WEQAS

Maintaining
Quality in
Laboratory
Medicine

Glycated Haemoglobin Scheme Guide

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UKAS PROFICIENCY TESTING 4301

UKAS CALIBRATION 4301

Contents

1. Scheme details and repertoire

1.1 Source Material and Serum Integrity

Different material is supplied every alternate month.

1 ***Stable lyophilised human whole blood*** supplied in 0.1ml ampoules, distributed every two months. These samples are pooled donations. A customised batch last approximately 12 months and consists of eight levels covering the pathological range. The samples have a long shelf life of 1 to 2 years at 4C and once reconstituted are stable for 48 hours. They provide an assessment of a laboratory's assay performance over time whereby each level is analysed on 3 occasions over the course of a year (every 4 months). This is an important factor for all assays where treatment management is based on determining the significance of change in a patient's result over time.

2 ***Fresh EDTA whole blood*** from individual diabetic patients and healthy volunteers supplied as 0.3ml sterile aliquots distributed every alternate month. These samples reflect the wide range of HbA1c seen in the Type 2 diabetic patients. Samples from normal healthy volunteers are also distributed to ensure that the concentration near the diagnostic "cut off" of 48 mmol/mol Hb, used for the diagnosis of diabetes, is achieved.

The degree of carbamylation (from urea), acetylation (from salicylate), iron status, other glycated products and degree of lipaemia will affect different methods in different ways. Carbamylated Hb is one of the major interfering substances. At normal urea levels, there is approximately 0.4% carbamylated Hb and this increases by between 1 and 2% at urea concentrations of 15 to 30mmol/l. Both HPLC and electrophoresis methods give falsely elevated results. The fresh whole blood samples are from diabetic volunteers and will be subject to the same potential interferences listed above.

Haemoglobin in fresh EDTA whole blood samples deteriorate over time resulting in abnormal chromatograms with varying and unpredictable effects on the different analytical methods. These samples have therefore a short shelf life and can only be distributed on one occasion.

3 Samples from patients with known ***Hb variants*** are also distributed on a regular basis. Measurement of HbA_{1c} is dependent on the haemoglobin circulating being predominantly HbA. Hb variants will produce different effects with different methods. Being able to identify and account for abnormal haemoglobins is even more important when using HbA1c for diagnosis.

1.2 List of Analytes and Frequency of Distribution

Frequency: Every month

Four lyophilised samples are sent out every two months with two fresh patient samples sent out on alternative months.

Analyte	**Approximate. Range Covered**	**Units**
HbA1c	35-80	mmol/mol Hb

2. Instructions for use

Although every effort is made to ensure that the material is free from any known infectious agent, the samples should be handled as for clinical specimens.

2.1 Fresh Whole blood samples

The samples should be well mixed prior to use and assayed within 3 days of dispatch**.**

2.2 Lyophilised Samples

1. Remove metal cap.
2. Lift rubber stopper carefully.
3. Add 0.1ml distilled water.
4. Replace stopper and allow to stand for 15 minutes.
5. Hold vial under an angle of 45 and swirl gently for 30 seconds, the liquid will flow round the bottom of the vial without touching the stopper and become homogeneous.
6. Allow vial to stand for 15 minutes.
7. Repeat step 5.
8. Process the sample as whole blood for the assay used.

Storage and Stability

The stability of the reconstituted product is 48hrs when the vials are properly stoppered after use and stored at 2 to 8ºC.

3 Standardisation of HbA1c

3.1 Reference Methods

3.1.1 National Glycohaemoglobin Standardization Programme (NGSP). The NGSP HbA1c standardization program began in 1996 and was modelled on the Cholesterol Reference Method Laboratory Network program. Its purpose was to standardize glycated haemoglobin test results so that clinical laboratory results were comparable to those reported in the Diabetes Control and Complications Trial (DCCT) where relationships to mean blood glucose and risk for vascular complications had been established.

3.1.2 The International Federation of Clinical Chemistry Working Group (IFCC-WG) on HbA1c Standardization was established in 1965 to develop a reference system for HbA1c which included the definition of the analyte, development of a primary reference material, development of primary reference methods and establishment of reference network laboratories. Two reference methods were developed which specifically measure the glycated N-terminal residue of the beta-chain. The principle is that in a first step haemoglobin is cleaved into peptides by a proteolytic enzyme and thereafter the specific glycated and non-glycated N-terminal peptides of the beta-chain are measured by HPLC and either mass spectrometry or capillary electrophoresis. These reference methods were approved by the IFCC in July 2001.

Over the last decade, the relationship between HbA1c results from the NGSP network and the IFCC network has been evaluated and a master equation has been developed IFCC-HbA1c (mmol/mol) = [DCCT-HbA1c (%) - 2.15] x 10.929**. (NGSP = [0.09148 * IFCC] + 2.152).** This relationship will continue to be monitored and any changes will be investigated. The NGSP certification process, will continue to be directly traceable to the DCCT reference and now also the IFCC reference method.

For HbA1c the target values in the WEQAS Scheme are assigned using IFCC secondary reference methods in an IFCC network laboratory.

