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Evaluation of Hemoglobin A_{1c} Measurement by Capillarys 2 Electrophoresis for Detection of Abnormal Glucose Tolerance in African Immigrants to the United States

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Abstract

BACKGROUND—Hemoglobin A_{1c} (HbA_{1c}) is used to monitor long-term glycemic control in individuals with diabetes, guide therapy, predict the risk of microvascular-complications, and more recently to diagnose diabetes. An automated liquid-flow-capillary-electrophoresis method was recently developed to measure HbA_{1c} using the Capillarys 2 Flex Piercing instrument.

METHODS—Analytical evaluation was performed at 2 clinical centers. A clinical analysis was conducted in 109 African-born individuals, 24% of whom have variant hemoglobin (HbAS or HbAC). Abnormal glucose tolerance (which includes both diabetes and prediabetes) was defined as 2 h glucose ≥ 140 mg/dl (7.8 mmol/l) during an oral glucose tolerance test.

RESULTS—Interlaboratory CVs were ≤ 2.1%. The method showed satisfactory correlation with 2 other analyzers that measure HbA_{1c} by high-performance liquid chromatography. Neither labile HbA_{1c}, carbamylated hemoglobin, uremia, bilirubin nor common hemoglobin variants (HbC/HbS/HbE) interfered. Forty-five individuals (41%) had abnormal glucose tolerance. The sensitivity of HbA_{1c} for diagnosing abnormal glucose tolerance was 38%, 36% and 42% for total, normal and variant hemoglobin groups, respectively.

CONCLUSIONS—The analytical performance of HbA_{1c} on the Capillarys 2 is suitable for clinical application. Variant hemoglobin in Africans did not interfere with the detection of abnormal glucose tolerance by HbA_{1c} measured on the Capillarys 2.

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Keywords

Hemoglobin A1c; Capillarys 2; Diabetes; Abnormal Glucose Tolerance; Capillary Electrophoresis; Sickle Cell; Africans

Introduction

Hemoglobin A_{1c} (HbA_{1c}) is the reaction product of glucose with the NH_2-terminal valine of the β-chain of hemoglobin. In individuals with diabetes, HbA_{1c} is used to monitor long-term glycemic control, guide therapy, and predict the risk of microvascular complications. More recently, HbA_{1c} has been advocated by several influential diabetes organizations for the diagnosis of diabetes [1,2]. It is therefore vital that methods used to measure HbA_{1c} are accurate, precise, reproducible and subject to minimal interference.

Initial assays to measure glycated hemoglobin were limited by a lack of comparability, resulting in very wide variability in results among laboratories [3]. The implementation of standardization activities by the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has considerably improved HbA_{1c} accuracy and precision [4]. Notwithstanding this progress, further improvement is required given the integral importance of HbA_{1c} measurement for the diagnosis and management of diabetes.

Recently, Sebia (Lisses, France) developed an automated liquid-flow capillary electrophoresis (CE) method to measure HbA_{1c} on the Capillarys 2 Flex Piercing instrument. This is the first CE technology approved by the U. S. Food and Drug Administration for HbA_{1c} measurement. The instrument provides complete automation for fast separation and quantification of HbA_{1c} using the principle of liquid-flow capillary electrophoresis in free solution. With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer with a specific pH, as well as by electrolyte pH and electroosmotic flow. Hemoglobins are detected by absorption spectroscopy at the cathodic end of the capillary [5].

Sickle cell trait (HbAS) occurs in about 0.08% of US newborns [6]. The prevalence is 6–8% in African-Americans [6,7] and as high as 40% in certain areas of Africa [8]. Hemoglobin C trait (HbAC) is the second most common variant in African Americans and prevalence reaches 15% in areas of West Africa [9]. Importantly, populations of African descent are also at particularly high risk for diabetes (prevalence of 13.3% to 14.9%), which is twice that of non-Hispanic Whites [10]. Although the diagnostic utility of HbA_{1c} for diabetes has been investigated in several ethnic groups, data are lacking for populations of African descent with high prevalence of both diabetes and hemoglobin variants. The accuracy of HbA_{1c} methods can be adversely affected by the presence of hemoglobin variants [11, 12]. Therefore, to assess Africans an accurate HbA_{1c} method that is not affected by hemoglobin variants, such as HbAS or HbAC, is necessary.

