CAT
Critically Appraised Topic

Titel: Comparison of Methods of analysis for HbA1c

Author: Mae Jeraldine de Vera
Supervisor: Steven Pauwels, Sara Vijgen, Silvie Nickmans
Search/methodology verified by: Steven Pauwels
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CLINICAL BOTTOM LINE

Diabetes mellitus is one of the major health problems worldwide with an increasing prevalence and is expected to further increase overtime. HbA_{1c} is an approved blood test for the monitoring therapy as well as diagnostic parameter for diabetes. This parameter represents the long-term glycemic status with a cut-off value of 6.5%. In 2022 Jessa hospital reported > 10,000 HbA_{1C} analysis excluding quality control results. Currently, this parameter is measured using the ADAMS A1c HA-8180V (Arkray, Kyoto, Japan) which utilizes a reversed phase cation exchange (HPLC) technique. However, the contract with the current provider/supplier will end in 2025 and recently, the core-laboratory has switched to Abbott Alinity ci-series analysers which is possible for HbA_{1c} measurement. Utilizing this new platform would be more advantageous for the laboratory than a separate machine, prompting us to do a literature study for the HbA_{1c} analysers. Comparison of four NGSP certified analysers were done namely: Abbott Architect c, Roche Cobas c513, ADAMS A1c HA-8180V and Capillarys 2 Flex Piercing. Overall performance of the four analysers were interpreted as acceptable to excellent with an CV of <2%, bias of < 3mmol/mol and coefficient correlation of 0.995 to 0.999 (please refer to Table 2 and Table 3 for detailed results). Hb derivatives (labile Hb and carbamylated Hb) >10% showed interference using the Abbott Architect c and Roche Cobas c513 while common Hb variants (HbS, HbC, HbD, HbE) did not have any interference in all the analysers except for ADAMS A1c HA-8180V. Further, a concentration of >15% HbF also caused interference with the Sebia analyser. Although further experiment is recommended for the evaluation of HbA_{1c} in Abbott Alinity ci-series, these results showed strong correlation and high comparability of results for HbA_{1c} measurement between analysers using different techniques. Limitation per method of analysis should be taken into consideration and be applied individually in the laboratory setting.

CLINICAL/DIAGNOSTIC SCENARIO

Introduction

Diabetes mellitus is defined as a chronic disorder characterized by insulin deficiency and hyperglycemia by the National Glycohemoglobin Standardization Program (NGSP) (1). Complications can develop in the eyes, kidneys, peripheral nerves, heart and blood vessels. This disease is highly prevalent, affecting approximately 422 million people worldwide according to the report of World Health Organization (WHO) in 2014. Type 2 diabetes accounts for 90% of diabetes cases in the world and is the cause of 2.6% of the world's blindness. In Belgium, exact figures on diabetes are lacking, but according to a Sciensano report of 2014, the prevalence was estimated at 6.33%. Approximately 1/3 of type 2 diabetes mellitus patients in Belgium are not aware that they have the disease, therefore, the prevalence is probably higher (2). In the US, the estimated economic cost of diabetes increased from $245 billion in 2012 to $327 billion in 2017.

The diagnosis of diabetes includes the measurement of two biomarkers: glucose and hemoglobin A 1C (HbA 1C). Glucose is measured for the diagnosis of diabetes using the fasting plasma glucose (FPG), random plasma glucose (RPG) or the oral glucose tolerance test (OGTT), which reflects the immediate glycemic status. HbA 1C on the other hand, is used for monitoring the long-term glycemic status as well as planning for treatment strategies to lower the risk of

complications by achieving ≤53mmol/mol HbA 1C (4). In 2011 the WHO recommended HbA 1C tobe used as a diagnostic criteria (≥ 6.5% or 48 mmol/mol) for diabetes provided that the laboratory uses a method that is NGSP certified and standardized to the Diabetes Control and Complications Trial (DCCT) assay (4). There is therefore a necessity for a high quality measurement of this parameter.

Hemoglobin (Hb) is composed of four globin chains, adult hemoglobin (HbA) consists of two α and two β chains and is the most abundant form (approximately 97%). Hemoglobin A2 consists of two α and two δ chains, a minor form seen both after birth and in adults (2.5%). Fetal Hb (HbF) consists of two α and two γ chains, is most predominant at birth and a minor form in normal adults (<2%) (7,8). Approximately 6% of the HbA is glycated, with HbA 1c as the primary component. HbA 1c is formed by a specific non-enzymatic covalent chemical binding of glucose to the N-terminal valine of the β-chain of hemoglobin. Glycation of hemoglobin on other sites of the alpha or beta chains is known as glycohemoglobin (GHb). The total GHb is therefore the total measurement of HbA 1c and Hb glycated with glucose at other sites which can be measured using the affinity chromatography method.

Based on the update of NGSP, there are 5 methods for HbA1c quantification: HPLC ion exchange, capillary electrophoresis, affinity chromatography, immunoassay and enzymatic assays. For HbA1C quantification, more than 300 instruments or machines are certified, from more than 80 manufacturers. In our laboratory, we are currently using the Arkray ADAMS A1c HA-8180V analyzer by Menarini. It is an automated HPLC system with two measurement modes (variant mode and fast mode) which detect HbA_{1c}, HbF, HbS, HbC and can flag HbD and HbE. A throughput of 40 samples per hour can be achieved with an analysis of 90 seconds per sample on the variant mode and 48 seconds per sample for the fast mode. Whole blood sample is used for testing approximately 14µl per analysis but samples < 0.5 ml can be added with a diluent for testing. In 2022, the lab performed approximately 11,700 analysis of HbA_{1c} in Jessa hospital, excluding quality controls. The current contract with Menarini will end around September 2025. Hence, we wanted to compare the available methods for HbA_{1c} quantification for future reference.

QUESTION(S)

1) What are the methods of measurement for HbA1C quantification?
2) What are the current criteria for standardization of HbA1c quantification?
3) What are the analytical considerations in the comparison of these methods?
4) What is the effect of hemoglobin variants in HbA1c quantification in the four methods?

SEARCH TERMS

1) MeSH Database (PubMed): MeSH term: " "
2) PubMed Clinical Queries (from 1966; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi): Systematic Reviews; Clinical Queries using Research Methodology Filters (diagnosis + specific, diagnosis + sensitive, prognosis + specific)
3) Pubmed (Medline; from 1966), SUMSearch (http://sumsearch.uthscsa.edu/), The National Institute for Clinical Excellence (http://www.nice.org.uk/), Cochrane (http://www.update-software.com/cochrane)
4) International organizations: e.g. National Committee for Clinical Laboratory Standards (NCCLS; http://www.nccls.org/), International Federation of Clinical Chemistry (IFCC; http://www.ifcc.org/ifcc.asp), American Diabetes Association (ADA; http://www.diabetes.org/home.jsp)
5) *UpToDate Online*