3.1.3 2010 Consensus Statement on the Worldwide Standardization of HbA1c

The American Diabetes Association, the European Association for the Study of Diabetes, the International Diabetes Federation, the International Federation of Clinical Chemistry and the International Society for Paediatric and Adolescent Diabetes recently published an updated consensus statement regarding HbA1c standardization (the original consensus statement was issued in 2007). The recommendations are:

1. HbA1c test results should be standardised worldwide, including the reference system and results reporting.
2. The IFCC reference system for HbA1c represents the only valid anchor to implement standardisation of the measurement.
3. HbA1c results are to be reported by clinical laboratories worldwide in SI (Système International) units (mmol/mol – no decimals) and derived NGSP units (% - one decimal), using the IFCC-NGSP master equation (DCCT units).
4. HbA1c conversion tables including both SI (IFCC) and NGSP units should be easily accessible to the diabetes community.
5. Editors of journals and other printed material are strongly recommended to require that submitted manuscripts report HbA1c in both SI (IFCC) and NGSP/DCCT units.
6. The reportable term for glycated haemoglobin is HbA1c, although other abbreviations may be used in guidelines and educational material (A1C).
7. The above consensus recommendations apply through 2011, when they will be discussed again at the next consensus meeting at the IDF meeting in Dubai December 2011.

3.2 Change in units for HbA1c - UK Consensus Statement

From 1st October 2011 all UK laboratories changed to reporting in SI units of millimoles of HbA1c per mole of Hb (mmol/mol). Prior to that date laboratories provided two sets of results (% and mmol/mol) when reporting a patient's HbA1c value. To reinforce this change in the UK, from December 2011, WEQAS no longer accepted results as Hb %.

4 HbA1c in the Diagnosis of Diabetes Mellitus

4.1 WHO Recommendation (Summary)

A WHO expert consultation was held from 28 to 30 March 2009. A systematic review was conducted on the use of HbA1c as a diagnostic test for diabetes mellitus. The evidence was summarized and its quality evaluated using the GRADE methodology.

HbA1c can be used as a diagnostic test for diabetes providing that stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement.

An HbA1c of **48 mmol/mol (6.5%)** is recommended as the cut point for diagnosing diabetes. A value of less than 48 mmol/mol (6.5%) does not exclude diabetes diagnosed using glucose tests.

4.2 Use of Haemoglobin A1c (HbA1c) in the diagnosis of diabetes mellitus in the UK - The implementation of World Health Organisation (WHO) guidance 2011

Summary
An expert group have discussed the WHO report. The group agree that the WHO requirements are met in the UK. HbA1c is not suitable for use in everyone. Do not use HbA1c to diagnose diabetes in pregnancy.

The test
Analysis of venous HbA1c in UK laboratories participating in national quality assurance schemes currently fulfils WHO requirements. HbA1c should usually be measured on a laboratory venous blood sample. Point of Care HbA1c should not be used for diagnosis unless the healthcare staff have been appropriately trained and the HbA1c method used can demonstrate an internal quality control and external quality assessment performance that matches that of a laboratory method. Confirm a point-of-care diabetes diagnosis with laboratory venous HbA1c.

Most patients
HbA1c ≥48 mmol/mol can be used to diagnose diabetes in most situations. In patients without diabetes symptoms repeat venous HbA1c in the same lab within 2 weeks. If the second sample is <48 mmol/mol (6.5%) treat as high risk of diabetes and repeat the test in 6 months or sooner if diabetes symptoms develop. In symptomatic adults with relatively slow onset of symptoms a single result ≥48 mmol/mol will suffice.

Situations where HbA1c must not be used as the sole test to diagnosis diabetes
HbA1c reflects glycaemia over the preceding 2 – 3 months so may not be raised if blood glucose levels have risen rapidly. Examples:

ALL symptomatic children and young people
Symptoms suggesting Type 1 diabetes (any age)
Short duration diabetes symptoms
Patients at high risk of diabetes who are acutely ill
Taking medication that may cause rapid glucose rise e.g. corticosteroids, antipsychotics
Acute pancreatic damage/pancreatic surgery

Do an immediate quality-assured finger-prick capillary glucose test. Check blood/urine ketones if available. If glucose is >11.0 mmol/l seek same-day specialist diabetes advice. For children and teenagers phone the specialist paediatric diabetes team same day. Send same day laboratory venous glucose, adding HbA1c to exclude stress hyperglycaemia and/or for baseline, but do not delay seeking advice whilst awaiting the result.

Presence of factors that influence HbA1c and its measurement
See Annex 1 from WHO report. Discuss the patient with your local laboratory or specialist diabetes team or use glucose testing. Factors include abnormal haemoglobins, anaemia, altered red blood cell lifespan.

