Evaluating new HbA1c methods for adoption by the IFCC and NGSP reference networks using international quality targets

Evaluation of three different HbA1c methods

Erna Lenters-Westra[1,2] & Emma English,[3]

[1]Department of Clinical Chemistry, Isala, Zwolle, The Netherlands

[2]European Reference Laboratory for Glycohemoglobin, location Isala, Zwolle, The Netherlands

[3] Faculty of Medicine and Health, University of East Anglia, Norwich Research Park, Norwich, England

Corresponding author:

Erna Lenters-Westra
Isala
Dr. van Heesweg 2
8025 AB Zwolle
The Netherlands

Words:3581
Tables:2
Figures:3
Supplemental data:5

Abstract

Background: As a reference laboratory for HbA_{1c} it is essential to have accurate and precise HbA_{1c} methods covering a range of measurement principles. We report an evaluation of the Abbott Enzymatic (Architect c4000), Roche Gen.3 HbA_{1c} (Cobas c513) and Tosoh G11 using different quality targets.

Methods: 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 using certified evaluation protocols.

Results: Each of the evaluated HbA_{1c} methods had CVs <3% in SI units and <2% in NGSP units at 46 mmol/mol (6.4%) and 72 mmol/mol (8.7%) and passed the NGSP criteria when compared with 6 Secondary Reference Measurement Procedures (SRMP). Sigma was 8.6 for Abbott Enzymatic, 3.3 for Roche Cobas c513 and 6.9 for Tosoh G11. No clinically significant interference was detected for the common Hb-variants for the 3 methods.

Conclusion: All 3 methods performed well and are suitable for clinical application in the analysis of HbA_{1c}. Partly based on the result of this study the Abbott Enzymatic method on the Architect c4000 and the Roche Gen.3 HbA_{1c} on the Cobas c513 are now official, certified IFCC and NGSP SRMPs in the IFCC and NGSP networks. Sigma metrics quality criteria, presented in a graph distinguish between good and excellent performance.

Keywords: HbA1c, sigma metrics, IFCC, diabetes, method evaluation

Introduction

Diabetes represents a huge global health burden and is a leading cause of morbidity and mortality worldwide [1].It is estimated that up to 50% of people with diabetes are currently undiagnosed, and this is a particular issue in hard to reach settings such as rural communities. The ability to identify and effectively treat people with diabetes is dependent on accurate and timely diagnostic testing, most commonly provided by hospital clinical laboratories, using a range of methods.

Recently the World Health Organization advocated the use of HbA_{1c} testing for the diagnosis of Type 2 diabetes however, there must be stringent quality control procedures in place to ensure accurate and precise test results and methods must be aligned to the international reference measurement procedure [2].

Whilst the Diabetes Control and Complications Trial (DCCT) and UK Prospective Diabetes Study (UKPDS) were seminal trials of the time, more recently treatment targets for people with diabetes have become more individualised with the needs of the patient at the core of decision making [3]. With a patient centred approach it is essential that methods for detecting and monitoring diabetes are both accurate and precise to enable high quality, consistent care.

The IFCC Task Force on Implementation of HbA_{1c} standardization (TF-HbA_{1c}) recently advocated sigma-metrics as the model of choice to set and evaluate quality targets for HbA_{1c} [4]. In the laboratory sigma-metrics is a quality management strategy that provides a universal benchmark for process performances. Sigma-metrics places analytical characteristics (bias and imprecision) within the framework of clinical requirements (Total Allowable Error (TAE)). The risk is defined in sigma units: a sigma of 2 implies a 5% risk to fail the TAE. The TF-HbA_{1c} has set default risk levels of 2σ for routine laboratories and 4σ for laboratories performing clinical trials [4]. These targets can be universally applied to commercially available HbA_{1c} methods, with comparison to the IFCC Primary Reference Measurement Procedure (PRMP) via the SRMPs as the correct way to determine bias.

The European Reference Laboratory for Glycohemoglobin (ERL) is responsible for the production of IFCC secondary reference material which enables manufacturers to be traceable to the IFCC PRMP and thus meet the requirement of the WHO and international consensus for the global standardisation of HbA_{1c}. Currently the ERL consists of 7 IFCC certified and 5 NGSP certified SRMPs for the determination of HbA_{1c} in 2 laboratories and is therefore able to evaluate any new HbA_{1c} method at the highest level [5, 6].

The aim of this paper was to evaluate the Roche Gen.3 Tina-quant HbA_{1c} method on the Cobas c513 and the Abbott Architect Enzymatic method on the Architect c4000 for adoption by the IFCC and NGSP reference networks as certified SRMPs using international quality targets. Beside these 2 methods, we also evaluated the Tosoh G11.

Material and methods

Abbott Enzymatic method on the Architect c4000 (Abbott Enzymatic)

The Architect c4000 is a routine chemistry analyzer with photometric, potentiometric and turbidimetric methods available. The instrument is not specifically dedicated to HbA_{1c} and has a maximum sample throughput of up to 800 tests per hour. The total run-time for HbA_{1c} is 10 minutes. The enzymatic method principle has been described before and consists of two separate steps: measurement of glycated dipeptide, obtained by enzymatic cleavage, and measurement of total hemoglobin [7, 8]. Samples can be run using either the whole blood mode or the hemolysate mode, if there is not sufficient whole blood available, using a manual pre-dilution step. The instrument does not have closed tube sampling.

The reagents are ready for use (350 tests per reagent cartridge) and are stable for 50 days onboard the instrument which needs calibration every 50 days provided there is no change of reagent lot number. During the precision study three different reagent lot numbers were used so the instrument was calibrated 3x using a single calibrator lot number.

Roche Tina-quant Gen.3 on Cobas c513 (Roche c513)

The Cobas c513 is the successor of the Integra 800 and is a fully dedicated instrument for HbA_{1c}. The sample throughput is 400 patient results per hour which doubles the throughput compared with the Integra 800. The ready to use reagent is available in a large kit size (500 test per reagent cartridge) suitable for handling high workloads.

The HbA_{1c} determination is based on a turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood. Total hemoglobin is measured bichromatically during the preincubation phase of the immunological reaction. Samples can be run using the whole blood mode with closed tubes sampling and the hemolysate mode for small blood volumes [9].

Onboard stability of the reagent is 4 weeks. The method needs to be calibrated every 28 days or when there is change of reagent lot number. The calibration is a reagent lot specific, which means that not every calibrator can be used with the same values for every reagent lot. During the precision study the instrument was calibrated 3x using the same lot number for the calibrator and reagent. During the method comparison study the instrument was calibrated once using the same lot number for the calibrator and the reagent.

Tosoh G11

The Tosoh HLC-723G11 variant mode (software version V02.00) uses cation exchange HPLC to separate hemoglobin components by different ionic charge. The various fractions of hemoglobin, including HbA_{1c}, are quickly (30 seconds per sample) separated into 6 peaks and assayed. A step gradient of three different salt concentrations is used for peak separation and elution. The Tosoh G11 is the successor of the Tosoh G8 and has a reduced run time of 60 seconds. As a direct result of the shortened run time there is no longer a specific Hb-variant window. The G11 only has a H-VO window (HbAD, HbAS, HbAC all appear in this window), P-HV3 (HbAE) and POO (for Unknown Hb-variants).

The reagents are stable for 90 days after opening. The instrument requires calibration every 30 days. During the precision and method comparison study the instrument was calibrated once. Only one lot number of reagent and calibrator was used.

Precision study

Two samples with an HbA_{1c} value of approximately 48 mmol/mol (6.5%) and 75 mmol/mol (9.0%) were used, according to the CLSI EP-5 protocol (duplicate measurements twice per day for 20 days), to investigate assay imprecision. Aliquots were made from patient samples and stored at minus 80 °C until analysis [10].