RELEVANT EVIDENCE/REFERENCES

A. Guidelines and Recommendations (most recent topics on top))

B. Systematic Reviews and Meta-analyses

C. Reviews

D. Original Articles

E. Reference Works, Handbooks and Databases

F. Posters, "grey literature", presentations

APPRAISAL

1. What are the methods of measurement for HbA1C quantification?

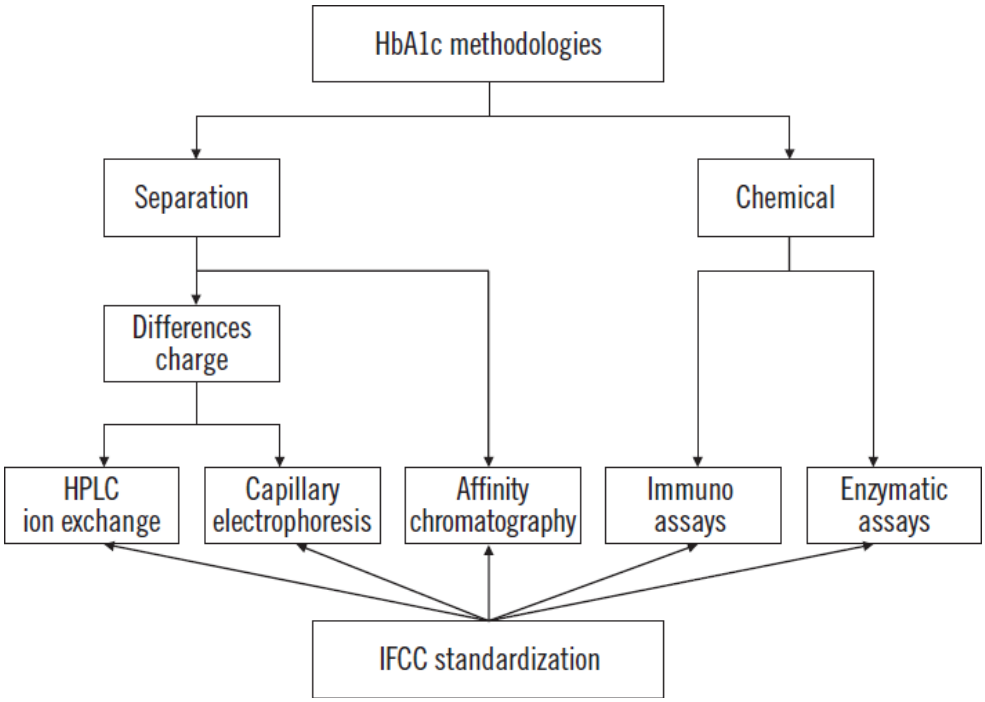


Figure 1: Methods of measurement for HbA1c and traceability to IFCC-RMP.

Currently there are two major analytical principles for HbA1c measurement (Figure 1). The separation technique allows separation of the glycated and non-glycated hemoglobin. This technique includes the high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and affinity chromatography (AC). The chemical technique on the other hand measures the HbA1c based on the chemical reaction to glycated N-terminal valine of the β-chain of the hemoglobin. This method of measurement includes immunoassays(IA) and enzymatic assays (EA).

Separation method

Ion exchange HPLC: utilizes cation exchange HPLC to separate the different hemoglobin components by their different ionic charge. Separation of these components is very quick, approximately 30 seconds per sample with the use of three different salt concentrations for peak separation and elution (6). The most recently developed machine that employs this method is the Tosoh HLC-723G11, a successor of the Tosoh G8, which reduces the run time from 60 seconds to 30 seconds per sample. Due to the shortened run time, separation of the hemoglobin variants are shown in three windows instead of specific Hb peaks: H-VO window includes HbAD, HbAS and HbAC, P-HV3 window includes HbAE and POO window for the

unknown Hb variants. The reagents for the Tosoh G11 are stable for 90 days after opening and require calibration every 30 days.

Capillary electrophoresis: uses capillaries to separate the hemoglobin variants based on their surface charge under high power voltage in a alkaline buffer solution (pH 9.4). Hemoglobin variants can be traced with this method due to the difference in runtime as well as the surface charge of the hemoglobin. Hemoglobin variants are detected in the following order (from the cathode to anode): A2, C, E, S, D, F, A0, A and A_{1c}. The presence of hemoglobin variants might co-elute with the native fraction of HbA which in return can cause inaccurate estimation of HbA_{1c}. For Sebia Capillarys 2 Flex Piercing, whole blood sample is used for analysis with a maximum sample throughput of 38 test per hour and calibration every 2 months.

Affinity chromatography: measures the total glycated Hb including HbA_{1c} and glycated Hb at other sites. Alere afinion is the most common machine utilizing this type of analysis, which is based on the specific reaction between *m*-aminophenylboronic acid and the *cis*-diol group of glucose bounded to Hb. This method showed the least interference of Hb variants in the measurement of HbA_{1c} in comparison to HPLC and enzymatic method.

Chemical method

Enzymatic method: this is a routine chemical analyzer that utilizes either photometric, potentiometric or turbidimetric methods. The machine is not dedicated for the measurement of HbA_{1c} alone. For the Abbott Architect c4000, the enzymatic principle is based on two steps: 1) measurement of glycated dipeptide and 2) the measurement of total hemoglobin. The maximum sample throughput is up to 800 analysis per hour with a total run time of 10 min per sample. The sample can either be whole blood or hemolysate, as well as possibility of manual predilution of whole blood if sample is insufficient. The reagents for Abbott Architect are ready for use with 350 tests per reagent cartridge, which are stable for 50 days and require calibration every 50 days provided that there is no change of lot number.

Immunoassay: the Cobas c513 analysis is based on turbidimetric inhibition immunoassay for hemolyzed whole blood with measurement of total hemoglobin during the preincubation phase. Samples can be whole blood mode as well as hemolysate for small volume samples. Total sample throughput is doubled with Cobas c513 from 200 tests of the Integra 800 to 400 tests per hour. The reagent is ready to use kit which contains 500 test per cartridge and an onboard stability of 4 weeks. This method requires calibration every 28 days, which is reagent lot specific.

Table 1. 15 Most used analyzers for HbA1c quantification

1	Abbott Architect c Enzymatic	Abbott Park, Illinois, U.S.A.
2	Alere Afinion	Abbott Park, Illinois, U.S.A.
3	Arkray ADAMS A1c HA-8180V (Menarini)	Adams Arkray, Kyoto, Japan
4	Beckman HbA1c Advanced B00389 Manual Application on DxC 700 AU AU system	Beckman Coulter Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA
5	Beckman HbA1c Advanced B93009 Online Application on DxC 700 AU	Beckman Coulter Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA
6	Beckman Synchron System Unicel DxC	Beckman Coulter Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA
7	Bio-Rad D-100 (A1c program)	Bio-Rad Laboratories N.V.3, Winninglaan 9140 Temse, Belgium
8	Bio-Rad Variant II Turbo 2.0	Bio-Rad Laboratories, Milan, Italy
9	Ortho-Clinical Vitros	1 International Business Park The Synergy #01-12 Singapore 609917
10	Roche Cobas c513	Roche Diagnostics, Germany
11	Sebia Capillarys 2 Flex Piercing	Capillarys 2FP, Sebia Lisses, France

12	Siemens DCA Vantage	1717 Deerfield Road Deerfield, Ilinois, USA
13	Siemens Atellica	1717 Deerfield Road Deerfield, Ilinois, USA
14	Siemens Dimension	1717 Deerfield Road Deerfield, Ilinois, USA
15	Tosoh G8 ver. 5.24, 5.28	Tosoh Bioscience, Tokyo, Japan

NGSP: Certified Methods and Laboratories update 06/17/2022

2. What are the current criteria for standardization of HbA1c quantification?

The landmark for standardization started with the Diabetes Control and Complications Trial (DCCT), completed in 1993, which established the risk of development or progression of chronic complication with diabetes related to the glycemic status of patients. The purpose of NGSP is to standardize the HbA_{1c} levels to the risk outcome of diabetic patients while the IFCC will standardize the HbA_{1c} results in correlation to the IFCC reference measurements. All analyzers in the market calibrate their methods against the IFCC requirements.