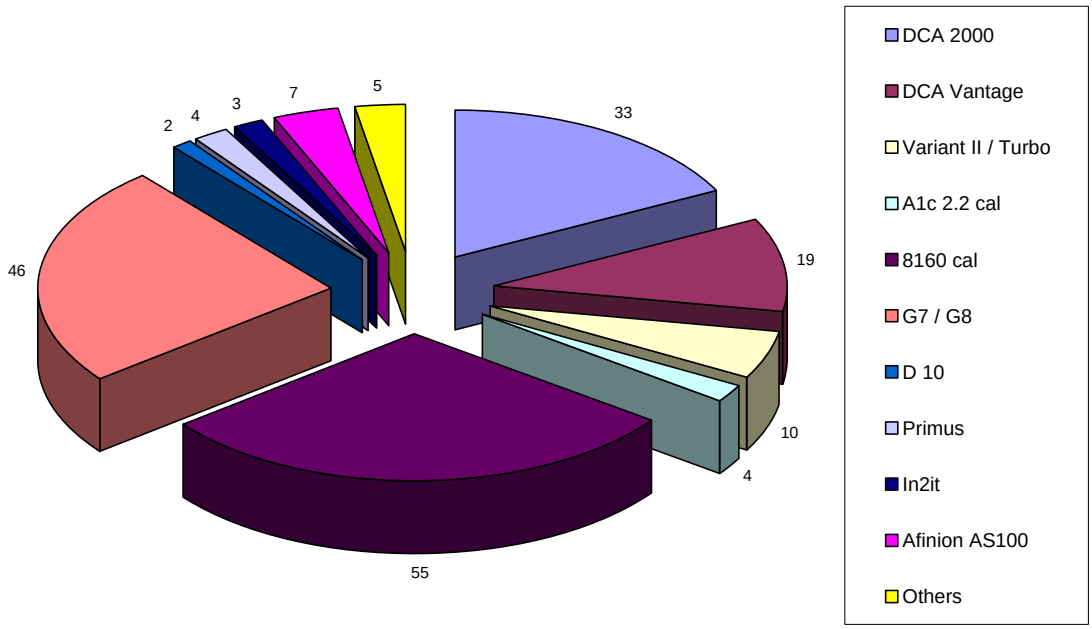
5. Statistical Analysis

Please refer to the accompanying Participants Manual for full details on statistical analysis and interpretation of results.

5.1 WEQAS Performance criteria

For HbA1c performance criteria are based on biological variation and clinical need. Acceptable performance has therefore been defined as ± 7% from the IFCC target value.

5.2 Distribution of analysers used in the UK – November 2010



5.3 Method Performance

5.3.1 Method Summary report.

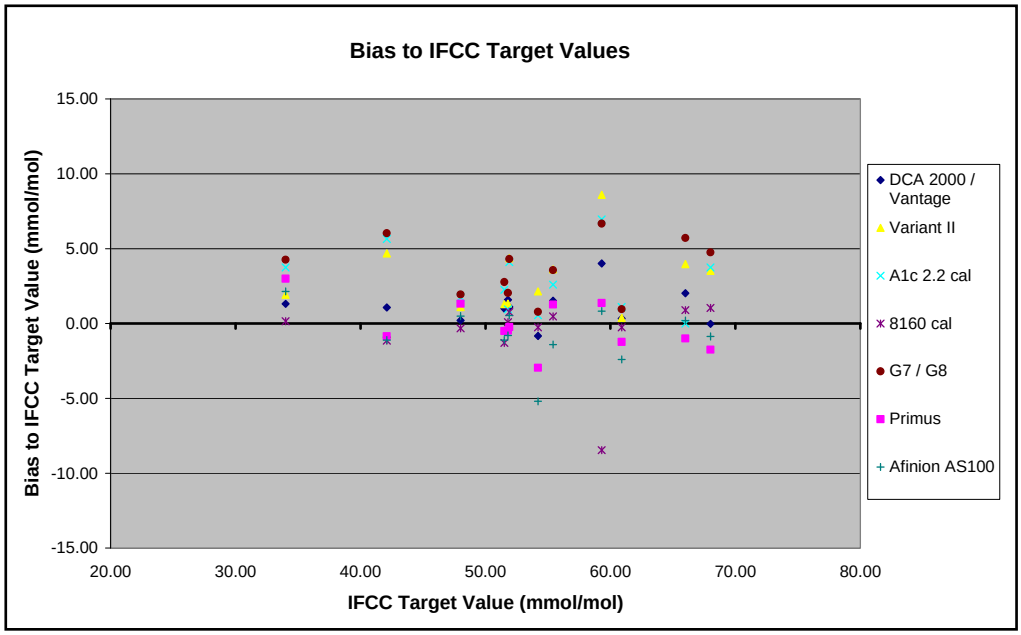
The data represents a typical distribution of results for two fresh whole blood samples. This table is provided for each distribution.

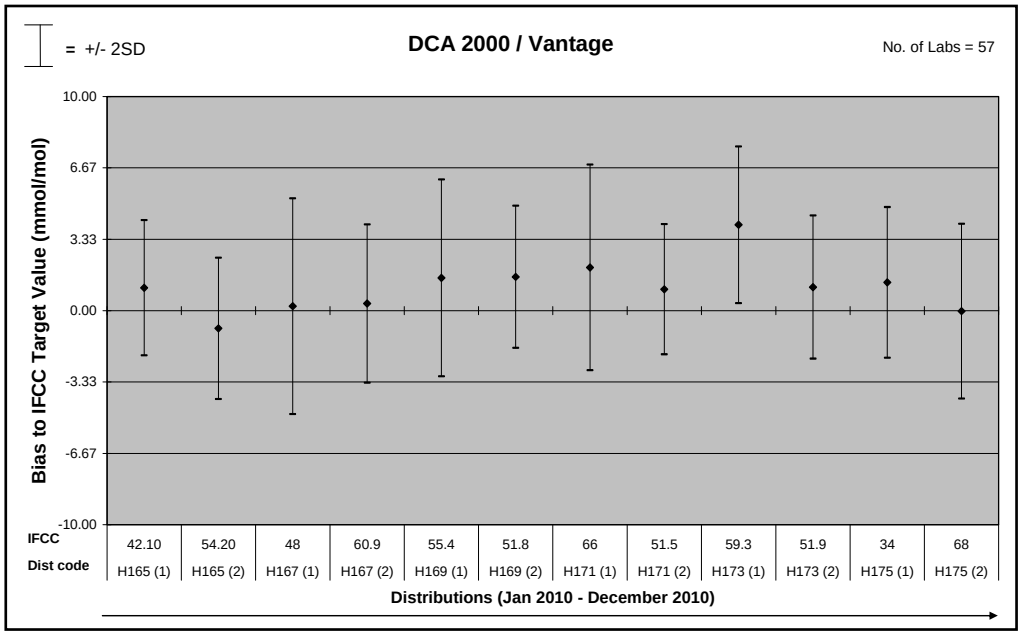
Distribution:	H173
Distribution Date:	21-Sep-10
Analyte:	HbA1c IFCC (mmol/mol)