Materials and Methods

Participating centers

Technical evaluation was performed at the Diabetes Diagnostic Laboratory using NGSP Secondary Reference Laboratory methods (NGSP SRL9 and SRL3), University of Missouri (Center 1), and Department of Laboratory Medicine, National Institutes of Health (NIH, Center 2). Clinical evaluation was conducted at the NIH.

Characteristics of the CAPILLARYS 2 Flex Piercing analyzer

Sample analyses were carried out following the manufacturer's instructions (4).

Capillarys 2 Flex Piercing is a multipurpose instrument using 8 capillary tubes for multiple and simultaneous hands-free electrophoretic separations at a high throughput. The separation of the different hemoglobin fractions takes place in 8 silica capillary tubes of 25 μm internal diameter, and migration is performed at a high voltage of 9.4 kV under tight temperature control of 34°C using a Peltier device. Hemoglobins are directly detected at a specific absorption wavelength of 415 nm at the cathodic end of the capillary.

Analytical evaluation

Blood samples used in this portion of the study were left over from samples routinely submitted for HbA_{1c} measurement. Imprecision, trueness and linearity studies were performed following Clinical and Laboratory Standards Institute (CLSI) EP-5, EP-9 and EP-6 protocols, respectively. Tosoh G8 and Bio-Rad D10 (both NGSP-certified; G8 is also an NGSP Secondary Reference Laboratory method) were the comparison methods for trueness. Sample pools of high and low HbA_{1c} concentrations were run alternately to assess carry over.

For all interference studies (namely labile HbA_{1c}, carbamylated hemoglobin, uremia, bilirubin, and total hemoglobin), a difference of ±0.3% (3.3 mmol/mol) HbA_{1c} from the target was used as an acceptable limit [13]. Methodological details of the analytical evaluation are in the supplement.

Hemoglobin variants for analytical evaluation

The possible interference of variant hemoglobins was assessed. Samples with HbAS (n=5), HbAC (n=5) or HbAE (n=1) were analyzed from frozen whole blood aliquots on both the Trinity ultra^2 (NGSP SRL3, Trinity Biotech, Kansas City, MO) and Capillarys 2; these methods have previously been shown to compare well in samples without variants (30). The Trinity ultra^2 boronate affinity method was selected as a comparison method because these hemoglobin variants do not interfere with HbA_{1c} measurement by this method [14]. In addition, HbSS (n=4), HbEE (n=2), HbCC (n=2), HbDD (n=1), and HbSC (n=6) samples were analyzed by the Capillarys 2.

Clinical study

This is a substudy of the Africans in America Study [15–17], which is an ongoing, prospective study of adults who were born in equatorial Africa, but resided in the US at the

time of the study. The group evaluated consisted of 109 consecutively enrolled adults who came to the NIH Clinical Center between January 2012 and August 2014. Participants stated at enrollment they were healthy and denied any history or symptoms of diabetes. Informed written consent was obtained from all participants. The study protocol was approved by the NIDDK Institutional Review Board.

At the first outpatient visit a history and physical examination were conducted. Anemia, iron deficiency and renal, hepatic and thyroid dysfunction were ruled out by routine blood tests at the screening visit. Glucose homeostasis was evaluated at the second and third visits.. At Visit 2, following a 12 h fast, fasting plasma glucose (FPG), HbA1c, and a 2h plasma glucose during a standard oral glucose tolerance test (OGTT) using 75 g dextrose were obtained. At Visit 3, an insulin modified-frequently sampled intravenous glucose tolerance test (IM-FSIGT) was performed in 80 participants as described previously [18]. Twenty-nine participants did not undergo an IM-FSIG; 11 participants had an increased 2h plasma glucose during their OGTT consistent with the diagnosis of diabetes and per protocol did not proceed to the IM-FSIGT, while 18 participants declined the IM-FSIGT. The insulin sensitivity index (S_I) (a measure of insulin resistance) and the acute insulin response to glucose (AIRg) (a measure of β-cell secretion) were determined as a previously described [19].