For standardization, a central primary reference laboratory (CPRL) sets the initial calibration and is responsible for the monitoring and back up of the primary and secondary reference laboratories (PRL/SRL). The manufacturers work with the SRL to standardize their methods with the DCCT reference values with a yearly proficiency testing (1).

The NSGP/DCCT certification includes an assessment of 40 patient samples compared to a SRL result. Criteria for passing the certification is shown in Figure 1. The bias from the NGSP target and variability should be ±2SD with an absolute mean bias ranging from 0 to 0.37%.

Figure 2. NGSP certification criteria (16):

Certification type	Certification criteria 1996–1998	Certification criteria 1999–2012	Certification criteria 2013–2018	Certification criteria 2019	Monitoring protocol
Manufacturer Level II Lab	EP-5 precision (≤5%) EP-9: 95%CI for predicted bias must overlap ±5% of SRL at 6% and 9% HbA1c	EP-5 precision (≤5% to ≤4% in 2002, dropped in 2007) Bland/Altman assessment of agreement: 95% CI of differences within ±1.0% HbA1c in 1999 to ±0.75% in 2010	37 of 40 results within ±7% in 2013 to ±6% in 2014	36 of 40 results within ±5%	None
Level I Lab	EP-5 precision (≤3%) EP-9: 95%CI for predicted bias must overlap ±3% of SRL at 6% and 9% HbA1c	EP-5 precision (≤3%, dropped in 2007) 95% CI of differences within ±0.75% HbA1c in 1999 to ±0.70% in 2010	38 of 40 results within ±7% in 2013 to ±6% in 2014	37 of 40 results within ±5%	10 samples quarterly

SRL: secondary reference laboratories

The IFCC criteria are based on the sigma metrics, consisting of a combination of target measurements specifically defined by the IFCC using the total allowable error (TAE). This allowes the combination of the effects of the bias and the imprecision to be measured on a single measurement. The criteria requires the total error to not be more than 5mmol/mol (0.46%) at a 50mmol/mol (6.7%) of HbA_{1c} concentration (9).

Laboratories in the US participate in a sample proficiency testing survey conducted by the College American Pathologist (CAP) by testing five samples three times per year. Based on the

latest CAP survey of 2023, 86% of laboratories assessed are using a between-lab CV's of (0.7% - 3.9%) (11).

In Belgium, laboratories participate in the Sciensano EKE for the evaluation of the accuracy, CV and linearity of HbA1c methods. The latest criteria for the year 2022 consist of the following parameters:

Figure3: Sciensano criteria for EKE evaluation in IFCC unit (18)

Parameter	Excellent	Goed	Acceptabel	Slecht	Onacceptabel
Afwijking Doelwaarde	<2 mmol/mol	2 – 2.9 mmol/mol	3 – 3.9 mmol/mol	4 – 4.9 mmol/mol	≥ 5 mmol/mol
Reproduceerbaar-heid (CV)*	<2%	2.0 – 2.99%	3.0 – 3.99%	4.0 – 4.99%	≥ 5%
Lineariteit (r)	>0.9970	0.9950-0.9970	0.9900- 0.9949	0.9800- 0.9899	<0.9800

Sciensano criteria for EKE evaluation of accuracy, CV and linearity

Figure 4: Sciensano criteria for EKE evaluation in NGSP unit (18)

Parameter	Excellent	Goed	Acceptabel	Slecht	Onacceptabel
Afwijking Doelwaarde	<0.2%	0.2 - 0.29%	0.30 - 0.39%	0.40 - 0.49%	≥ 0.50%
Reproduceerbaar-heid (CV)*	<1.4%	1.4 – 1.99%	2.0 – 2.99%	3.0 – 3.99%	≥ 4%
Lineariteit (r)	>0.9970	0.9950-0.9970	0.9900- 0.9949	0.9800-0.9899	<0.9800

Sciensano criteria for EKE evaluation of accuracy, CV and linearity

3. What are the analytical considerations in the comparison of these methods?

<u>Abbott Architect enzymatic assay (10, 6, 22)</u>

- Preanalytical considerations
  The influence of the anticoagulant used during blood collection was evaluated by analyzing samples on a EDTA and lithium heparin tubes. Values obtained from a EDTA tubes were systematically lower ($p<0.001$) than those obtained from lithium heparin tubes, however this difference is very limited and did not exceed 2mmol/mol. Sedimentation of the red blood cells was tested by analyzing sample every 30 mins for 2 hours with agitation between assays. Results showed that sedimentation did not have any impact on the HbA_{1c} levels of the samples.
- Precision
  The precision data of the studies conducted in 2017 and 2018 showed a coefficient of variation (CV) of 0.9 – 1.1% IFCC unit (0.6 – 0.7% NGSP unit) and 1.37% IFCC unit (1.13 NGSP unit) respectively. These results coincide with the findings of the European HbA_{1c} Trial of 2018 which demonstrated an excellent reproducibility with a between lab CV of 1.6% IFCC unit (1.1% NGSP unit).
- Accuracy (bias)
  Calculated biases were lower than 3mmol/mol for the 2018 study and lower than 1.1 mmol/mol for the 2017 study. These findings are also in accordance to the finding of European HbA_{1c} Trial of 2018 which demonstrated a bias of -0.1 – 0.0 for the whole blood sample. However, there was a noted high bias of 5.8 mmol/mol (0.53%) with lyophilized hemolysate sample using this machine due to a standardization issue for Abbott enzymatic assay specifically in Austria.
- Correlation with HPLC (12)
  Comparison of Abbott Architect c was done with HPLC machine of Tosoh G8 and capillary electrophoresis from Sebia (Minicap Flex Piercing). This showed a correlation

coefficient between 0.995 - 0.999 which is considered to be a good to excellent correlation.