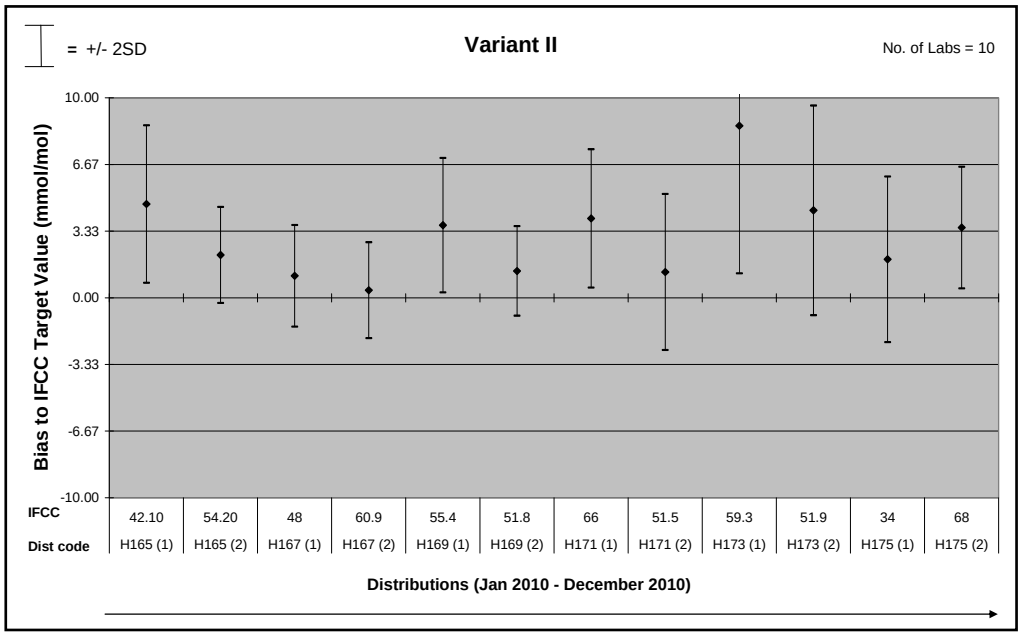
Method	Instrument		1	2
		Overall Mean	60.35	53.75
		Overall SD	6.68	2.25
		Overall Number	182	177
		Reference Value	59.3	51.9
HPLC		Method Mean	59.2	54.43
		Method SD	8.01	2.16
		Number	114	112
	Variant II / Turbo	Instrument Mean	67.9	56.27
		Instrument SD	3.69	2.62
		Number	10	10
	A1c 2.2 cal	Instrument Mean	66.25	56
		Instrument SD	2.28	1.22
		Number	4	4
	8160 cal	Instrument Mean	50.84	52.66
		Instrument SD	1.65	0.91
		Number	50	50
	G7 / G8	Instrument Mean	65.98	56.21
		Instrument SD	1.44	0.99
		Number	45	44
Affinity		Method Mean	60.07	51.44
		Method SD	2.57	1.84
		Number	15	16
	Primus	Instrument Mean	60.67	51.67
		Instrument SD	2.62	1.89
		Number	3	3
	In2it	Instrument Mean	55.67	49.67
		Instrument SD	2.05	1.25
		Number	3	3
	Afinion AS100	Instrument Mean	60.13	52.44
		Instrument SD	2.26	2.11
		Number	8	9