Method comparison

The CLSI EP-9 protocol was performed with 80 frozen samples with HbA_{1c} values over a clinically relevant range (27 mmol/mol (4.6%) to 86 mmol/mol (10.0%)) and the data were used to investigate the bias between the investigated methods and 6 IFCC and NGSP SRMPs (n=80, 16 samples per day for 5 days, duplicate measurements) [11]. HbA_{1c} value assignment for the patient samples was performed with 6 IFCC SRMPs (4 of which are also NGSP SRMPs):

Isala, Zwolle

- Roche Tina-quant Gen.2 HbA_{1c} on Integra 800, immunoassay, IFCC and NGSP certified (Roche Diagnostics, Rotkreuz, Switzerland)
- Premier Hb9210, affinity chromatography HPLC, IFCC and NGSP certified (Trinity Biotech, Bray, Ireland)
- Tosoh G8, cation-exchange HPLC, IFCC certified (Tosoh Bioscience, Tessenderlo, Belgium).

Queen Beatrix Hospital, Winterswijk

- Premier Hb9210, affinity chromatography HPLC, IFCC certified (Trinity Biotech, Bray, Ireland)
- Menarini HA8180V, cation-exchange HPLC, IFCC and NGSP certified (Menarini Diagnostics, Florence, Italy)
- Sebia Capillarys 2 Flex Piercing, IFCC and NGSP certified (Sebia, Paris, France).

To check bias, independently of the chosen SRMP, the results of the investigated instruments in the EP-9 procedure were compared with the mean of the 6 SRMPs and medical decision point analysis was performed at 48 mmol/mol (6.5%) and 75 mmol/mol (9.0%). The 6 SRMPs were calibrated with IFCC secondary reference material placing them one step higher in the traceability chain, than when using calibrators supplied by the manufacturer.

IFCC monitoring program

The Roche c513 and the Abbott Enzymatic were both candidates to become an official SRMP in the IFCC and the NGSP network. To become IFCC certified the methods must demonstrate traceability to the IFCC Reference System by participation in the IFCC monitoring program. This monitoring program consists of 24 interconnected samples (12 samples in duplicate). One sample is analyzed every two weeks, and the results submitted via the website. Values are assigned by all of the approved laboratories of the IFCC Network (n=21) [5]. The 24 samples from the IFCC monitoring program were analyzed in one run by the evaluated methods.

Linearity

Linearity was assessed using the CLSI EP-6 protocol [12]. After adjustment for Hb concentration, patient samples with a low HbA_{1c} value (27 mmol/mol (4.6%)) and a high HbA_{1c} value (148 mmol/mol (15.7%)) were mixed in incremental amounts to generate a series of samples over a broad HbA_{1c} concentration range (n=11). The theoretical HbA_{1c} value and the measured values were compared.

Hemoglobin Variant Interferences

Interference from common Hb-variants HbAS, HbAC, HbAD, HbAE, increased A_2 (β-thall) and HbF was investigated. Five samples of each variant, with different HbA_{1c} values were analyzed in one run. The specific variants were identified using cation-exchange HPLC (Menarini HA8180V, Diabetes Mode) and capillary electrophoresis (Sebia Capillarys 2 Flex Piercing, Hemoglobin program). HbA_{1c} values were assigned using an IFCC calibrated boronate affinity HPLC (Premier Hb9210).

The percentage HbF was determined using the Sebia Capillarys 2 Flex Piercing and, HbA_{1c} values of the samples with HbF were assigned using an IFCC calibrated cation exchange HPLC (Menarini HA8180V, Diabetes Mode). The percentages of HbF in the 5 HbF samples were: 4.6%, 6.2%, 15.0%, 18.0% and 39.0%. The investigated Hb variant can be considered as not causing an interference if the results of the Hb variant fall within the deviation of the non-variant samples distributed around the regression line. A mean relative difference exceeding ± 10% in SI units compared to the assigned value was defined as clinical significant.

Other interferences

Four samples with 12.9%, 9.1%, 5.4% and 3.4% carbamylated hemoglobin were made according to a previously published method [13].

The plasma of 6 patient samples with triglyceride concentrations of 5.2, 8.1, 9.3, 10.1, 14.6 and 15.6 mmol/L and plasma of 3 samples containing 164, 215 and 409 µmol/L bilirubin were used to re-suspend pooled red cells from samples with an HbA_{1c} value of approximately 48 mmol/mol (6.5%). The samples were measured in singleton together with the original pooled sample.

Investigating the effect of using fresh versus frozen samples in both hemolysate and whole blood modes and sedimentation of red blood cells

Aliquots were made from 9 samples with HbA_{1c} values ranging from, approximately, 26 mmol/mol (4.5%) to 103 mmol/mol (11.6%) and stored at minus 80°C for 2 days. After 2 days hemolysates were made from the frozen samples and from the primary samples which were kept at +4°C . The same fresh whole blood samples were used to investigate the influence of sedimentation of the red blood cells whilst samples were on the analyzer awaiting analysis. The whole blood samples were thoroughly mixed before loading and analyzed (T=0). After 30, 60, 90, 120, 150 and 180 minutes the samples were analyzed again without mixing and the results were compared with the T=0 sample. All samples were analyzed in a single run (fresh whole blood using the whole blood mode, hemolysates made from both frozen blood and the whole blood samples using the hemolysate mode) and compared with each other. The student 2-tailed t-test for paired samples was used to check for statistically significant difference between the results obtained in hemolysate and the whole blood mode and at different times. A P value <0.05 was considered significant.

Analytical performance criteria

Sigma metrics

The Total Allowable Error (TAE) for HbA_{1c} has been set by the TF-HbA_{1c} as a default of 5 mmol/mol (0.46% DCCT) at an HbA_{1c} level of 50 mmol/mol (6.7% DCCT) which corresponds with a relative TAE of 10**%** ((5/50)*100%) in SI units (6.9% DCCT units ((0.46/6.7)*100%)) with risk levels of 2σ for routine laboratories and 4σ for laboratories performing clinical trials [4].

Medical decision point analysis

When 2 methods are statistically identical, the 95% CI for each y MDP includes the corresponding x MDP. For example: 48 mmol/mol, the diagnostic cut-off value for the diagnosis of diabetes falls within 46.5 to 48.1 mmol/mol, the 95% CI around the calculated y so both methods are statistically identical.

IFCC monitoring criteria

The analytical performance is considered excellent if the mean deviation from the assigned value is <1.9 mmol/mol, CV < 2% and linearity >0.9950.

NGSP Manufacturer Certification Criteria

Thirty seven of 40 results need to be within 6% (relative) of an individual NGSP SRMP to pass certification [14].

Statistics

Calculations were performed using Microsoft® Excel 2010 (Microsoft Corporation). Statistical analyses were performed using Analyse-It® (Analyse-It Software) and EP Evaluator Release 9 (Data Innovations) [15].

For the duplicates in the IFCC monitoring program, CV was calculated with the following formula:

$$\mathrm{CV_a} = \frac{\sqrt{\frac{\sum(\Delta)^2}{n}}}{\bar{x}\sqrt{2}} \times 100\%$$

where CV_a is the analytical CV, Δ is the difference between duplicates, n is the number of duplicates, and $\bar{x}$ is the mean of the duplicates.

Results

Precision studies

The imprecision results of the EP-5 protocol are detailed in Table 1.Each of the evaluated HbA_{1c} methods had CVs <3% in SI units and <2% in NGSP units at 46 mmol/mol (6.4%) and 72 mmol/mol (8.7%).

Method comparisons

In the EP-9 study the Roche c513 and the Tosoh G11 both had a mean bias of approximately -2 mmol/mol (0.2%) and the Abbott Enzymatic method had a mean bias of -0.5 mmol/mol (-0.05%) compared with the mean of the 6 SRMPs (Figure 1A-C and). Medical decision point analysis for the Abbott Enzymatic method at 48 mmol/mol compared to the mean of the 6 SRMPs was 47.5 mmol/mol (95% CI: 47.4 to 47.6) and at 75 mmol/mol 74.4 mmol/mol (74.2 to 74.5). For Roche c513 it was 46.3 mmol/mol (46.1 to 46.5) and 72.1mmol/mol (71.8 to 72.4). For the Tosoh G11 it was 46.2 mmol/mol (46.1 to 46.4) and 72.4 (72.2 to 72.6). Supplemental Table 1 details the results of individual method comparisons with each of the included SRMPs.