Cobas c513 analyser (13, 6, 24)

- Pre-analytical factors
  Red blood cell sedimentation was assessed by incubating samples for 24 hours without agitation. The relative bias did not exceed 5% and even lesser when samples were stored in 4°C compared to room temperature. Lastly, the biases were close to 0% when samples were homogenized before re-analysis.
- Precision study
  Precision study for Roche c513 conducted in 2013 and 2017 showed a CV of < 1.4% IFCC and <2.1% IFCC units respectively. At the same time, precision study for Roche c501 module reported in 2019 was 0.9 – 1.13%. These values showed a good to excellent precision for both modules. In the European HbA_{1c} Trial of 2018 however, precision results of Roche machines did not perform good. Whole blood samples and lyophilized hemolysate demonstrated a precision of 4.4 – 4.9% IFCC unit (3.0 – 3.3% NGSP unit). These results were attributed to the variable performance of the different modules of Roche in different countries as well as the communicability issues of the samples. Based on the results, Roche performed good in Sweden, Nederland and UK but had poor results in Switzerland and Turkey.
- Accuracy
  Accuracy results for Roche c513 in 2013 and 2017 demonstrated < 3mmol/mol and 1.7 mmol/mol IFCC units respectively, while for the Roche c501, results showed a accuracy of 0.06%. These results coincide with the findings of the European HbA_{1c} Trial of 2018 with a bias range of -0.01 – -0.09 mmol/mol IFCC unit.
- Correlation with HPLC and capillary electrophoresis
  Comparison with two HPLC methods namely, Variant II and D-100 Bio-Rad Laboratories. Comparison between Cobas c513 and D-100 showed a linear regression of y = 0.96 +2.54, slope 0.94 – 0.97 (95% CI: 1.49 – 3.42). Further comparison of Cobas c513 and Variant II showed an y = 1.0 + 1.57, slope of 0.99 – 1.02 (95% CI: 0.78 – 2.50). The Bland-Altman plot showed a 5% outliers for D-100 and 2% of outliers for Variant II which indicate slight constant and proportional errors but no significant deviation for linearity. Comparison with Sebia electrophoresis and Abbott Architect c showed an r value of 0.995 which can be interpreted as good correlation.

ADAMS A1c HA-8180V (17, 24)

- Precision and Linearity
  The study in 2011 showed a with-in run CV of 0.2 % for both the low and high valued samples, while between run CV was 0.4% for the low valued samples and 0.2% for the high valued sample. The more recent study in 2019 comparing HA-8180V with Capillary 3 Tera (Sebia) showed a CV between 0.46 and 0.52%. Both results demonstrated an excellent reproducibility compared to the 2% requirement cut-off by IFCC.
- Accuracy
  The study conducted in 2011 showed a maximum deviation of 0.8 mmol/mol (0.1% NGSP) with a relative bias range of -2mmol/mol - +2 mmol/mol in IFCC units (-0.2% to +0.2% relative range in NGSP units). While the study in 2019 demonstrated a bias of 0.03%. These results showed an excellent accuracy for this analyser even with a 12 years time difference.

- Correlation
  Comparison with HPLC method from Tosoh 8 showed a slope of 1.022 (CI 1.003 to 1.038), intercept of -1.15 (CI -2.27 to -0.03) in IFCC units while a slope of 1.030 (CI 1.010 to 1.049) and an intercept of -0.209 (CI -0.356 to -0.062) in NGSP unit. Statistically speaking, these results showed a borderline significant difference between ADAMS HA-8180V compared to the reference system, however, this difference did not exceed 0.8mmol/mol in IFCC units and 0.1% in NGSP units which is not clinically significant. Comparison with Capillary Tera showed a correlation coefficient of 0.996 ($p < .0001$) showing a significantly strong correlation. In addition, Passing-Bablok regression analysis with 95% CI showed an intercept of y = 0.000(-0.336–0.000) and a slope of 1.000(1.000–1.040).

Capillarys 2 Flex Piercing (14, 22)

Sebia

- Pre-analytical factors (19)
  Comparison between capillary blood sample vs venous blood in potassium EDTA tubes tested with Capillary 2 Flex Piercing and Tosoh G8 (Tosoh Corp., Tokyo, Japan) showed a bias of ≤ 1mmol/mol and ≤ 4mmol/mol respectively. Storage of capillary blood samples at room temperature for at least 5 days before analysis showed a bias of less than mmol/mol and a good agreement with venous blood results. Samples with high level HbA_{1c} stored at 30°C had exceeded 3mmol/mol after 5 days and increased further to 6mmol/mol after 8 days.
- Precision and Linearity
  Studies evaluating Sebia Minicap Flex Piercing Analyser conducted in 2013 and 2018 demonstrated a CV <3.4% IFCC unit (<2.1% NGSP unit) and 1.67% IFCC unit (1.017% NGSP unit) respectively. These values fulfill the precision requirements of the IFCC in and interpreted as acceptable. Linearity results showed a range between 19mmol/mol (3.9%) to 161mmom/mol (16.9%) and 32–156 mmol/mol (5.1%–16.4%). Values for the linear regression demonstrated excellent results for linearity with the equation y (measured HbA 1c , mmol/mol) = 1.007 × (expected HbA1c values, mmol/mol) – 2.950 and y = −0.15 (95% CI, −6.13 to 5.83) + 1.00 (0.94 to 1.06), with r coefficients = 0.999, showing excellent correlation.
- Accuracy
  For the 2013 study there was no difference of more than 3 mmol/mol compared to the IFCC target values noted, indicating a good accuracy. However, the 2018 study showed a good accuracy on the medium (54 mmol/mol [7.1%]), and high (115 mmol/mol [12.7%]) valued samples but had a increased bias with low valued samples (32 mmol/mol [5.1%]) of 9.38%.
- Correlation
  Comparison was done with Variant II demonstrated a coefficient of correlation r value of 0.996 with a few outliers, mainly by samples with higher HbA_{1c} concentrations. The linear regression equation is y (HbA1c Capillarys 2 Flex Piercing) = 0.9452 × (HbA1c Variant II) + 1.7279. Comparison of values obtained from the two machines showed 3.4% discordant results from one another, with Variant II giving higher values (over 90mmol/mol (10.4%)).

Table 2. Bias, CV and Linearity per analyzer

	Bias	CV	Linearity (r)
Abbott Architect c	<3 mmol/mol	0.73% – 1.37% IFCC 0.60% – 1.13% NGSP	0.995 – 0.999
Roche Cobas c513	<3 mmol/mol	1.4 – 2.1% IFCC 1.1 – 1.5% NGSP	0.995
ADAMS A1c HA-8180V	0.03 – 0.8 mmol/mol	0.46% - 0.52% IFCC 0.2% - 0.7% NGSP	0.996
Sebia Capillarys 2	< 3mmol/mol	1.67% - <3.4% IFCC 1.017% - <2.1% NGSP	0.996 – 0.999

Table 3. Interpretation

	Bias	CV	Linearity (r)
Abbott Architect c	Good - Excellent	Excellent	Good - Excellent
Roche Cobas c513	Good - Excellent	Good - Excellent	Good
ADAMS A1c HA-8180V	Excellent	Excellent	Good
Sebia Capillarys 2	Good	Acceptable - Excellent	Good - Excellent

4. What is the effect of hemoglobin variants in HbA1c quantification in the four methods?

The most common Hb variants worldwide in descending order of prevalence are HbS, HbE, HbC, and HbD. All of these variants are found at the β-chain of hemoglobin, with HbS and HbC close to the N terminus, which can affect the quantification of HbA_{1c} in some (but not all) immunoassay methods. While HbE and HbD are structurally further away from the N terminus, causing less interference during analysis with immunoassay. All four variants are known to affect the ionic charge of the Hb molecule which in turn can cause interference for HbA_{1c} quantification using HPLC and electrophoresis. Studies examining elevated HbF levels on HbA_{1c} quantification revealed variable results, however these studies did not use a reference method known to be free of interference from HbF. Most current HPLC methods can separate HbF, however, concentrations >10-15% can still cause interference resulting to a falsely low result of HbA_{1c}.