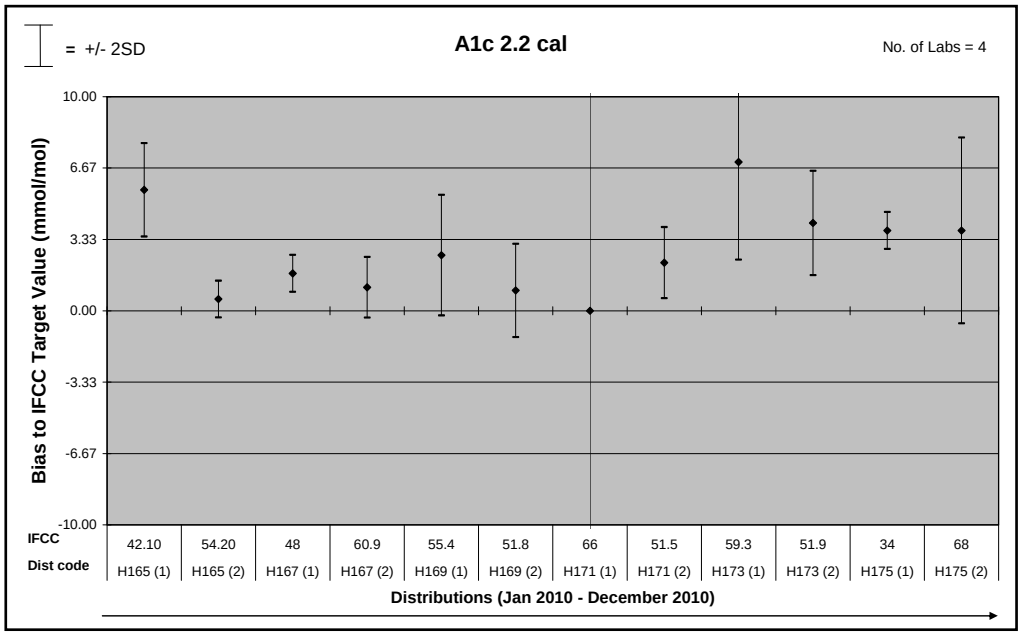
5.3.2 Accuracy

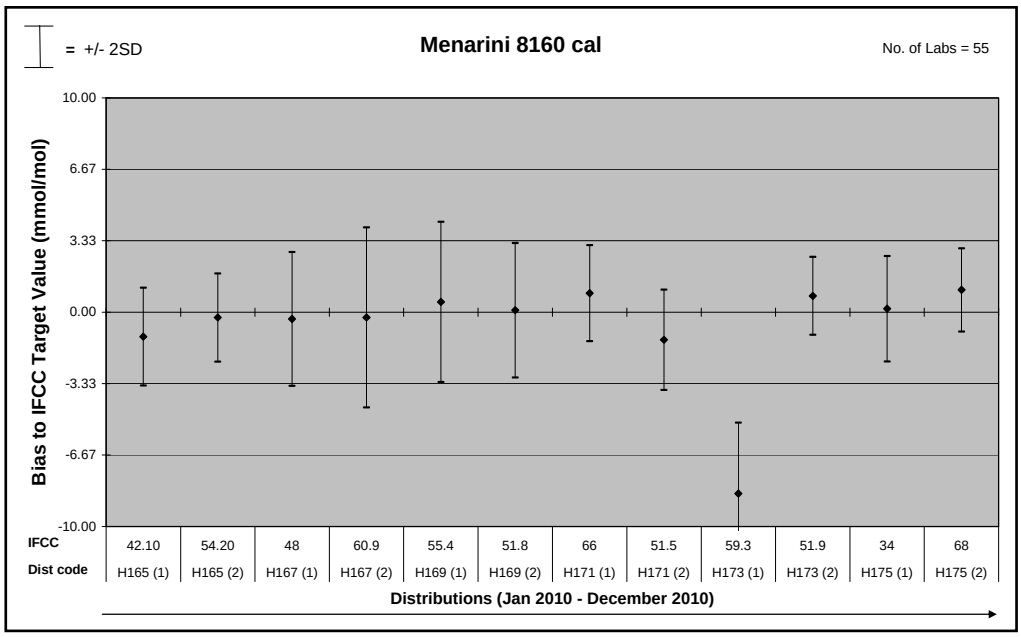
The data represents the method bias compared with the IFCC reference values for the patient samples analysed from January 2010 to November 2010.