Linearity and interferences

All 3 methods were linear up to 140 mmol/mol (15%) (Supplemental Figure 1A-C). All three methods showed no clinically significant interference from the common Hb-variants (HbAS, HbAC, HbAD, HbAE and elevated A2). HbF > 6.2% interfered with the Abbott Enzymatic and the Roche c513 methods but not with the Tosoh G11 (Figure 2A-C and supplemental Table 2). Carbamylated Hb up to 12.9% showed no clinically significant interference on the Abbott Enzymatic and the Roche c513 method. The Tosoh G11 showed no clinically significant interference with HbCarb of 3.4% but HbCarb of 5.4% showed clinically significant interference and no results were given at an HbCarb of 9.1% and 12.9% because of a "total plate too low" flag. The three investigated methods showed no clinically significant interference of total bilirubin up to 409 µmol/L and triglycerides up to 15.6 mmol/L (Supplemental Table 3).

The effect of using fresh versus frozen samples in both hemolysate and whole blood modes and sedimentation of red blood cells

There was no statistical difference between frozen samples and whole blood samples or samples analyzed using either the hemolysate mode or the whole blood mode. Results of samples which had been stood for 3 hours without mixing showed no statistical difference to those which were mixed just prior to analysis. (Supplemental Table 4).

Analytical Performance Criteria

Sigma metrics

All 3 methods had a sigma> 3 using the precision results, from the EP-5 protocol, at an HbA_{1c} value of 46 mmol/mol (6.4%) and the bias calculated at 48 mmol/mol (6.5%) compared to the mean of the 6 SRMPs. Sigma's calculated using the results of the IFCC monitoring program were > 6 for all 3 methods (Figure 3 and Table 2). Sigma's for the Abbott Enzymatic method, compared with the 6 individual SRMPs, ranged from 8.4 to 10.0, for the Roche c513, from 3.2 to 4.4 and for the Tosoh G11 from 7.0 to 8.7 (Supplemental Table 1).

Medical decision point analysis

All 3 methods showed statistically significant difference at 48 mmol/mol (6.5%) and 75 mmol/mol (9.0%) but clinically seen the differences were very small and therefore acceptable.

IFCC monitoring criteria

When using the criteria and samples of the IFCC monitoring program the 3 methods showed excellent performance with a mean deviation from the target value of <1.9 mmol/mol, CV < 2.0% in SI units and linearity >0.9950. In addition, using this protocol the sigma values were > 6 for each method (Figure 3 and Table 2).

NGSP criteria

All 3 methods passed NGSP manufacturer criteria compared with the 6 individual SRMPs (Supplemental Table 1). Pass/fail calculations were based on passing with 74/80 samples.

The 3 methods also passed the NGSP Secondary Reference Method Certification Criteria which includes precision (EP-5) and comparison with all SRMPs in the NGSP network (data not shown) [16].

Discussion

Overall, each of the three methods performed well meeting the essential performance criteria detailed by the IFCC Task Force on Implementation of HbA_{1c} Standardization, guidance on sigma metrics targets for routine laboratories (sigma > 2). The Abbott enzymatic method and the Tosoh G11 also met the more stringent criteria for methods used in clinical trials (sigma > 4). A small shift in results after some calibrations of the Roche c513 resulted in a higher CV in EP-5 protocol and a small bias (CV was 2.0% at 46 mmol/mol, bias was -2 mmol/mol) which may have contributed to the slightly lower sigma value observed. Sigma's calculated from the results of IFCC monitoring program were >4 as these CV's were not influenced by calibrations. CV has a bigger impact on the calculation of sigma than bias [17]. The Abbott Enzymatic method showed the most robust performance with minimal bias and a very stable CV even with several different calibrations and different reagent lot numbers. This is in line with results of this method in the College of American Pathologist External Quality Scheme [18].

Reducing the run time for the Tosoh G11 had no influence on the analytical performance in general. However, the disadvantage of shortening the run time is that it is no longer possible to distinguish the different Hb-variants from each other as the retention times are very close to each other. Ion-exchange methods in particular have shown tendencies to show variable interferences over time due to software/reagent changes. The recent publications of Rohlfing, et. al. [19] and Lenters-Westra [20] shows this very clearly. Shortening the run time of the Tosoh G11, like all cation exchange methods, has the potential to make the instrument vulnerable to interference from Hb-variants and other substances such as carbamylated Hb. Carbamylated Hb up to 3.4% did not interfere with the Tosoh G11 but an HbCarb of 5.4% showed an clinically significant interference. This might be a problem with patients with diabetes and advanced kidney disease. However, many patients are not allowed to become that uremic any longer so this may only be an issue in poorer and underdeveloped health systems. In addition to potential analytical interferences, such as carbHb, patients with end stage renal failure are likely to have multiple clinical factors that may affect the validity of HbA_{1c} such as anaemia and the use of Epo, which can only be accounted for with good clinical information on the patient. The Tosoh G11 showed no clinically significant interference with the common Hb-variants but HbAC was borderline. The mean relative difference of the 5 HbAC samples was 9.6% but 2 out of the 5 HbAC samples had a difference >10%. Historically HbAE has been a problem with the Tosoh analysers but interestingly showed no interference with the Tosoh G11. This is remarkable as, unlike the HbD peak, the HbE peak does not separate from the HbA0 peak. This means that the instrument incorporates an adjustment factor, which worked with the samples we investigated but might not work with all samples containing HbAE.

Whilst all methods passed the NGSP criteria for manufacturer certification (where methods are compared against one NGSP SRMP rather than the mean of all), the Abbott enzymatic method performed very well with no samples more than ±6% of the designated SRMP. The results of the Roche c513 show that it is possible to pass the manufacturer certification criteria whilst failing to meet the sigma metrics criteria ($\sigma > 4$) for laboratories engaged in clinical trials.

When an offline calibration, using IFCC secondary reference materials, is applied to the results, the small shifts in results, seen with changes in manufacturer’s calibrants, are negated, resulting in sigma values >4. The IFCC secondary reference material is one step higher in the traceability chain than the calibrators of the manufacturer and therefore more accurate. However this is not practical in a routine laboratory setting. Each of the methods included in this study are produced by manufacturers that can demonstrate traceability of their calibrators to the IFCC primary reference measurement procedure, which complies with ISO 17511:2003 standards detailing how to assure the metrological traceability of patient sample values. This also complies with WHO criteria for the diagnosis of type 2 diabetes (T2DM) using HbA_{1c} [2]. In addition the MDP analysis at 48 mmol/mol offers reassurance to clinicians that the instruments perform well at this level.

The Abbott Enzymatic method on the Architect c4000, the Roche Gen.3 HbA_{1c} on the Cobas c513 and the Tosoh G11 were shown to perform well and are suitable for clinical application in the analysis of HbA_{1c}. In addition, a critical aim of the study was to assess the suitability of the Abbott Enzymatic and the Roche c513 as candidate SRMPs, and based on most of the data shown here, they are now official, certified IFCC and NGSP SRMPs [5, 6].

Acknowledgments: The authors would like to thank Abbott Diagnostics, Roche Diagnostics and Tosoh Bioscience for the contribution of the instruments and reagents for this evaluation. The authors would also like to thank Agnes den Ouden for her contribution of this evaluation by analysis of the samples.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: Tosoh Bioscience financially supported the study.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

Legends

Figure 1 (A-C)
HbA_{1c} results in SI units for (A) Abbott Enzymatic on Architect c4000, (B) Roche Gen.3 HbA1c on Cobas c513 and (C) Tosoh G11 compared to the mean HbA_{1c} results from 6 IFCC Secondary Reference Measurement Procedures.

Figure 2 (A-C)
Interference from common Hb-variants (n=5 per Hb-variant) by Abbott Enzymatic (A), Roche c513 (B) and Tosoh G11 (C)

Figure 3
Sigma metrics results for the Abbott Enzymatic on Architect c4000 (A and AA), Roche Gen. 2 HbA1c on Cobas c513 (B and BB) and the Tosoh G11 (C and CC)) based on the CV in EP-5 at 46 mmol/mol and bias at 48 mmol/mol compared to the mean of 6 Secondary Reference measurement Procedures and (A,B and C) and the results of the IFCC monitoring program (AA, BB and CC).