Hemoglobin, hematocrit, mean corpuscular volume (MCV), and reticulocyte count were analyzed in EDTA-anticoagulated whole blood using a Sysmex XE-5000 analyzer. Glucose, total bilirubin, direct bilirubin, AST, ALT, blood urea nitrogen, creatinine, iron, transferrin, ferritin, vitamin B12, folate and insulin were analyzed in serum or plasma using Siemens Dimension Vista analyz or Roche Cobas 6000 analyzer. HbA_{1c} was measured using whole blood aliquots frozen at −80°C on Sebia Capillarys 2 Flex Piercing system at Center 2. Hemoglobin electrophoresis was performed on Helena Zip-Zone electrophoresis instrument to identify variant hemoglobin in 88 consecutively enrolled participants. Identities of hemoglobin types were confirmed by comparison to known samples of HbA, HbS and HbC. Other than hemoglobin electrophoresis and HbA_{1c}, all laboratory measurements were performed on the day of the visit. Abnormal glucose tolerance (which comprises both diabetes and prediabetes) was defined according to ADA criteria as 2h glucose ≥ 140 mg/dl (7.8 mmol/l) during an OGTT [1].

Statistical analysis

Student's *t*-test and the χ^2 test were applied to compare the metabolic and demographic characteristics of individuals with normal or variant hemoglobins. Diagnostic sensitivity and specificity were calculated based on ADA criteria for pre-diabetes and diabetes [1], namely $HbA_{1c} \geq 5.7\%$ (39 mmol/mol) or FPG ≥ 100 mg/dl (5.6 mmol/l). Sensitivity and specificity values were compared via chi-square test when applicable. All statistics were performed with STATA 13.0 (StataCorp), Prism GraphPad 5.0 (GraphPad Software) or EP Evaluator Release 10 software (David G. Rhoads Associates). Statistical significance was defined as a *P* value ≤0.05.

Results

Analytical performance

Within-run coefficients of variation (CVs) were 1.1–2.2% for NGSP units/ 1.3%–3.7% for SI (IFCC) units (center 1) and 0.9%–2.0% for NGSP units/ 1.0%–3.4% for SI units (center 2), while between-run CVs were 0.0%–0.7% for NGSP units/ 0.0%–0.9% for SI units (Table 1). Total intra-laboratory CVs were ≤ 2% for NGSP units/ ≤ 4% for SI units for all samples in both centers. The inter-laboratory CVs were 1.1–2.1% for NGSP units/ 1.3%–3.7% for SI units.

HbA_{1c} values correlated well between the Capillarys 2 Flex Piercing instruments at the 2 centers and also with those of the comparison methods (Tosoh G8 and Bio-Rad D10), with minimal bias (Fig. 1 and supplemental Fig. 1). Linear regression analysis yielded the following: y (HbA_{1c} Capillarys 2 center 1) = 1.033 × (HbA_{1c} Tosoh G8) −0.34, r=0.997, S_{yx}=0.16; y (HbA_{1c} Capillarys 2 center 2) = 1.049 × (HbA_{1c} Tosoh G8) − 0.38, r=0.996, Syx=0.19; and y (HbA_{1c} Capillarys 2 center 2) = 1.084 × (HbA_{1c} Bio-Rad D10)−0.67, r=0.995, S_{yx}=0.22. Results of the Capillarys 2 were comparable between centers with mean bias = 0.09% (1.0 mmol/mol) HbA_{1c} (y (HbA_{1c} Capillarys 2 center 1) = 0.982 × (HbA_{1c} Capillarys 2 center 2) ± 0.05, r=0.996, Syx=0.18). The HbA_{1c} values obtained with the Capillarys 2 Flex Piercing at each center also correlated well with the NGSP secondary reference laboratory method (r ≥ 0.995 for both centers) and mean bias was −0.09% (−1.0 mmol/mol) HbA_{1c} (Capillarys 2 center 1 vs. Tosoh G8, NGSP SRL9) and 0.00% (0 mmol/mol) HbA_{1c} (Capillarys 2 center 2 vs. Tosoh G8, NGSP SRL9). Ninety-five % (center 1) and 97 % (center 2) of single Capillarys 2 results were within ±6% of the SRL9 mean (NGSP manufacturer certification criteria require that 37/40 or 92.5%, results be within ±6% of the SRL mean) [20, 21].

