 6.9 mmol/L (Tosoh G8 IE HPLC analyser)

Figure 3

Probability density functions for HbA_{1c} in patients presenting for OGTT and from the comparison of analytical methods in patients with diabetes
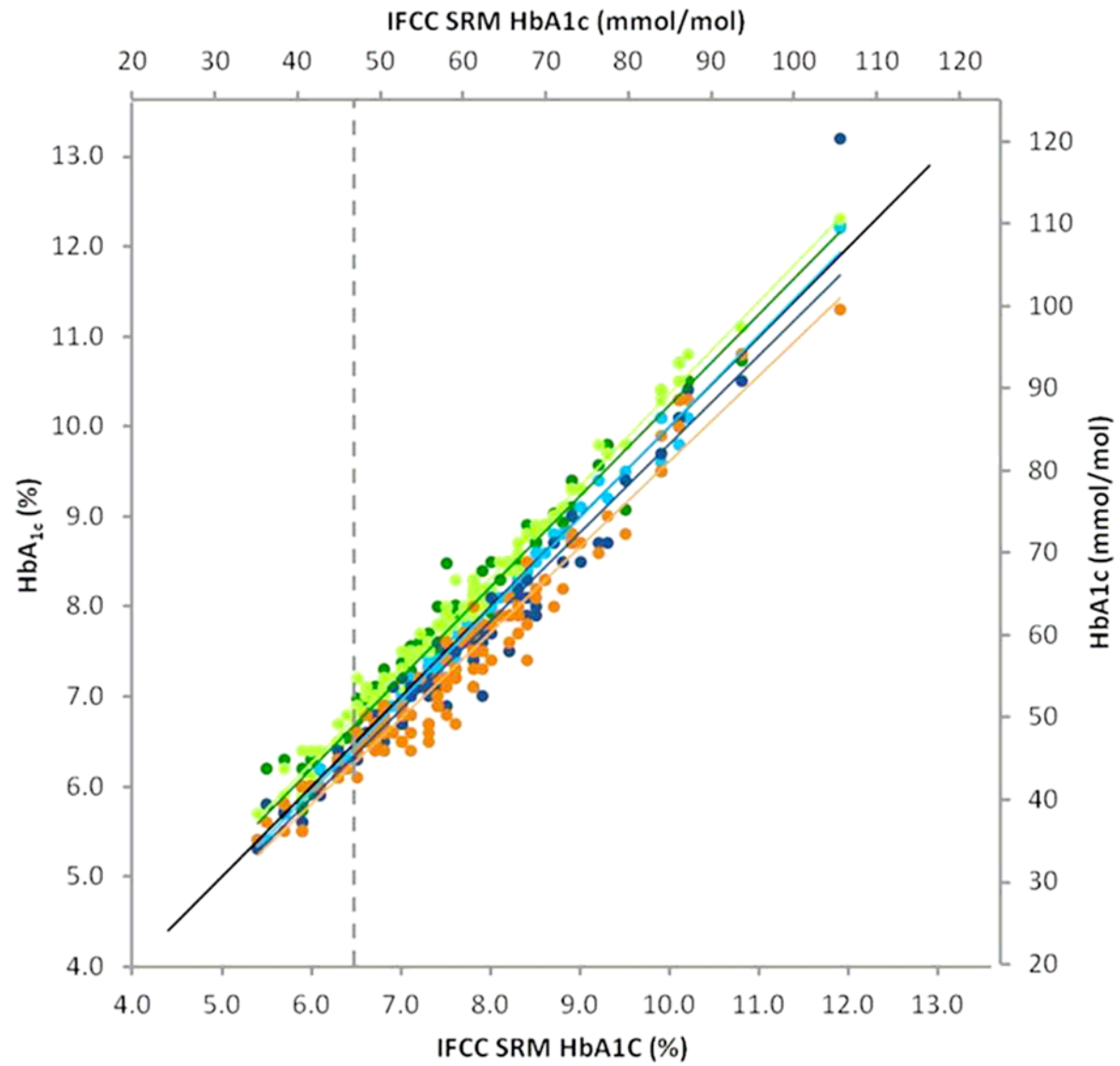
Colours of symbols as per Figure 1a