For heterozygote Hb variants erythrocyte survival is normal and HbA_{1c} quantification is not affected, however this is not the case for homozygote variants. The effect of homozygosity which include HbSS (sickle cell anemia), HbCC (also known as HbC disease), HbSC (sickle-hemoglobin C disease), HbD (Punjab disease) and HbEE (homozygous E disease) should be interpreted with caution. These types of variants are known to shorten the lifespan of erythrocytes causing a falsely lower HbA_{1c} regardless of the method of analysis. Alternative tests are recommended for glucose monitoring such as glycated serum protein or glycated albumin.

<u>Abbott Architect enzymatic assay (10)</u>
Labile glycated hemoglobin (LA_{1c}) lower than 10.1% and carbamylated Hb (cHb) lower than 3.7% do not interfere with HbA_{1c} quantification. The analytical interference for bilirubin was noted up to 453µmol/L, and up to 11.2mmol/L for triglyceride. Common Hb variants tested did not show interference, however, these results included very limited samples and further studies on

a larger scale were recommended. The same findings were noted with a more recent study by Lenters-Westra et al in 2017 and demonstrated interference with HbF at >6.2%.

Cobas c513 analyser (13)

There was no analytical interference noted with a bilirubin of 352µmol/L and triglyceride of 20.6mmol/L. Hb variants tested were Hb AC, AD, AE, AS, respectively, showing no significant interference with HbA_{1c} measurement. Samples with elevated levels of cHb and labile HbA_{1c} were not included in the study. The same findings were demonstrated by the study of Lenters-Westra et al and showed also no interference with cHB as well as HbF.

ADAMS A1c HA-8180V (17)

Interference of hemoglobin variants was tested by using 165 samples known to contain Hb variants A2, C, D, E, F, or S. This test was compared to the reference HPLC method (Tosoh G8) which is free of Hb variant interferences. Results showed that HbA_{1c} quantification were accurate without any significant interference from the tested Hb variants.

Capillarys 2 Flex Piercing (19)

Influence of bilirubin and triglyceride on HbA1c was studied and analytical interference was tested for Labile A1c, cHb, HbS, HbD, HbF, HbC and HbE. No interference was noted in HbA_{1c} quantification for normal and elevated samples up to 26 mmol/L of triglyceride and 280 µmol/L of bilirubin. All samples tested with labile A_{1c} and cHb were within ±1 mmol/mol of the baseline HbA_{1c} values. Chromatograms showed a good separation of the tested Hb variants, with no interference and a good quantification.

Table 4. Effect of Hb derivatives and serum indices on HbA_{1c}

	LHb	cHb	Bil	TAG
Abbott Architect c	Yes > 10.1%	Yes > 10%	453 µmol/l	11.2mmol/L
Roche Cobas c513	-	Yes > 10 - 15%	352 µmol/L	20.6 mmol/L
ADAMS A1c HA-8180V	No*	No	268 µmol/L	-
Sebia Capillarys 2	No**	No	280 µmol/L	26mmol/L

Bil – bilirubin; TAG – triglyceride; * 1 mmol/mol difference from the target value;
**within ±1 mmol/mol from the measured baseline HbA_{1c}

Table 5. Effects of frequently encountered Hb variants

	Method	*HbC*	*HbS*	*HbE*	*HbD*	*HbF*
Abbott Architect c	Enzymatic	No	No	No	No	-
Roche Cobas c513	Immunoassay	No	No	No	No	No
ADAMS A1c HA-8180V	HPLC	No	No	Yes*	Yes**	-
Sebia Capillarys 2	Electrophoresis	No	No	No	No	Yes > 15%

NGSP: Factors that interfere with HbA_{1c} test results update 06/17/2022
*small peak at the edge of A_o – gives unreportable results;
**extra peak in S/C window – reported as abnormal separation

Table 6. Overall comparison

	Abbott Architect c	Roche Cobas c513	ADAMS A1c HA-8180V	Sebia Capillarys 2
Method	Enzymatic assay	Immunoassay	Ion exchange chromatography	Capillary electrophoresis
Sample	Whole blood and hemolysate	Whole blood and hemolysate	Whole blood and hemolysate	Whole blood
Reagent kit	350 test stable for 50days	500 test stable for 28 days	400 test per kit stable until expiry date	38 test stable for 2 months
Sampling time	800 test per hour	400 test per hour	40 samples per hour	38 samples per hour in batch of 8
Analytical performance				
CV	Excellent	Good – Excellent	Excellent	Acceptable
Bias	Good – Excellent	Good – Excellent	Excellent	Good
Linearity (r)	Good – Excellent	Good	Good	Good - Excellent
Influence of Hb variants	No interference with HbC, HbS, HbE, HbD but no data over HbF	No interference with HbC, HbS, HbE, HbD and HbF	Interference with HbD and HbE	Interference with HbF >15%
Effect of labile Hb and cHb	Yes > 10%	Yes > 10-15 %	No	No
Chromatogram	No	No	Yes	Yes
Bidirectional coupling with Glims	Yes	Yes	Yes	Yes
Price comparison	+	-	++	++++

COMMENTS

The results are based on the latest literature available for the evaluation of the analyzers. Guidelines have evolved and become more strict for the standardization of HbA1c, therefore, further studies and experiments may be required to re-evaluate these analyzers. HbA1c analysis can be performed using the Alinity-ci series, however no study have been conducted using this model. The Architect c model has been mostly studied. There is no available stand-alone HbA_{1c} analyzer from Roche; the test is incorporated with other parameters in immuno-analyzers such as c513 which is not ideal for the lab setting in Jessa hospital. The Sebia Capillarys 2 can only process whole blood samples and dilution or use of hemolysate is not advised. Additionally, testing needs to be done per batch of 8, otherwise there will be a waste of materials and reagents per run. Lastly, Sebia Capillarys 2 costs more per analysis than the Abbott and Menarini analyzers.

TO DO/ACTIONS

1) Inform about the availability of HbA1c analysis in Alinity ci-series and conduct experiments to evaluate the performance of the analyzer and compare performance with the current method.
2) Possible renewal of contract with the current provider, compare advantages and disadvantages with Alinity ci-series

ATTACHMENTS

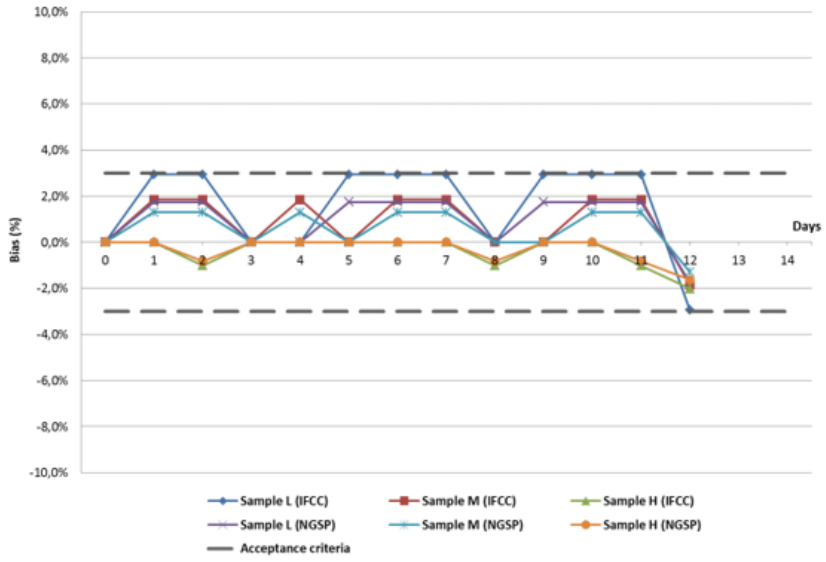
Attachment 1: Analytical performance evaluation for Architect c