References

1. Federation ID. IDF Diabetes Atlas, 7th edn. Brussels, Belgium International Diabetes Federation; 2015.

2. WHO guidelines Use of glycated hemoglobin A1c in the daignosis of diabetes www.who.int/diabetes/publications/diagnosis_diabetes2011/en/ (Accessed January 2017)

3. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2015;38:140-9.

4. Weykamp C, John G, Gillery P, English E, Ji L, Lenters-Westra E, et al. Investigation of 2 models to set and evaluate quality targets for Hb A1c: biological variation and sigma-metrics. Clin Chem. 2015;61:752-9.

5. Approved laboratories of the IFCC network laboratories for HbA1c 2016. www.ifcchba1c.net/network/approved (Accessed January 2017)

6. NGSP Network Laboratory Members 2016. www.ngsp.org/network.asp (Accessed January 2017)

7. Jaisson S, Desmons A, Renard B, Chevelle B, Leroy N, Gillery P. Analytical performances of a new enzymatic assay for hemoglobin A1c. Clin Chim Acta. 2014;434:48-52.

8. Teodoro-Morrison T, Janssen MJ, Mols J, Hendrickx BH, Velmans MH, Lotz J, et al. Evaluation of a next generation direct whole blood enzymatic assay for hemoglobin A1c on the ARCHITECT c8000 chemistry system. Clin Chem Lab Med. 2015;53:125-32.

9. Imdahl R, Röddiger R, Casis-Saenz E. Multicenter Evaluation of a New High-Throughput HbA1c Testing Platform. [Epub ahead of print] doi:107754 Clin. Lab. 2016.160633

10. Institute CaLS. Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline. NCCLS Document EP05-A. PA: CLSI Wayne; 1992.

11. Institute CaLS. Method Comparison and Bias Estimation using Patient Samples; Approved Guideline. CLSI Document EP09-A PA: CLSI Wayne; 1995.

12. Institute CaLS. Evaluation of the Linearity of Quantitative Measurement Procedures: A statistical Approach; Approved Guideline. CLSI Document EP06-A (2003). PA: CLSI Wayne; 2003.

13. 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.

14. NGSP Manufacturer Certification Criteria. www.ngsp.org/critsumm.asp. (Accessed January 2017)

15. EP Evaluator Data Innovations. www.datainnovations.com/products/ep-evaluator (Accessed January 2017).

16. NGSP Protocol Laboratory Network. www.ngsp.org/protln.asp. (Accessed January 2017).

17. Lenters-Westra E, English E. Understanding the Use of Sigma Metrics in Hemoglobin A1c Analysis. [Epub ahead of print] 2016 Clin Lab Med. doi: http://dx.doi.org/10.1016/j.cll.2016.09.006.

18. College of American Pathology External Quality Scheme for HbA1c 2016 www.ngsp.org/CAP/CAP16c.pdf. (Accessed January 2017).

19. Rohlfing C, Hanson S, Weykamp C.W., Siebelder C., Higgens T, Molinaro R., Yip P.M., Little R.R.. Effect of hemoglobin C,D,E and S traits on measurements of hemoglobin A1c by twelve methods. Clin Chim Acta 2016;455:80-3.

20. Lenters-Westra E. Common Hb-variants show no longer interference on the Tosoh G8 after an update of the software. Clin Chim Acta 2016;463:73-4.