The method was linear for HbA_{1c} results from 4.2% to 17.6% (22 to 169 mmol/mol, center 1) and 4.3% to 14.1% (23 to 131 mmol/mol, center 2), with correlation coefficients of 0.998 and 1.00, respectively, and the differences between measured and expected values were within ±6% (Supplemental Table 1, Supplemental Fig. 2).

The difference between the means of the high-low and the low-low sequence samples was 0.06% (0.3 mmol/mol), which is < 3 SD of the low-low values (1 SD of the low-low values= 0.07% (0.7 mmol/mol), indicating no significant carryover.

Evaluation of interference

There was no interference from labile HbA_{1c} (up to 11%, 97 mmol/mol), carbamylated hemoglobin, BUN (97 mg/dl, 34.6 mmol/l) or bilirubin (40 mg/dl, 684 μmol/l). No additional peaks (i.e. for labile HbA_{1c} or carbamylated hemoglobin) were observed on the Capillarys 2 electropherograms. Varying hemoglobin concentrations from 6.9 to 18.3 g/dl had no effect on HbA_{1c} measurement (Supplemental Table 2, Supplemental Fig. 3).

Hemoglobin variants

All the Capillarys 2 results with HbAS, HbAC and HbAE samples were within ±7% of the Ultra[2] results (Supplemental Table 3). Analysis of HbSS, HbEE, HbCC, HbDD, and HbSC samples with Capillarys 2 gave no results (data not shown). This was expected as these samples have no HbA and therefore no HbA_{1c}.

Clinical analysis of HbA1c on Capillarys 2 for the detection of abnormal glucose tolerance

Since the Capillarys 2 method accurately measured HbA_{1c} in individuals with HbAS or HbAC, we evaluated its ability to identify individuals with abnormal glucose tolerance in a population with a high prevalence of variant hemoglobin.

The characteristics of the study population are shown in Table 2. There was no significant difference in age or BMI between subgroups with normal and variant hemoglobin. Reticulocytes, iron, vitamin B12, folate, liver function, and renal function did not vary by hemoglobin status. Thus, factors reported to interfere with HbA_{1c} analysis or levels (i.e., uremia, anemia or iron deficiency) [22] were absent. The variant hemoglobin subgroup had significantly lower MCV ($P<0.001$). Among the 109 participants, 83 had normal hemoglobin. The Capillarys 2 detected variant hemoglobin in 26 (23 HbAS and 3 HbAC) (24%) of the participants. Hemoglobin electrophoresis was performed in 88 subjects and verified that the Capillarys 2 correctly identified all variant (19 HbAS and 2 HbAC) and normal hemoglobin. Importantly, hemoglobin status did not alter glucose homeostasis; FPG, 2h glucose, S_I (insulin resistance) or AIRg (β-cell function) were not significantly different (Table 2). Mean HbA_{1c} concentrations were essentially identical among the groups (Table 2).

Forty-five participants (41%) had abnormal glucose tolerance based on 2h glucose ≥ 140 mg/dl (7.8 mmol/l) (Table 2). Diabetes was detected in 11 (10%) participants, while 34 (31%) had pre-diabetes. The sensitivity of HbA_{1c} to detect abnormal glucose tolerance in the variant hemoglobin subgroup (42%) was comparable to the whole cohort (38%) ($P=0.81$) and was slightly higher than in the normal hemoglobin subgroup (36%) (P=NS) (Table 3). The sensitivity of HbA_{1c} was slightly, but not significantly, higher than that of FPG in the whole cohort (38% vs. 31%, P=NS), the subgroup with normal hemoglobin (36% vs. 33%, P=NS) and the subgroup with hemoglobin variants (42% vs. 25%, P=NS).