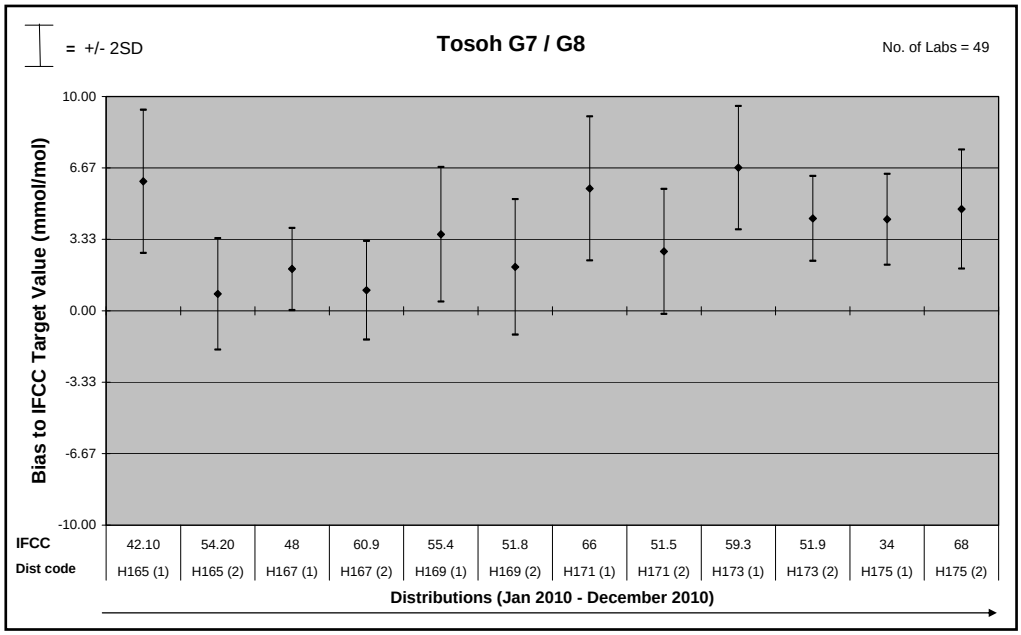


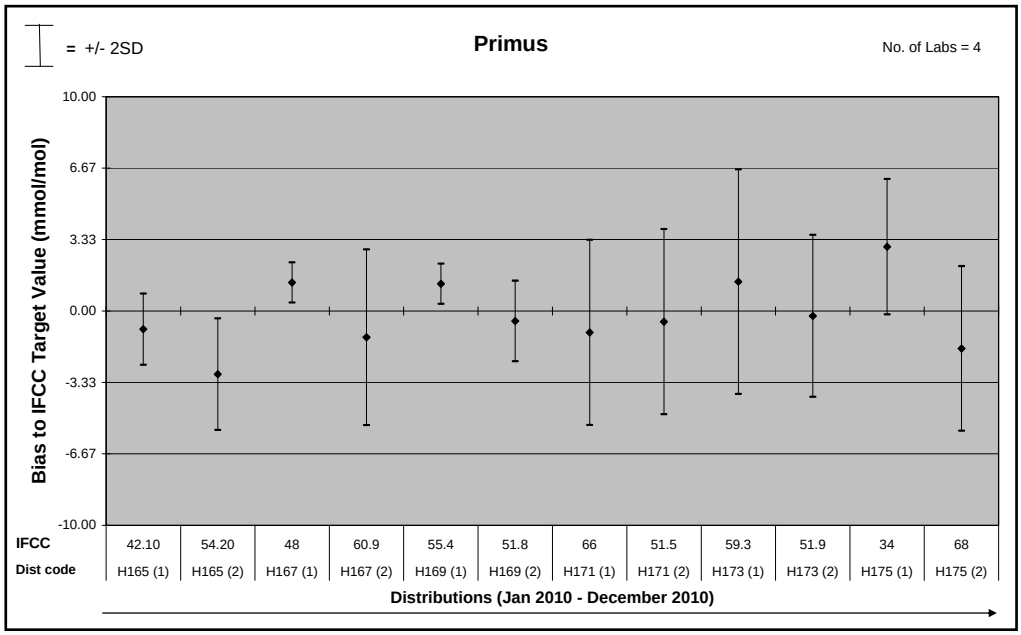


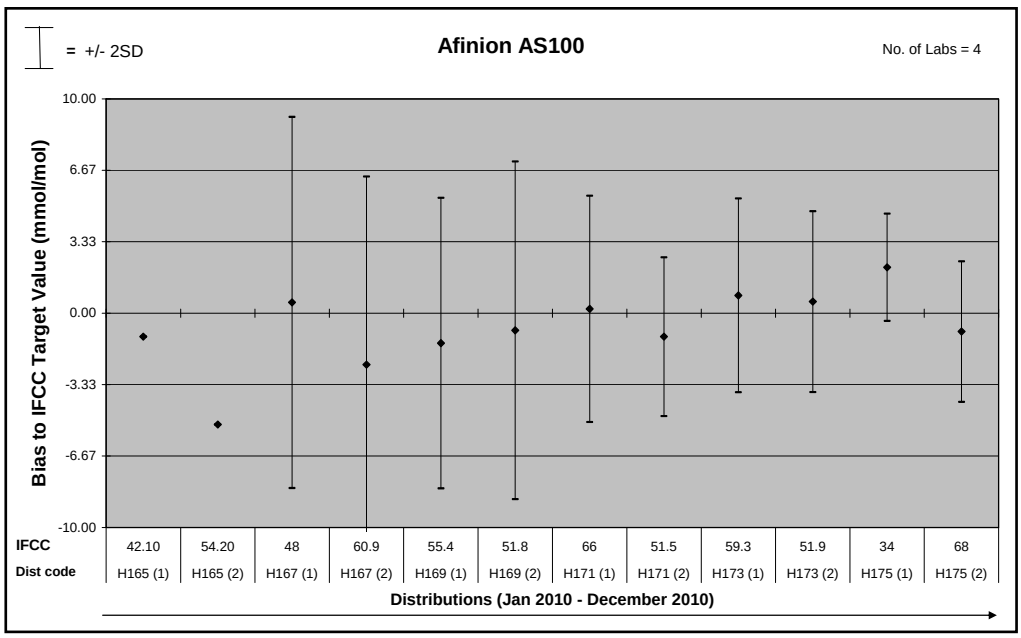

= +/- 2SD
Variant II
No. of Labs = 10
Bias to IFCC Target Value (mmol/mol)
10.00
6.67
3.33
0.00
-3.33
-6.67
-10.00
IFCC
42.10 54.20 48 60.9 55.4 51.8 66 51.5 59.3 51.9 34 68
Dist code
H165 (1) H165 (2) H167 (1) H167 (2) H169 (1) H169 (2) H171 (1) H171 (2) H173 (1) H173 (2) H175 (1) H175 (2)
Distributions (Jan 2010 - December 2010)