Precision study of Abbott Architect c vs Sebia Minicap Flex Piercing Analyser (22)

HbA1c whole blood sample	Low value (N=15)			Medium value (N=15)			High value (N=15)		
Method	EM	CE		EM	CE		EM	CE	
		C 1	C 2		C1	C2		C1	C2
Mean value									
IFCC (mmol/mol)	28	31	30	53	43	43	79	96	97
NGSP (%)	4.7	4.9	4.9	7.0	6.1	6.1	9.3	11.0	11.0
Intra-laboratory precision (%)									
IFCC units	1.97	2.54	2.83	1.40	1.83	0.99	0.73	0.66	1.00
		2.69 [b]			1.47 [b]			0.85 [b]	
NGSP units	1.74	1.10	1.72	1.00	1.29	0.69	0.65	0.63	0.88
		1.41 [b]			0.99 [b]			0.76 [b]	
Acceptance Criteria for total CV	<3% (for IFCC units); <2% (for NGSP units)								

EM – enzymatic method, CE – capillary electrophoresis, CV – coefficient of variation. For the capillary electrophoresis data are presented for each of the two capillaries separately (C1 and C2). [a]Mean repeatability (%), [b]Mean within-laboratory precision (%).

Accuracy results (22)



Stability of hemoglobin (Hb)A1c in specimens with low (L, 34 mmol/mol [5.3%]), medium (M, 54 mmol/mol [7.1%]), and high (H, 99 mmol/mol [11.2%]) HbA1c level measured via the enzymatic method during a 12-day period. Biases refer to International Federation of Clinical Chemistry (IFCC) and National Glycohemoglobin Standardization Program (NGSP) units.

Method comparison of three HbA_{1c} analyzers (6)

	Deming regression line mean six SRMPs	CV (%) EP-5 HbA_{1c} 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 prog**	**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 Cobas C513 TQ	Y=0.99X – 1.23	0.7	1.7	3.6	9.1
Tosoh G11	Y=0.95X+1.46	0.6	0.9	1.9	13.5

Attachment 2: Evaluation of analytical performance of Cobas c513

Precision study

		Coefficients of variation (%)			
Sample	**Mean value**	**Repeatability**	**Between-run**	**Between-day**	**Intermediate precision (total)**
			HbA_{1c}, %		
Sample 1	5.8	0.5	0.6	0.8	1.1
Sample 2	6.1	0.7	0.6	0.7	1.1
Sample 3	7.9	0.5	0.8	0.6	1.1
Sample 4	11.6	0.6	0.6	0.7	1.1
QC sample (low-level)	5.7	0.6	0.5	0.4	0.84
QC sample (high-level)	11.1	0.5	0.2	0.9	1.1
			HbA_{1c}, mmol/mol		
Sample 1	39.9	0.7	1.0	1.2	1.7
Sample 2	42.9	1.0	1.0	1.0	1.7
Sample 3	62.9	0.7	1.1	0.9	1.6
Sample 4	103.5	0.8	0.8	0.9	1.4
QC sample (low-level)	39.2	0.9	0.7	0.7	1.3
QC sample (high-level)	98.2	0.7	0.3	1.1	1.4

Acceptance criteria: the analytical goals usually admitted for precision performances of HbA_{1c} methods are intermediate precision CVs lower than 2% (6). HbA_{1c} - haemoglobin A_{1c}. QC - quality control.

Precision study for Capillary Tera, Roche c501, ADAM™ A1c HA-8180V (24)

	Total precision (%CV_{WL})	
Methods	Control level-1	Control level-2
CE	1.01%	0.82%
TINIA	1.13%	0.90%
EA	1.01%	0.85%
HPLC	0.46%	0.52%

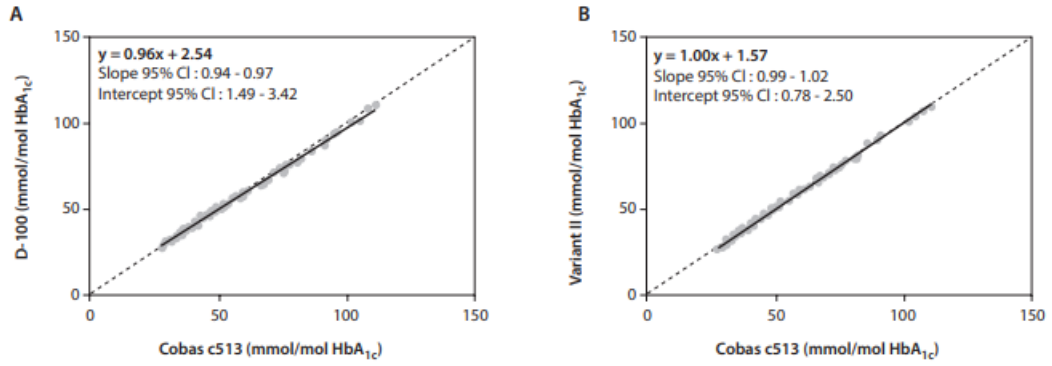
CE: Capillary electrophoresis; TINIA: Turbidimetric inhibition immunoassay; EA: Enzymatic assay; HPLC: High-performance liquid chromatography; %CV_{WL}: within-laboratory coefficient of variation.

Comparison of results using Cobas c513 analyzer with IFCC assigned value control samples

EAQ sample	IFCC target value	Measured value	Absolute bias (mmol/mol)	Relative bias (%)
		HbA_{1c}, mmol/mol		
Sample 1	31.4	32.1	+ 0.7	+ 2.2
Sample 2	38.7	38.9	+ 0.2	+ 0.5
Sample 3	49.6	49.5	- 0.1	- 0.2
Sample 4	58.5	59.3	+ 0.8	+ 1.2
Sample 5	69.0	69.8	+ 0.8	+ 1.2
Sample 6	78.3	80.6	+ 2.3	+ 2.9
Sample 7	88.6	91.6	+ 3.0	+ 3.4
Sample 8	99.2	100.4	+ 1.2	+ 1.2
		Mean	+ 1.1	+ 1.6
		HbA_{1c}, %		
Sample 1	5.0	5.1	+ 0.07	+ 1.5
Sample 2	5.7	5.7	+ 0.02	+ 0.3
Sample 3	6.7	6.7	- 0.01	- 0.1
Sample 4	7.5	7.6	+ 0.08	+ 1.0
Sample 5	8.4	8.5	+ 0.07	+ 0.9
Sample 6	9.3	9.5	+ 0.21	+ 2.3
Sample 7	10.3	10.5	+ 0.27	+ 2.6
Sample 8	11.2	11.3	+ 0.11	+ 0.9
		Mean	+ 0.10	+ 1.2

EAQ - external assurance quality. The measured value represents the mean of three HbA_{1c} determinations. The acceptance criterion for the evaluation of accuracy is absolute biases lower than 5 mmol/mol in IFCC units (12). HbA_{1c} - haemoglobin A_{1c}. IFCC - International Federation of Clinical Chemistry and Laboratory Medicine.