Fig. 2 shows the number of true positive, false positive and false negative cases of abnormal glucose tolerance by FPG or HbA_{1c}. Among these 45 participants, eight were identified by both FPG and HbA_{1c}, whereas 22 were not identified by either assay. Nine participants identified by HbA_{1c} were not detected by FPG and 6 identified by FPG were not detected by HbA_{1c}. HbA_{1c} and FPG falsely identified 5 and 2 cases, respectively, with 1 falsely detected by both.

Discussion

Considerable improvement has been made in the analytical performance of HbA_{1c} measurement since the NGSP and IFCC standardization schemes were implemented [4]. Sebia recently developed a new CE-based HbA_{1c} method on the Capillarys 2 Flex Piercing

system. Initial reports of its technical performance in Europe have appeared in the last 2 y [23–27]. CE is an established technique routinely used for protein analysis in clinical laboratories [28] and has also been used for high throughput analysis of hemoglobinopathies and thalassemia [29]. In addition, a CE method to measure HbA_{1c} was described previously [30]. The main advantages of the Capillarys 2 Flex Piercing system for HbA_{1c} measurement include the ability to detect hemoglobin variants, high throughput, full automation, small sample volume, and ability to use primary tubes. To the best of our knowledge, this is the first detailed analysis of the Sebia Capillarys 2 Flex Piercing system performed in the US.

Measurement trueness and precision for HbA_{1c} are essential as large, prospective clinical studies have demonstrated that a relatively small change in HbA_{1c} (e.g., from 9% to 8%) significantly reduces the risk of diabetes microvascular complications [31, 32]. The National Institute for Health and Care Excellence (NICE) recommends that treatment regimens for individuals with diabetes should be assessed on the basis of a minimal change in $HbA_{1c} \geq 0.5\%$ [33]. With the recent recommendation to use HbA_{1c} to diagnose diabetes [1,2], there is an even greater need for accurate and precise HbA_{1c} measurements.

One hundred samples were analyzed on the Capillarys 2 at the Diabetes Diagnostic Laboratory and the NIH Clinical Center for the evaluation of trueness. Then the data from the 2 centers were directly compared both to each other and to an NGSP secondary reference laboratory method. Analysis of the same samples in 2 independent laboratories provides information important for patient care as the results from a single sample should not differ substantially between laboratories. Comparing a single result to the NGSP mean result (as would be done for NGSP certification) showed that > 92.5% of Capillarys 2 results were within 6% of the NGSP mean at each center (95% at center 1 and 97% at center 2), indicating that these methods pass the NGSP criteria for manufacturer and both Level I and Level II Laboratory certifications [21] at each site. The precision met the NACB/ADA criteria of intra-laboratory CV<2% and inter-laboratory CV<3.5% [34] and was comparable to or slightly better than CVs shown in other studies [23–25]. These data reveal that the Capillarys 2 Flex Piercing system demonstrated excellent accuracy and precision to meet clinical requirements.

Many of the methods routinely used for HbA_{1c} measurement are subject to interference by hemoglobin variants [35]. For example, the accuracy of some HbA_{1c} methods can be adversely affected by the presence of hemoglobin C or S trait [11,12]. The current study detected no analytical interference from three common hemoglobin variants (HbAC, HbAS, or HbAE) on the Capillarys 2 Flex Piercing assay, validating recent results [36]. By using alkaline pH buffer, normal and abnormal (or variant) hemoglobins are detected in the following order, from cathode to anode: A2/C, E, S, D, F, A0, other Hb (including minor HbA1) and then A_{1c}. Common hemoglobin variants can be detected, but do not interfere with HbA_{1c} results. The instrument is able to generate automatic flags from samples with hemoglobin variants and/or beta-thalassemia. This feature enables easy classification and interpretation of HbA_{1c} results. Note that individuals with homozygous or compound heterozygous (e.g., HbSC) hemoglobin variants have no HbA and therefore no HbA_{1c}. Thus, Capillarys 2 does not provide a value in these individuals. By contrast, boronate affinity

methods (e.g., Ultra[2]), measure total glycated hemoglobin and calculate HbA_{1c}, and would give an HbA_{1c} result for these variant hemoglobins.