= +/- 2SD
A1c 2.2 cal
No. of Labs = 4
Bias to IFCC Target Value (mmol/mol)
10.00
6.67
3.33
0.00
-3.33
-6.67
-10.00
IFCC
42.10 54.20 48 60.9 55.4 51.8 66 51.5 59.3 51.9 34 68
Dist code
H165 (1) H165 (2) H167 (1) H167 (2) H169 (1) H169 (2) H171 (1) H171 (2) H173 (1) H173 (2) H175 (1) H175 (2)
Distributions (Jan 2010 - December 2010)

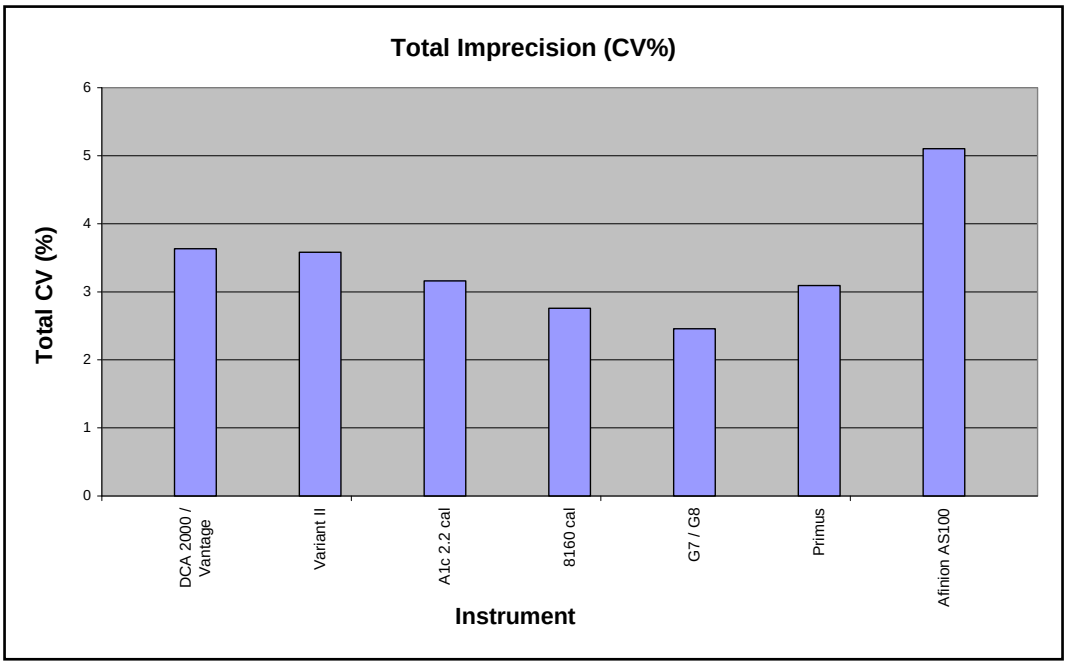

= +/- 2SD
Menarini 8160 cal
No. of Labs = 55
Bias to IFCC Target Value (mmol/mol)
10.00
6.67
3.33
0.00
-3.33
-6.67
-10.00
IFCC
42.10 54.20 48 60.9 55.4 51.8 66 51.5 59.3 51.9 34 68
Dist code
H165 (1) H165 (2) H167 (1) H167 (2) H169 (1) H169 (2) H171 (1) H171 (2) H173 (1) H173 (2) H175 (1) H175 (2)
Distributions (Jan 2010 - December 2010)


= +/- 2SD
Tosoh G7 / G8
No. of Labs = 49
Bias to IFCC Target Value (mmol/mol)
10.00
6.67
3.33
0.00
-3.33
-6.67
-10.00
IFCC
42.10 54.20 48 60.9 55.4 51.8 66 51.5 59.3 51.9 34 68
Dist code
H165 (1) H165 (2) H167 (1) H167 (2) H169 (1) H169 (2) H171 (1) H171 (2) H173 (1) H173 (2) H175 (1) H175 (2)
Distributions (Jan 2010 - December 2010)


= +/- 2SD
Primus
No. of Labs = 4
Bias to IFCC Target Value (mmol/mol)
10.00
6.67
3.33
0.00
-3.33
-6.67
-10.00
IFCC
42.10 54.20 48 60.9 55.4 51.8 66 51.5 59.3 51.9 34 68
Dist code
H165 (1) H165 (2) H167 (1) H167 (2) H169 (1) H169 (2) H171 (1) H171 (2) H173 (1) H173 (2) H175 (1) H175 (2)
Distributions (Jan 2010 - December 2010)


= +/- 2SD
Afinion AS100
No. of Labs = 4
Bias to IFCC Target Value (mmol/mol)
10.00
6.67
3.33
0.00
-3.33
-6.67
-10.00
IFCC
42.10 54.20 48 60.9 55.4 51.8 66 51.5 59.3 51.9 34 68
Dist code
H165 (1) H165 (2) H167 (1) H167 (2) H169 (1) H169 (2) H171 (1) H171 (2) H173 (1) H173 (2) H175 (1) H175 (2)
Distributions (Jan 2010 - December 2010)

5.3.3 Interlaboratory Variation



The data reflects the interlaboratory variation for the patient samples distributed from January 2010 to November 2010. The interlaboratory variation was calculated as the coefficient of variation for the samples for each *analyser* over this period. Acceptable interlaboratory variation (<5%) was observed for the majority of methods.