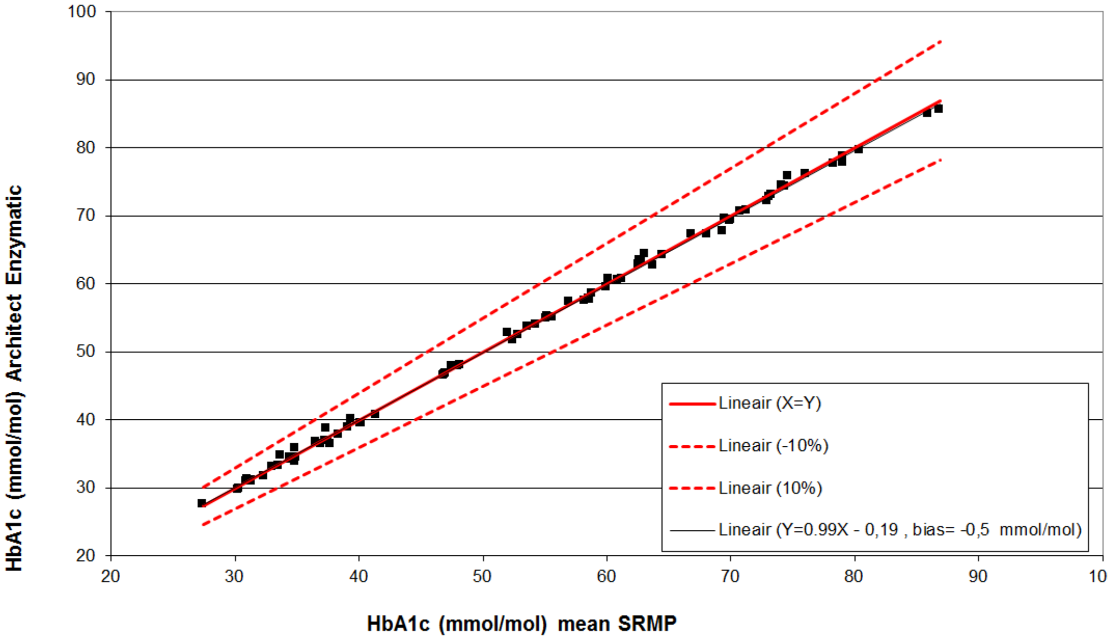


Figure 1A

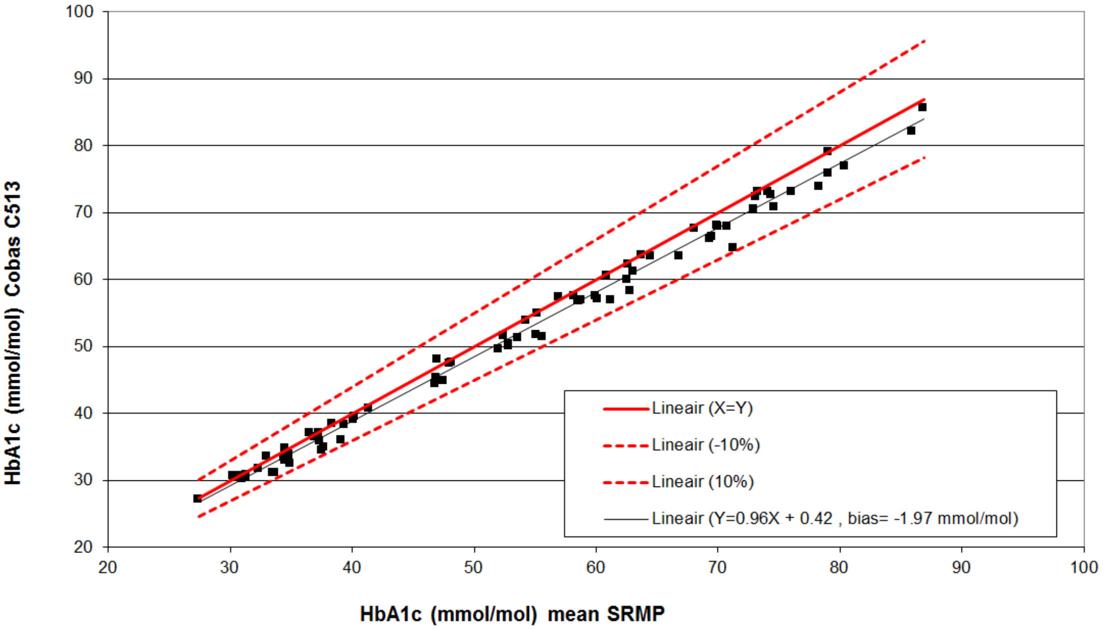


Figure 1B

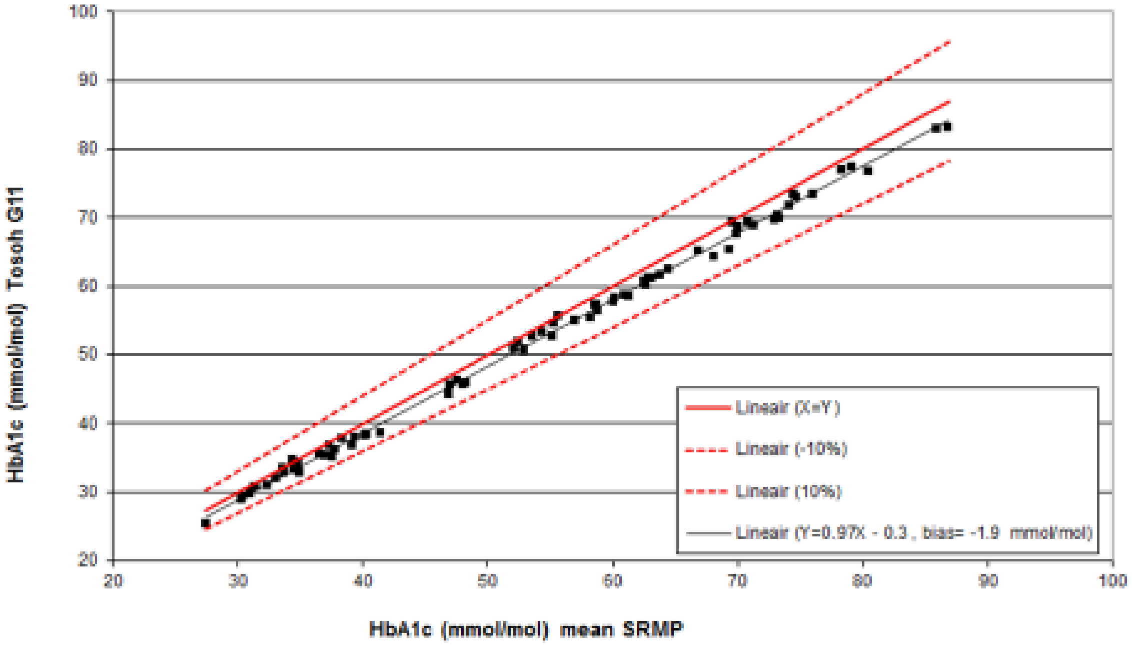


Figure 1C

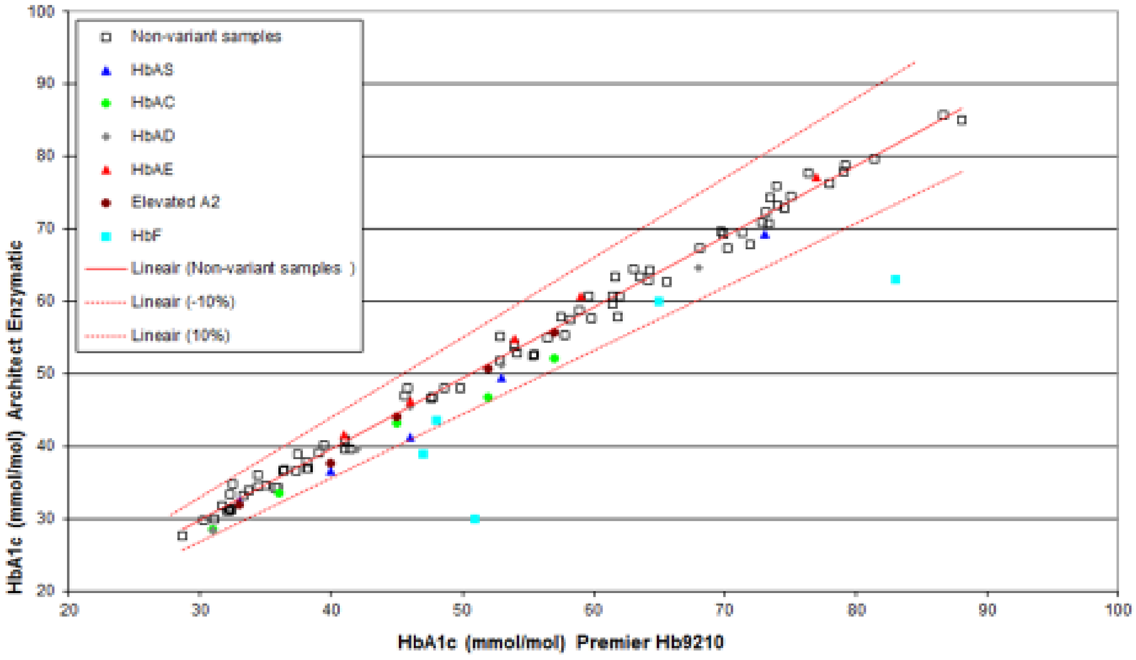


Figure 2A

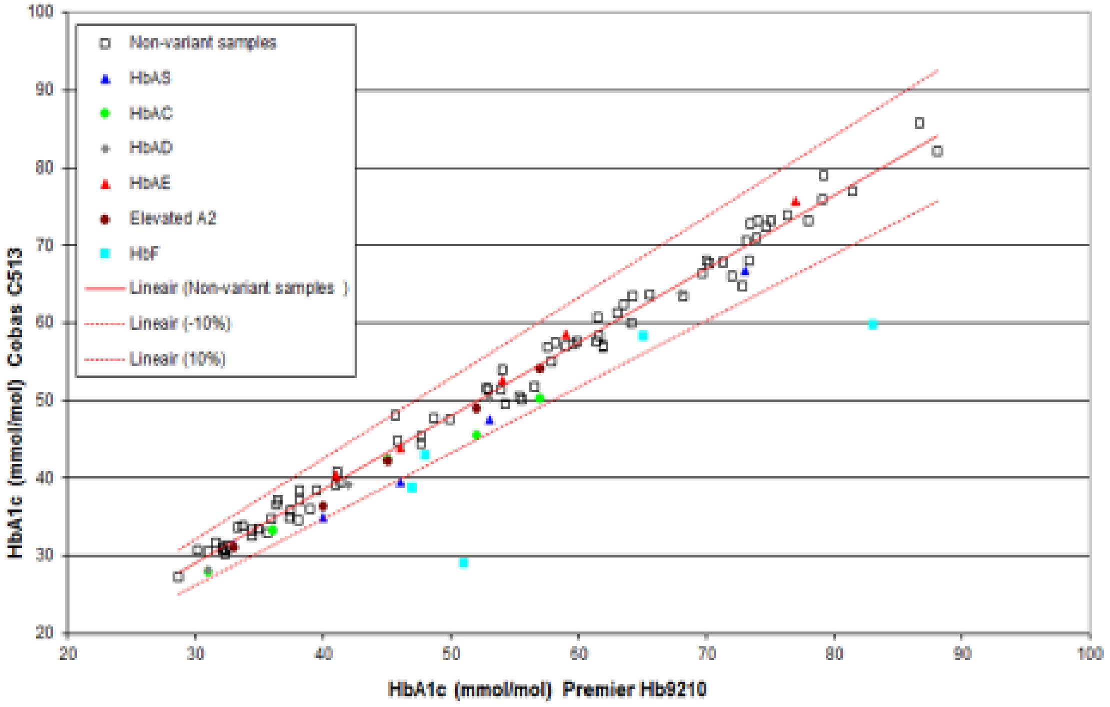


Figure 2B

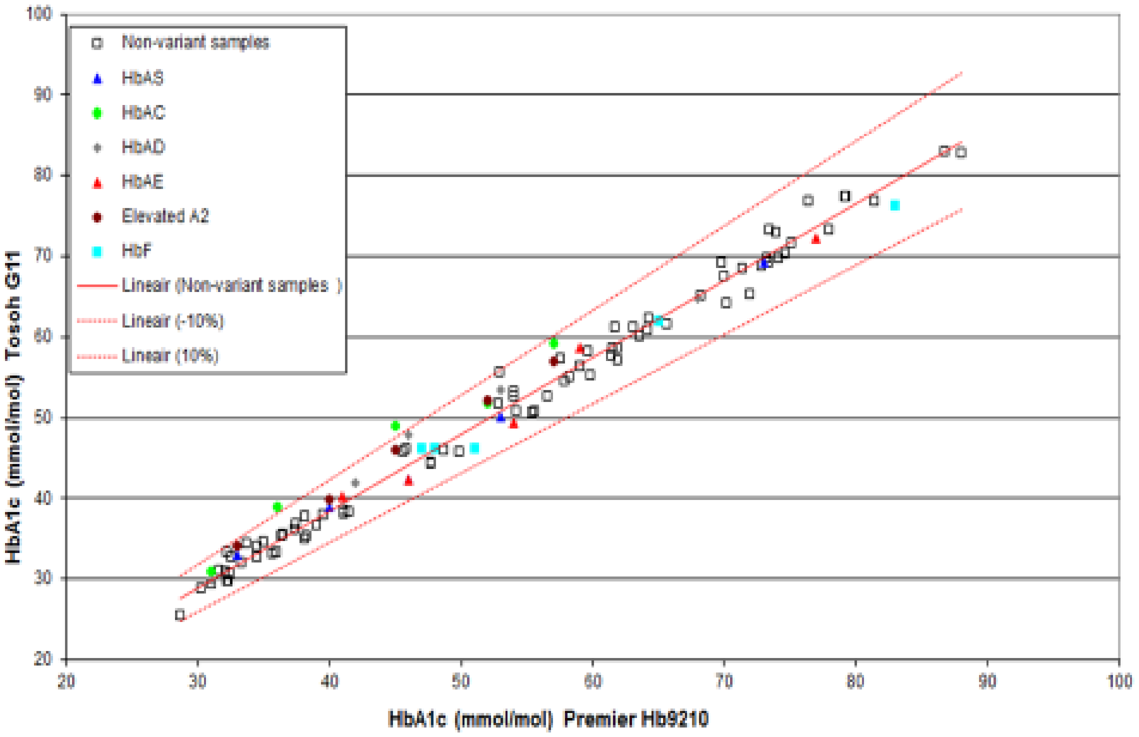


Figure 2C

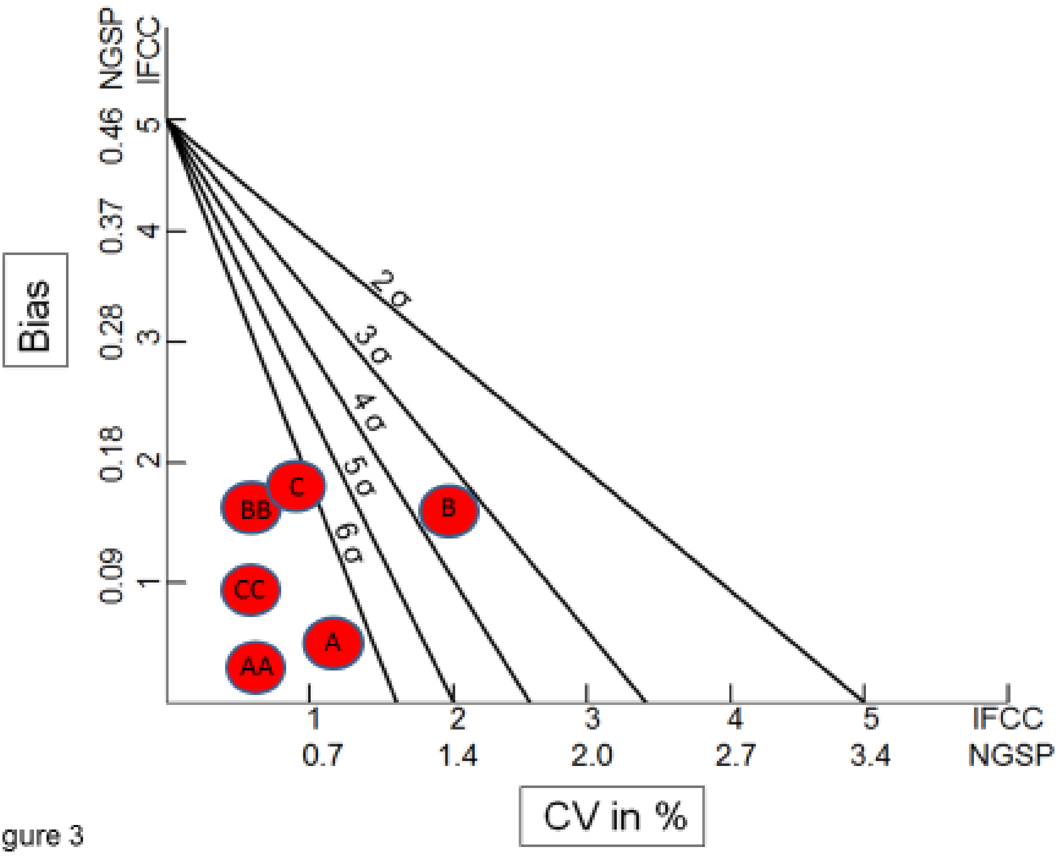