While several publications have evaluated analytical interference by hemoglobin variants (reviewed in Reference [35]), very little is known about their potential impact on the performance of HbA_{1c} for the diagnosis of diabetes or prediabetes. After documenting that the Capillarys 2 accurately measures HbA_{1c} in individuals with HbAS or HbAC, we evaluated its diagnostic performance in 109 asymptomatic African immigrants to the US. Laboratory analysis (see Table 2) revealed that the individuals did not have uremia, anemia or iron deficiency, conditions reported to interfere with HbA_{1c} [22].The sensitivity of HbA_{1c} on the Capillarys 2 for abnormal glucose tolerance in individuals with variant hemoglobin was slightly (but not significantly) higher than in those with normal hemoglobin. Analysis of additional individuals is required to ascertain if this initial observation is valid. Nevertheless, these data reveal that HbAS and HbAC do not impair the ability of HbA_{1c} measured on the Capillarys 2 to detect abnormal glucose tolerance.

Although not significant, there was a trend towards higher sensitivity of HbA_{1c} than FPG for abnormal glucose tolerance. These observations contrast with those of several prior studies. For example, the National Health and Nutrition Examination Survey (NHANES, 1988–2006) indicated that HbA_{1c} detects a much lower prevalence of diabetes than FPG in the US population [10]. In the Epidemiological Study on the Insulin Resistance Syndrome (DESIR) cohort HbA_{1c} identified significantly ($P<0.0003$) less diabetes in men than FPG: 5.3% and 7.5%, respectively [37]. Similarly, the number of individuals with diabetes identified by HbA_{1c} was 70% of that identified by FPG in the Insulin Resistance Atherosclerosis Study (IRAS) [38]. For detecting individuals at increased risk of diabetes the superiority of FPG (over HbA_{1c}) was less marked in African Americans than in whites [39]. Our data reveal that in immigrants to the US from equatorial African countries with a high prevalence of HbAS, HbA_{1c} is slightly better than FPG at identifying abnormal glucose tolerance. It seems reasonable to postulate that the performance of HbA_{1c} in Africa is likely to be similar to our observations.

A limitation of our study is the relatively small number of participants with both abnormal glucose tolerance and variant hemoglobin. A second limitation is the use of single measurements of FPG, 2h glucose and HbA_{1c}; 2 measurements are preferable [1]. However, the detailed characterization of insulin secretion and insulin resistance provides reassurance that glucose homeostasis was not different between the normal and variant hemoglobin groups. Finally, abnormal glucose tolerance was defined by a single 2 h glucose. OGTTs are limited by a lack of reproducibility [22]. Nevertheless, a single OGTT is frequently used in clinical studies to identify diabetes and pre-diabetes [40, 41].

Overall, the analytical performance of Capillarys 2 HbA_{1c} is within NACB/ADA [34] and NGSP [21] recommendations, has minimal interference, and is suitable for clinical application. To our knowledge, this is the first clinical investigation of HbA_{1c} on the Capillarys 2 Flex Piercing instrument. Our cross sectional, population-based data derived from African immigrants with a high prevalence of variant hemoglobin reveal that sickle

cell trait did not interfere with the ability of HbA_{1c} (measured using the Capillarys 2) to detect abnormal glucose tolerance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AIRg	Acute Insulin Response to Glucose
FPG	Fasting Plasma Glucose
NGSP	National Glycohemoglobin Standardization Program
OGTT	Oral Glucose Tolerance Test
S_I	Insulin Sensitivity Index
SRL	Secondary Reference Laboratory
WC	Waist Circumference