6 References

1 Kenny D, Fraser CG, Hyloft Petersen P, Kallner A. Strategies to set global analytical quality specifications in Laboratory Medicine-Consensus agreement. *Scan J Clin Lab Invest* 1999; 59:585

2 Lytken Larsen M, Fraser CG, Hyloft Petersen P. A comparison of analytical goals for haemoglobinA1c assays derived using different strategies. *Ann Clin Biochem* 1991; 28: 272-278

3 Peterson PH, Larsen ML, Horder M. prerequisites for the maintenance of a certain state of health by biochemical monitoring, in Harris EK, Yasaka T (ed): maintaining a healthy state within the individual. Amsterdam, Elsevier science publishers, 1987; ppl147-158.

4 Ricos C et al. Current databases on biological variation: pros, cons and progress. *Scand J Clin Invest* 1999; 59: 491-500.

5 Harris EK. Statistical principles underlying analytical goal setting in clinical chemistry. *Am J Clin Pathol*; 1979, 72: 374-382.

6 The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependant diabetes mellitus. *N Eng J med* 1993; 329: 977-986.

7 UK prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared in patients with type 2 diabetes. (UKPDS 33). *Lancet* 1998; 352: 837-853.

8 Baynes JW, Bunn HF, Goldstein D, Harris M, Martin DB, Peterson C, Wintherhaller K. National Diabetes Data Group: Report of the Expert Committee on Glucosylated Haemoglobin. *Diabetes care* 1984; 7: 606-606.

9 Marshall SM, Barth JH. Standardization of HbA1c measurements: a consensus statement. *Ann Clin Biochem* 2000; 37:45-46.

10 Goldstein DE, Little RR, England JD, Wiedmeyer HM, McKenzie EM. Methods for quantitating glycosylated haemoglobins: high performance liquid chromatography and thiobarbituric acid colorimetry. In: Clarke WL, Larner J, Pohl SL eds *Methods in diabetes research*, Vol 2: Clinical Methods. New York: John Wiley, 1986: 475-504.

11 Weycamp CW, Penders TJ, Muskiet FAJ, et al. Influence of haemoglobin variants and derivatives on glycohaemoglobin determination as investigated by 102 laboratories using 16 methods. *Clin Chem* 1993; 39: 1717-1723.

12 Thomas MA, Davies KW. Standardisation of HbA1c, current performance in the UK. *Clin Chem Lab Med* 1999; 37: (S299): T327 (abstract).

13 Little RR, Wiedmeyer H-S, England JD, et al. Interlaboratory Standardisation of measurements of glycohaemoglobins. *Clin Chem* 1992; 38: 2472-2478.

14 WeyKamp CW, Penders TT, Muskiet FAJ, Van der Slik W. Glycohaemoglobin: comparison of 12 analytical methods, applied to lyophilised haemolysates by 101 laboratories in an external quality assurance programme. *Ann Clin Chem*; 30: 169-174.

15 Little RR, Myers GL, Nathan D, Steffes MW, Gunter EW, Messenger LJ, et al. Implementation of the National Glycohaemoglobin Standardisation program (NGSP) in the US. *Clin chem. Lab Med* 1999; 37: (S186): M274 (abstract).

16 Hoelzel W, Miedema K. development of a reference system for international standardization of HbA1c / glycohaemoglobin determinations. *JIFCC 1996; 9: 62-67.*

17 Finke A, Kobold U, Hoelzel W, Weykamp C, Miedema K, Jeppsson JO. Preparation of a candidate primary reference material for the international standardisation of HbA1c determinations. *Clin Chem Lab Med* 1998; 36: 299-308.

18 Kobold U, Jeppsson JO, Dulfer Th, Finke A, Hoelzel W, Miedema K. Candidate Reference Methods for HbA1c based on Peptide Mapping. *Clin Chem* 1997; 43: 1944-1951.

19 Keppsson JO, Hoelzel W, kobold U, Finke A, Mauri P, Miedema K, et al. International Network of reference laboratories for the deteremination of HbA1c. *Clin Chem* 1998; 44 (S6): A22 (abstract).

20 Monitoring glycaemic control in the diabetic patient. Ed WG John. *Printed for the IFCC by Excerpta Medica Publications 2002*.

21 Hanas R, John G, and On behalf of the International HbA_{1c} Consensus Committee. 2010 Consensus Statement on the Worldwide Standardization of the Haemoglobin A1C Measurement. *Diabetes Care*. 2010 August; 33(8): 1903–1904.

22 Barth JH et al. Consensus meeting on reporting glycated haemoglobin and estimated average glucose in the UK: report to the National Director for Diabetes, Department of Health. *Ann Clin Biochem* 2008; 45: 343–4.

23 Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus. http://www.who.int/diabetes/publications/report-hba1c_2011.pdf

24 JohnWG, Hillson R, Alberti G. Use of Haemoglobin A1c (HbA1c) in the diagnosis of diabetes mellitus. -The implementation of World Health Organisation (WHO) guidance 2011 http://www.acb.org.uk/docs/Article%20Summary%20Use%20of%20HbA1c%20WHO%20guidance%20251111[1].pdf