Figure 3

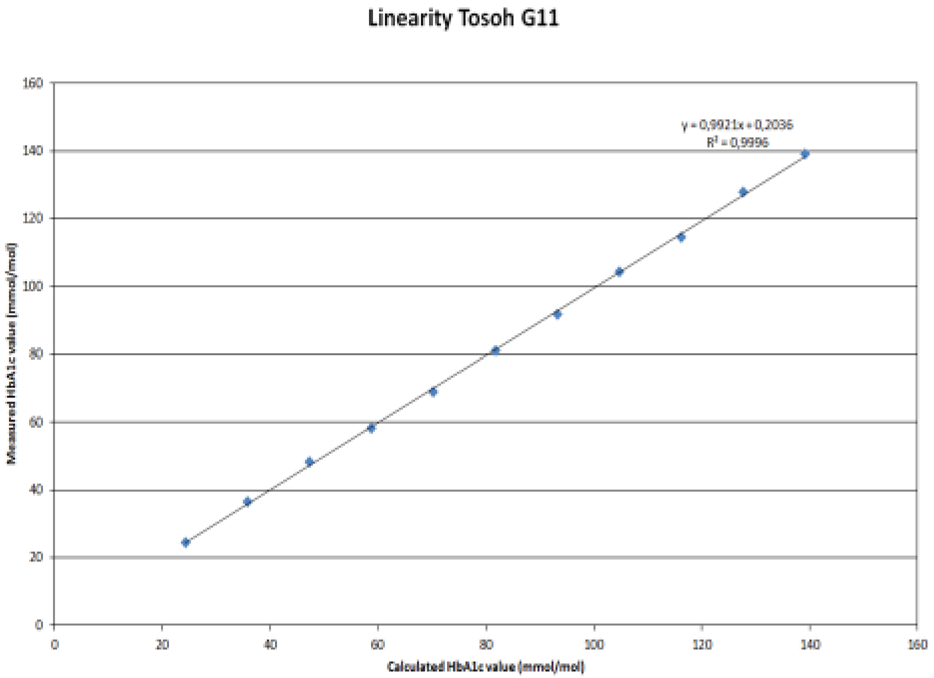
Table 1 EP-5 Imprecision results

	CV (%) SI units	CV(%) NGSP units
Abbott Enzymatic		
46.2 mmol/mol (6.38 % NGSP)	1.1	0.7
71.6 mmol/mol (8.70% NGSP)	0.9	0.6
Roche c513		
45.9 mmol/mol (6.35% NGSP)	2.0	1.3
71.9 mmol/mol (8.73% NGSP)	2.1	1.5
Tosoh G11		
45.8 mmol/mol (6.34% NGSP)	0.9	0.6
69.3 mmol/mol (8.50% NGSP)	0.6	0.4