27 | P a g e

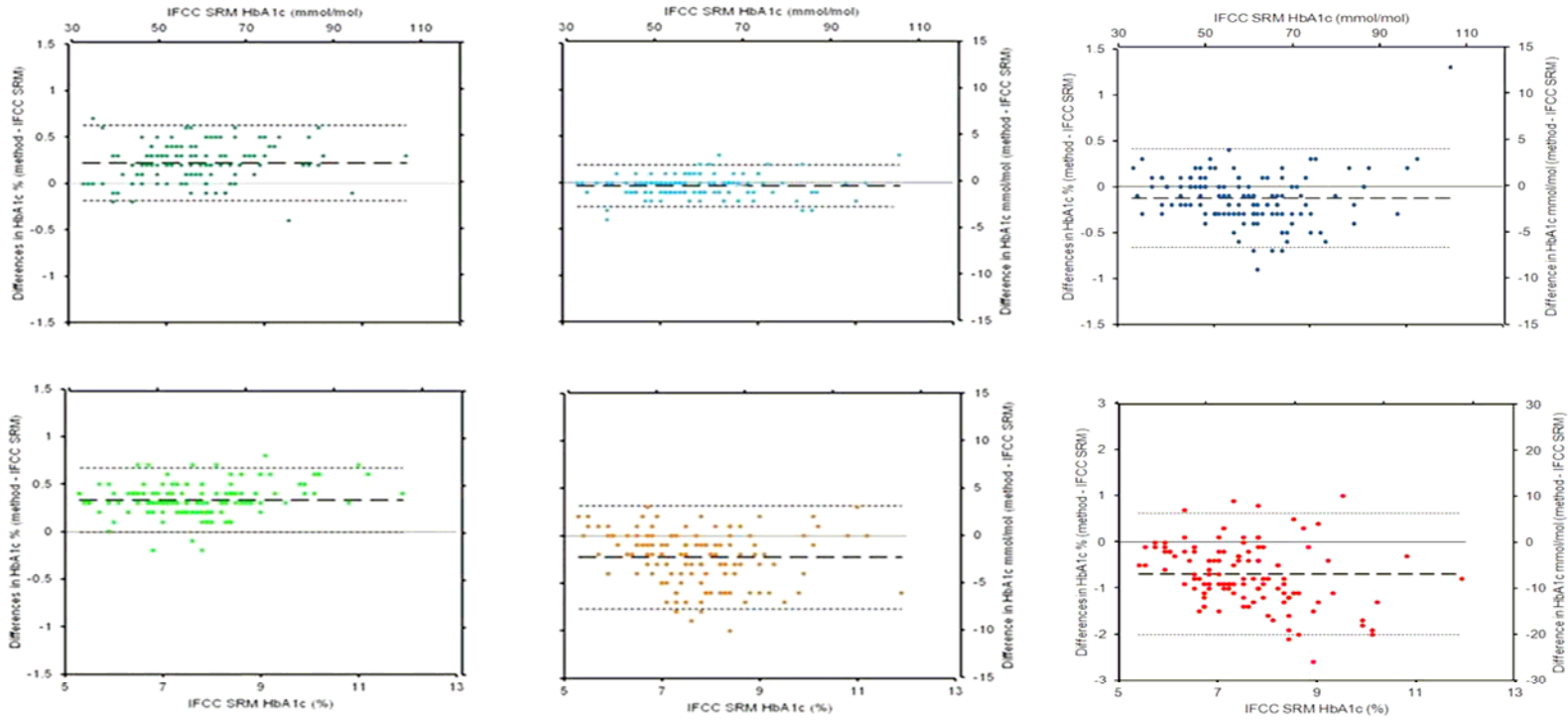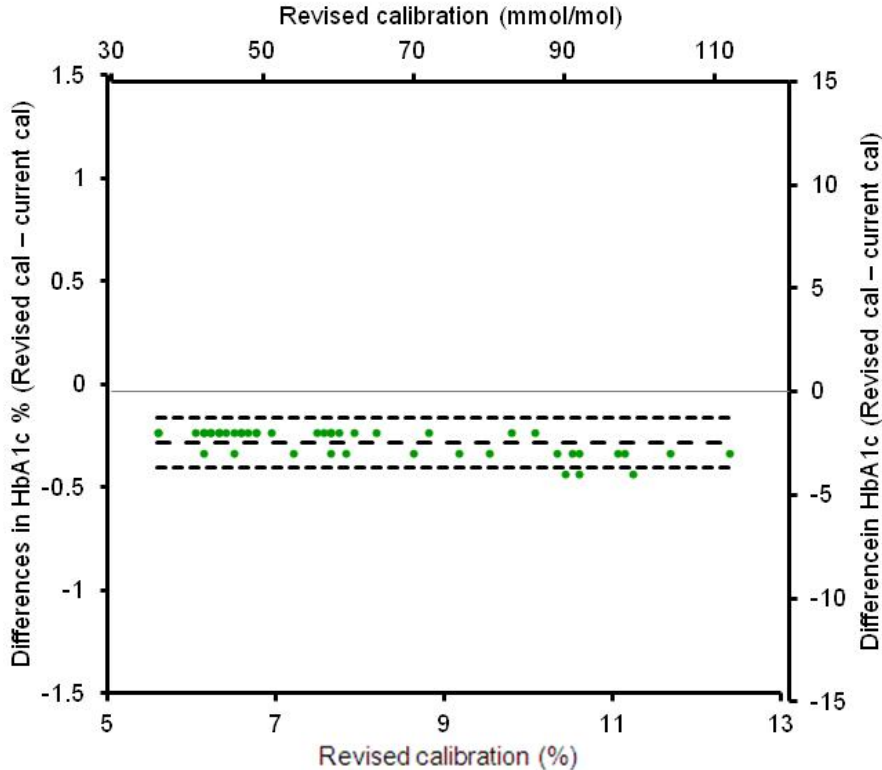**Figure 1a**

Figure 1b

Figure 1c

Figure 2

Figure 3