Correlation plot with linear regression line comparing results with Cobas c513 and Variant II analyzers (13)



Passing-Bablok regression analysis and Spearman rank correlation study of Capillary Tera, Roche c501, ADAM™ A1c HA-8180V (24)

	Method comparison	Intercept (95% CI)	Slope (95% CI)	Correlation coefficient* (95% CI)
Total	TINIA – CE	−0.070 (−0.225–0.100)	1.022 (1.000–1.042)	0.994 (0.993–0.995)
	EA – CE	−0.241 (−0.362–−0.100)	1.023 (1.000–1.040)	0.993 (0.991–0.994)
	HPLC – CE	0.000 (0.000–0.000)	1.000 (1.000–1.000)	0.996 (0.995–0.997)
Group 1 (HbA1c <6.5%)	TINIA – CE	−0.415 (−0.933–0.000)	1.077 (1.000–1.167)	0.945 (0.921–0.962)
	EA – CE	−0.100 (−1.083–−0.100)	1.000 (1.000–1.167)	0.934 (0.905–0.954)
	HPLC – CE	0.000 (0.000–0.000)	1.000 (1.000–1.000)	0.966 (0.951–0.977)
Group 2 (HbA1c 6.5 – 8%)	TINIA – CE	0.100 (−0.775–0.100)	1.000 (1.000–1.125)	0.965 (0.947–0.977)
	EA – CE	−0.667 (−1.283–−0.100)	1.083 (1.000–1.167)	0.942 (0.913–0.962)
	HPLC – CE	0.000 (0.000–0.000)	1.000 (1.000–1.000)	0.973 (0.959–0.982)
Group 3 (HbA1c >8%)	TINIA – CE	0.607 (0.100–0.971)	0.923 (0.905–1.000)	0.983 (0.973–0.999)
	EA – CE	0.000 (−0.223–0.000)	1.000 (1.000–1.022)	0.986 (0.978–0.992)
	HPLC – CE	0.000 (−0.336–0.000)	1.000 (1.000–1.040)	0.990 (0.983–0.994)

*All correlations are significant at the $p < .0001$. CE, Capillary electrophoresis; TINIA, Turbidimetric inhibition immunoassay; EA, Enzymatic assay; HPLC, High-performance liquid chromatography; CI, Confidence interval.

Attachment 3:

Precision study of ARKRAY HA-8180V analyzer

Parameter	Low HbA_{1c} level IFCC 39 mmol/mol NGSP 5.7%	High HbA_{1c} level IFCC 99 mmol/mol NGSP 11.2%
Within-run CV	0.2%	0.2%
Between-run CV	0.4%	0.2%
Between day CV	0.6%	0.2%
Total CV	0.7%	0.4%

[a]According to protocol NCCLS EP-5 using Menarini controls.

Comparison of results between ARKRAY HA-8180V analyzer and IFCC assigned values

Difference between measured and (assigned) values				
HbA_{1c} level	Measured	Assigned	Measured	Assigned
Low	29.5 mmol/mol	30.0 mmol/mol	4.8%	4.9%
Medium	60.2 mmol/mol	60.0 mmol/mol	7.6%	7.6%
High	90.8 mmol/mol	90.0 mmol/mol	10.5%	10.4%

[a]According to protocol NCCLS EP-9 on basis of 40 samples.

Attachment 4: Evaluation of analytical performance of Sebia Capillarys 2FP

Precision and accuracy tests on Tosoh G8 and Sebia Cap 2FP

	Center 1				Center 2				Center 3			
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
IFCC units, mmol/mol												
Mean	35.0	46.8	55.1	74.7	35.8	47.2	55.5	75.4	35.5	46.9	55.8	75.2
SD	1.2	0.8	1.3	1.5	1.1	0.9	1.2	1.6	0.8	1.1	1.2	1.5
CV%	**3.4**	**1.7**	**2.4**	**2.0**	**3.1**	**2.0**	**2.2**	**2.1**	**2.3**	**2.3**	**2.2**	**2.0**
NGSP units, %												
Mean	5.35	6.44	7.21	9.00	5.44	6.46	7.23	9.06	5.39	6.45	7.25	9.04
SD	0.11	0.08	0.12	0.13	0.10	0.10	0.12	0.15	0.09	0.10	0.11	0.14
CV%	**2.1**	**1.2**	**1.7**	**1.4**	**1.8**	**1.6**	**1.7**	**1.7**	**1.7**	**1.6**	**1.5**	**1.6**

Trueness of Capilllarys 2 Flex Piercing® HbA1c method—IFCC units (mmol/mol)

	Target value ± SD	Center 1		Center 2		Center 3	
		Mean	Bias	Mean	Bias	Mean	Bias
SQS1	31 ± 0.9	31.5	+0.5	31.5	+0.5	29.0	−2.0
SQS2	42 ± 1.0	42.0	0.0	42	0.0	42.0	0.0
SQS3	64 ± 1.1	63.0	−1.0	62.5	−1.5	64.5	+0.5
SQS4	84 ± 1.3	82.0	−2.0	85.5	+1.5	82.5	−1.5
SQS5	68 ± 1.0	66.0	−2.0	67.0	−1.0	66.5	−1.5
SQS6	89 ± 1.7	88.0	−1.0	88	−1.0	90.0	+1.0
SQS7	33 ± 1.0	32.5	−0.5	33.5	+0.5	32.5	−0.5
SQS8	41 ± 1.1	41.5	+0.5	43.5	+2.5	40.5	−0.5

Comparison study: results expressed in IFCC units (mmol/mol)

Samples	*N*	Center 1		Center 2		Center 3	
		r Passing–Bablok	Bias (95% CI)	*r* Passing–Bablok	Bias (95% CI)	*r* Passing–Bablok	Bias (95% CI)
HbA_2 <4% and HbF <1%	37	0.988 $y = 1.04x - 1.71$	0.3 (−0.5 to +1.1)	0.990 $y = 1.06x - 4.68$	−1.6 (−2.3 to −0.8)	0.988 $y = 1.09x - 3.82$	0.1 (−0.8 to +0.9)
HbA_2 >4% and HbF >1%	18	0.986 $y = 1.10x - 6.38$	−1.9 (−2.9 to −0.8)	0.988 $y = 1.00x - 3.23$	−3.6 (−4.5 to −2.6)	0.987 $y = 1.05x - 3.68$	−1.6 (−2.7 to −0.5)
HbA_2 >4% and HbF < 1%	15	0.992 $y = 1.11x - 5.04$	−1.3 (−2.0 to −0.6)	0.990 $y = 1.15x - 8.10$	−2.3 (−3.0 to −1.5)	0.986 $y = 1.00x - 1.00$	−1.3 (−2.2 to −0.4)
All samples	70	0.987 $y = 1.08x - 3.92$	−0.6 (−1.2 to −0.1)	0.990 $y = 1.06x - 4.97$	−2.3 (−2.8 to −1.7)	0.987 $y = 1.08x - 3.88$	−0.6 (−1.2 to −0.1)
All samples + interfering substances	83	0.985 $y = 1.07x - 4.28$	−1.0 (−1.5 to −0.5)	0.990 $y = 1.04x - 4.30$	−2.2 (−2.7 to −1.8)	0.985 $y = 1.07x - 3.40$	−1.0 (−1.5 to −0.4)