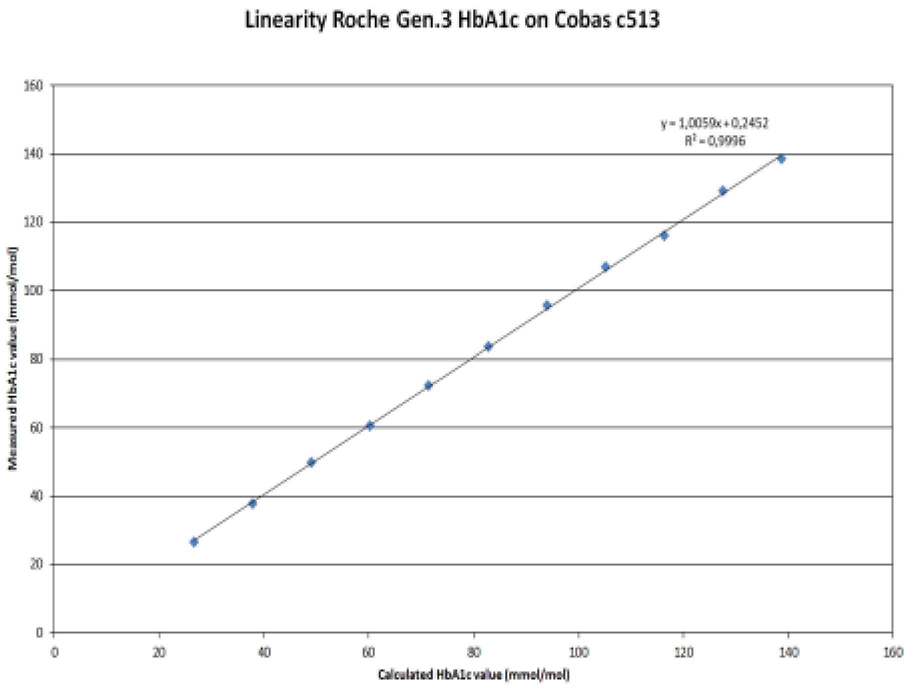
	Deming regression line Mean 6 SRMPs	CV (%) EP-5 HbA1c 46 mmol/mol	Abs. bias at 48 mmol/mol	Bias(%) at 48 mmol/mol	σ (TAE= 10%)
Abbott Architect Enzymatic	Y=0.99X – 0.19	1.1	0.5	1.0	8.2
Roche **Cobas C513 TQ**	Y=0.96X + 0.42	2.0	1.7	3.5	3.3
Tosoh G11	Y=0.97X – 0.30	0.9	1.8	3.8	6.9
	Deming regression line IFCC mon prog	CV (%) in IFCC mon program	Abs. bias at 48 mmol/mol	Bias(%) at 48 mmol/mol	σ (TAE= 10%)
Abbott Architect Enzymatic	Y=1.01X – 0.61	0.7	0.3	0.6	13.4
Roche		0.7	1.7	3.6	9.1

Cobas C513 TQ	Y=0.99X – 1.23				
Tosoh G11	Y=0.95X + 1.46	0.6	0.9	1.9	13.5

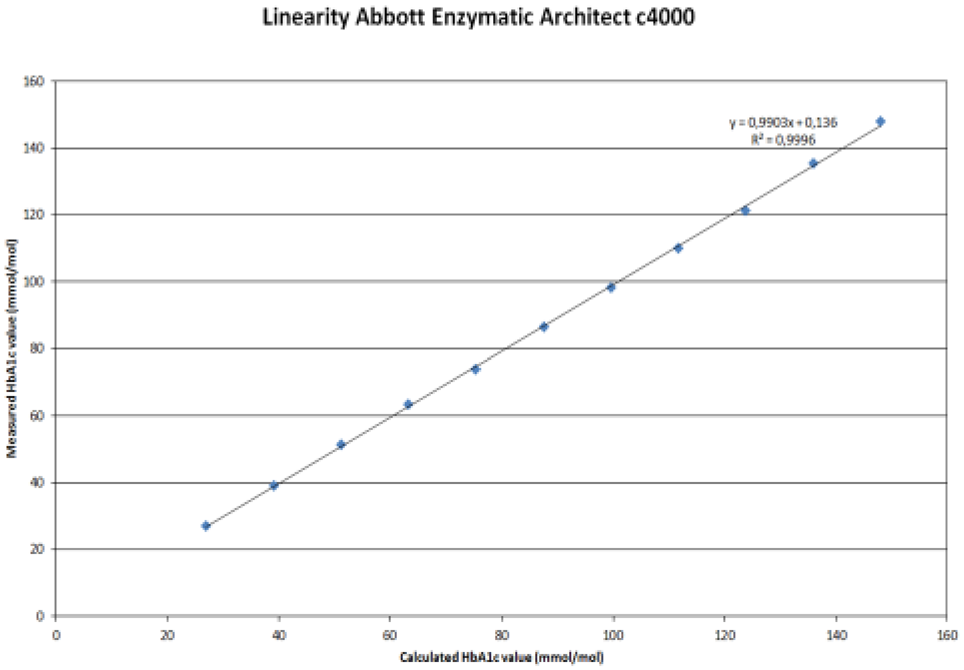
Table 2 Sigma calculated at 48 mmol/mol with TAE of 10% (σ=(TAE – B)/CV) using the method comparison results between investigated method and mean of 6 SRMPs and the results of the IFCC monitoring program.



Supplemental Figure 1C

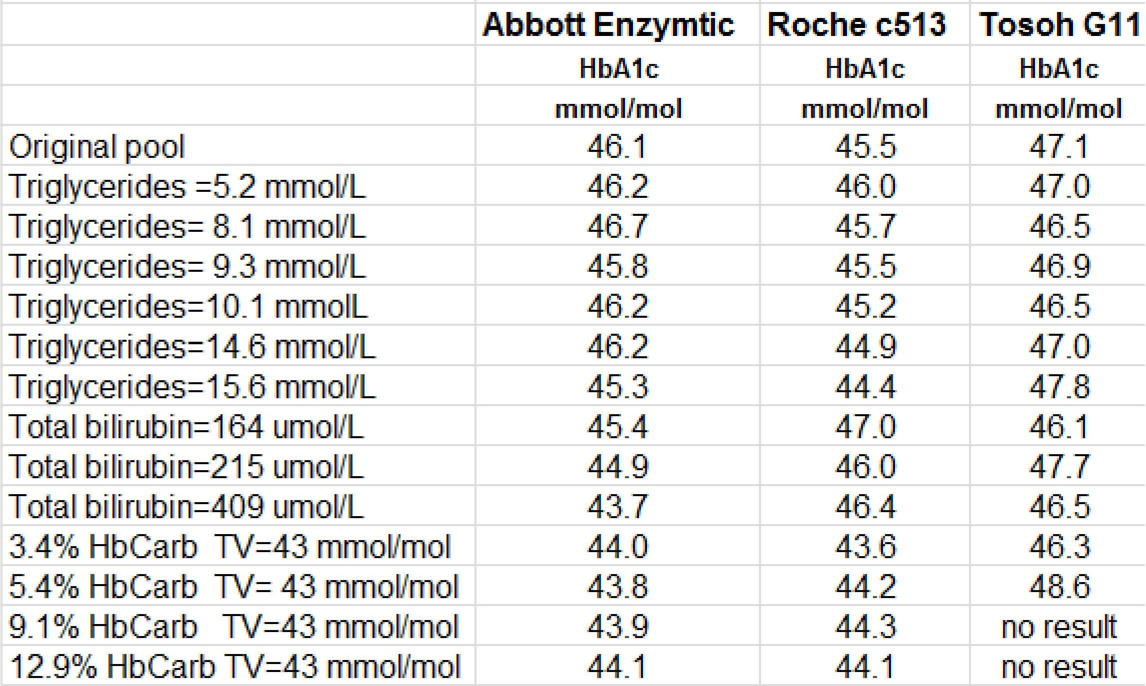

Linearity Roche Gen.3 HbA1c on Cobas c513

Supplemental Figure 1B


Linearity Abbott Enzymatic Architect c4000

Supplemental Figure 1A

Supplemental Table 3 Interferences



	Abbott Enzymtic	Roche c513	Tosoh G11
	HbA1c	HbA1c	HbA1c
	mmol/mol	mmol/mol	mmol/mol
Original pool	46.1	45.5	47.1
Triglycerides =5.2 mmol/L	46.2	46.0	47.0
Triglycerides= 8.1 mmol/L	46.7	45.7	46.5
Triglycerides= 9.3 mmol/L	45.8	45.5	46.9
Triglycerides=10.1 mmolL	46.2	45.2	46.5
Triglycerides=14.6 mmol/L	46.2	44.9	47.0
Triglycerides=15.6 mmol/L	45.3	44.4	47.8
Total bilirubin=164 umol/L	45.4	47.0	46.1
Total bilirubin=215 umol/L	44.9	46.0	47.7
Total bilirubin=409 umol/L	43.7	46.4	46.5
3.4% HbCarb TV=43 mmol/mol	44.0	43.6	46.3
5.4% HbCarb TV= 43 mmol/mol	43.8	44.2	48.6
9.1% HbCarb TV=43 mmol/mol	43.9	44.3	no result
12.9% HbCarb TV=43 mmol/mol	44.1	44.1	no result

TV=target value

		Deming regression lines	**Mean Bias**	**SEE**	**Out ± 6% SRM**	**NGSP Manfacturer criteria***	**Sigma**[#]
Abbott (Y)	vs Premier Isala (X)	Y=0.99X + 0.04	-0.05	0.12	0	Pass	9.1
Enzymatic	vs TQ Integra 800 Isala (X)	Y=0.99X + 0.05	-0.01	0.09	0	Pass	10.0
	vs Tosoh G8 Isala (X)	Y=1.01X – 0.10	-0.04	0.08	0	Pass	8.9
	vs Premier SKB (X)	Y=0.98X + 0.03	-0.08	0.12	0	Pass	8.4
	vs Menarini HA8180 SKB (X)	Y=0.99X - 0.00	-0.05	-0.06	0	Pass	9.1
	vs Sebia SKB (X)	Y=0.99X - 0.03	-0.06	0.11	0	Pass	8.7
Roche (Y)	vs Premier Isala (X)	Y=0.95X + 0.19	-0.18	0.15	5	Pass	3.6
C513	vs TQ Integra 800 Isala (X)	Y=0.96X + 0.18	-0.15	0.14	1	Pass	4.0
	vs Tosoh G8 Isala (X)	Y=0.97X + 0.06	-0.18	0.13	2	Pass	3.5
	vs Premier SKB (X)	Y=0.94X + 0.18	-0,21	0.14	3	Pass	3.2
	vs Menarini HA8180 SKB (X)	Y=0,95X + 0.14	-0.18	0.13	2	Pass	3.5
	vs Sebia SKB (X)	Y=0.96X +0.11	-0.20	0.11	1	Pass	4.4
Tosoh (Y)	vs Premier Isala (X)	Y=0.96X + 0.10	-0.17	0.15	2	Pass	7.8
G11	vs TQ Integra 800 Isala (X)	Y=0.97X + 0.10	-0.14	0.10	0	Pass	8.7
	vs Tosoh G8 Isala (X)	Y=0.98X – 0.05	-0.17	0.05	0	Pass	7.5
	vs Premier SKB (X)	Y=0.96X + 0.09	-0.21	0.15	3	Pass	7.0
	vs Menarini HA8180 SKB (X)	Y=0.97X + 0.05	-0.17	0.07	0	Pass	7.5
	vs Sebia SKB (X)	Y=0.97X + 0.01	-0.19	0.12	0	Pass	7.3

Supplemental Table 1 EP-9 (n=80) results in DCCT units and calculations of NGSP certification criteria

* 37 (74) of 40 (80) results need to be within 6% (relative) of an individual NGSP SRMP to pass certification.

[#] Sigma calculated using CV of EP-5 and bias at HbA1c value of 6.5% and total allowable error of 7.0% (σ=(TAE – B)/CV).

Supplemental Table 4 The effect of using fresh versus frozen samples in both hemolysate and whole blood modes and sedimentation of red blood cells

Abbott Enzymatic Architect c4000

	Hemolysate mode		Whole blood mode						
	Frozen whole blood	Fresh whole blood	T=0	T=30	T=60	T=90	T=120	T=150	T=180
1	26,4	26,5	25,9	26,6	26,7	26,9	26,6	26,7	26,7
2	30,3	30,6	30,2	30,6	30,6	30,6	30,6	30,8	30,9
3	37,3	37,3	36,0	36,9	37,0	37,3	37,2	37,2	37,3
4	47,3	46,1	46,9	48,0	48,1	48,3	48,0	48,1	48,4
5	59,4	59,8	59,5	60,3	60,5	60,5	60,6	60,4	60,9
6	65,6	65,7	65,7	66,4	67,0	66,9	67,0	67,0	67,1
7	77,9	78,9	79,5	80,8	81,2	81,3	81,4	81,3	81,5
8	90,2	90,5	90,3	91,3	92,3	92,3	92,5	92,3	92,6
9	103,4	103,5	104,0	104,8	105,9	105,7	106,0	106,0	106,2
X-mean	**59,7**	**59,9**	**59,8**	**60,6**	**61,0**	**61,1**	**61,1**	**61,1**	**61,3**
p value		0,99	0,99	0,95	0,93	0,92	0,92	0,92	0,91

Roche Cobas c513

	Hemolysate mode		Whole blood mode							
	Frozen whole blood	Fresh whole blood	T=0	T=30	T=60	T=90	T=120	T=150	T=180	after 24 hours
1	26,3	26,5	26,5	26,8	26,9	27,4	27,5	27,4	26,8	27,4
2	30,4	30,9	31,2	30,9	31,1	31,3	31,5	31,6	31,5	32,0
3	36,4	36,4	36,7	38,0	37,9	37,7	38,1	38,2	38,2	39,1
4	46,5	46,6	47,0	49,1	49,9	50,1	49,6	50,2	49,5	50,4
5	57,8	58,4	60,3	61,3	62,3	61,0	61,3	61,3	60,5	60,4
6	63,4	64,5	65,4	67,9	67,9	67,8	68,4	67,2	67,6	66,2
7	78,5	79,7	80,5	76,8	76,7	76,3	76,1	76,2	76,2	76,8

8	88,9	89,0	91,0	87,0	86,5	85,6	87,6	87,1	85,9	88,3
9	100,2	101,3	102,3	99,3	98,8	100,4	99,3	99,3	100,1	114,8
X-mean	**58,7**	**59,3**	**60,1**	**59,7**	**59,8**	**59,7**	**59,9**	**59,8**	**59,6**	**61,7**
p value		0,97	0,95	0,97	0,98	0,98	0,99	0,98	0,97	0,90

Tosoh G11

	Hemolysate mode		Whole blood mode						
	Frozen whole blood	Fresh whole blood	T=0	T=30	T=60	T=90	T=120	T=150	T=180
1	23,1	23,5	23,7	23,7	23,7	22,9	23,3	22,9	23,2
2	29,1	29,2	29,3	29,1	28,9	29,3	29,4	29,4	29,3
3	37,0	37,6	38,8	38,6	38,2	38,4	39,0	38,7	38,5
4	47,5	48,3	48,2	48,3	47,7	47,6	48,1	48,0	47,8
5	57,7	58,4	58,4	58,4	58,6	58,0	58,2	58,3	58,4
6	65,0	65,8	65,9	65,7	65,6	65,7	65,9	66,0	65,4
7	76,8	76,9	77,4	76,6	76,5	76,9	76,6	77,0	76,7
8	88,3	87,3	88,0	88,1	87,5	88,2	88,2	87,8	88,0
9	99,0	99,2	98,9	99,1	99,6	99,6	99,6	99,3	99,7
X-mean	**58,2**	**58,5**	**58,7**	**58,6**	**58,5**	**58,5**	**58,7**	**58,6**	**58,6**
p value		0,98	0,98	0,99	0,98	0,99	1,00	0,99	0,99