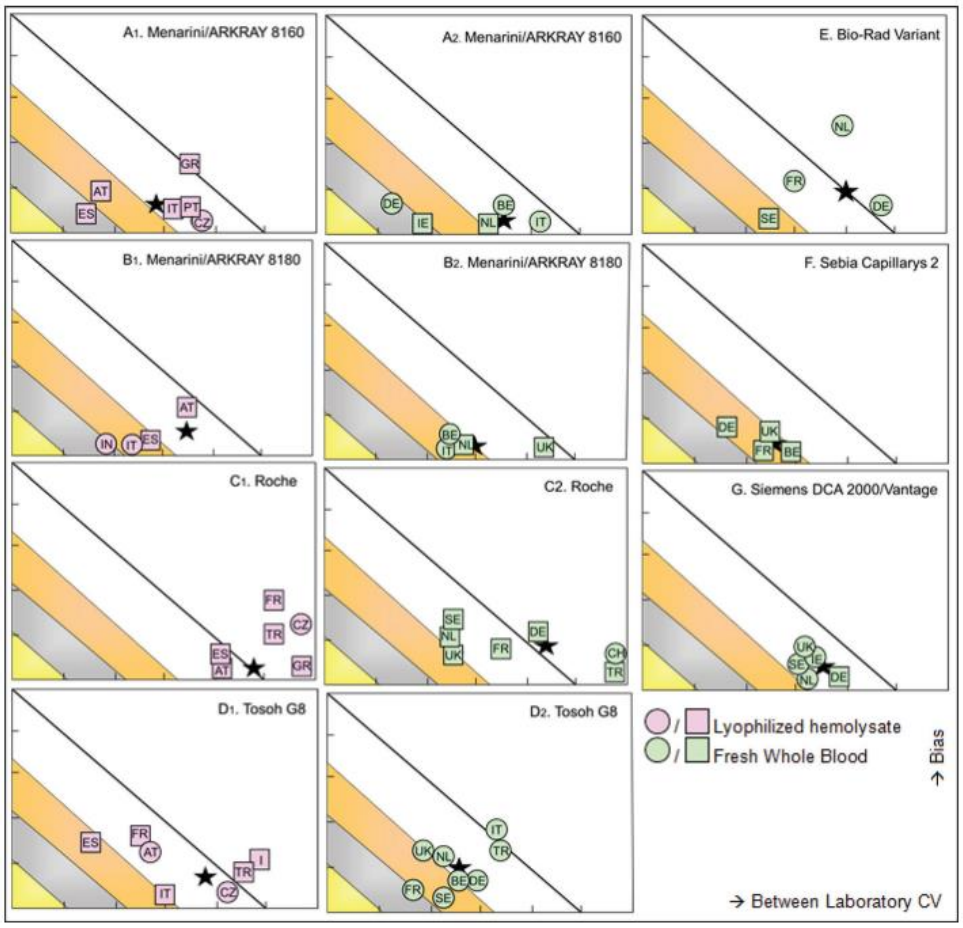
European HbA1c Trial to Investigate the Performance of HbA1c Assays of 2018 (21)

Table 2. Summary per manufacturer of number of participating labs, bias, and BLCV in fresh WB and LH.

	Fresh WB			LH		
Manufacturer	n	IFCC bias, mmol/mol (BLCV)	NGSP bias, % (BLCV)	n	IFCC bias, mmol/mol (BLCV)	NGSP bias, % (BLCV)
Abbott Architect Enzymatic	21	−0.1 (1.6%)	−0.01 (1.1%)	24	−4.0 (6.0%)	−0.37 (4.0%)
Abbott Architect Immuno	6	−1.8 (4.0%)	−0.16 (2.8%)			
Abbott Other	6	+1.9 (4.6%)	+0.18 (3.0%)			
Alere Afinion	76	−0.7 (3.4%)	−0.06 (2.2%)			
Beckman Coulter AU	26	−0.6 (5.6%)	−0.06 (3.8%)	7	+1.6 (6.5%)	+0.15 (4.4%)
Beckman Coulter UC DxC	15	−1.0 (3.5%)	−0.10 (2.4%)			
Bio-Rad D10	53	+0.8 (4.8%)	+0.07 (3.2%)	37	−1.2 (5.2%)	−0.11 (3.5%)
Bio-Rad D 100	11	−0.8 (1.8%)	−0.08 (1.2%)	16	−0.3 (1.9%)	−0.03 (1.2%)
Bio-Rad Variant	86	+0.9 (4.0%)	+0.08 (2.6%)	38	+1.3 (4.8%)	+0.12 (3.2%)
Medinor	6	−4.7[a] (14.6%)	−0.43 (9.9%)			
Menarini HA-8160	91	+0.4 (3.4%)	+0.04 (2.3%)	87	−0.6 (2.9%)	−0.06 (2.0%)
Menarini HA-8180	82	+0.4 (3.0%)	+0.03 (2.0%)	72	−0.7 (3.5%)	−0.06 (2.4%)
Not Known	123	0.0 (5.3%)	0.00 (3.6%)	14	−0.8 (8.1%)	−0.07 (5.4%)
Roche	288	−0.9 (4.4%)	−0.08 (3.0%)	100	−0.1 (4.9%)	−0.01 (3.3%)
Sebia Capillarys 2	57	−0.4 (2.6%)	−0.04 (1.8%)	45	−1.4[a] (2.5%)	−0.14 (1.7%)
Sebia Capillarys 3	8	0.0 (2.3%)	0.00 (1.6%)	9	−1.3 (2.1%)	−0.12 (1.4%)
Sebia Minicap	10	−0.8 (2.5%)	−0.08 (1.7%)			
Siemens Advia	15	+3.5[a] (4.8%)	+0.32 (3.2%)			
Siemens DCA/Vantage	158	+0.6 (3.6%)	+0.06 (2.4%)	6	+4.0 (3.6%)	+0.38 (2.4%)
Siemens Dimension	47	0.0 (4.0%)	0.00 (2.7%)	17	+0.4 (4.7%)	+0.04 (3.1%)
Siemens Other	13	−0.3 (4.2%)	−0.03 (2.8%)			
Tosoh G7	27	+1.1 (5.6%)	+0.10 (3.8%)	33	−0.4 (4.7%)	−0.04 (3.2%)
Tosoh G8	234	+1.0[a] (2.6%)	+0.09 (1.8%)	85	−0.7 (3.9%)	−0.07 (2.6%)
Trinity Premier Hb9210	27	+1.2 (3.8%)	+0.10 (2.5%)	16	−0.8 (3.7%)	−0.08 (2.5%)

[a] Significant different from target ($P < 0.05$).

European HbA1c Trial of 2018: Performance per manufacturer per country



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