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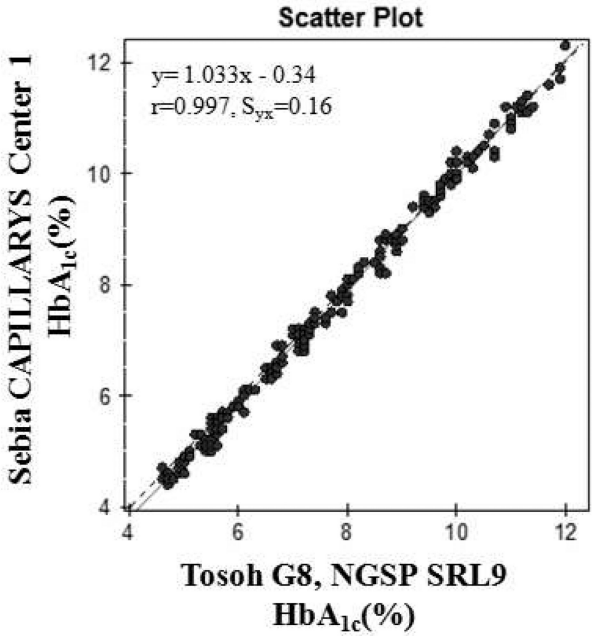
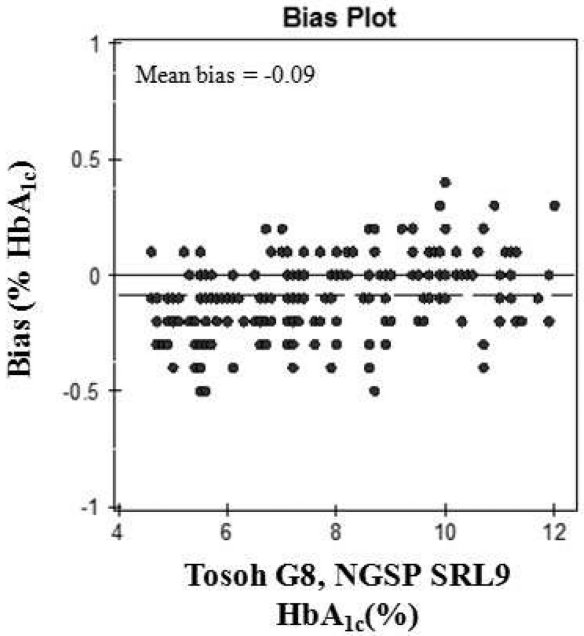
Highlights

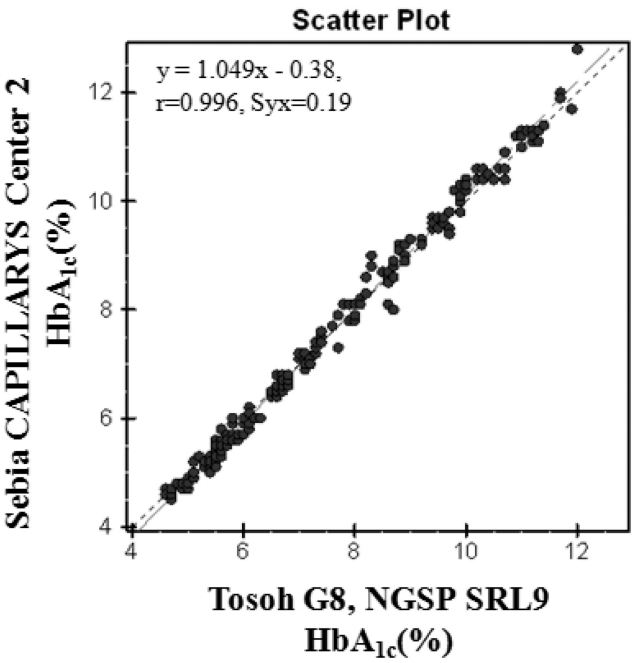
- Analytical performance of Capillarys 2 is within NACB/ADA and NGSP recommendations
- HbAS did not interfere with Capillarys 2 HbA_{1c} to detect abnormal glucose tolerance
- Capillarys 2 HbA_{1c} is suitable for Africans with high prevalence of hemoglobinopathy

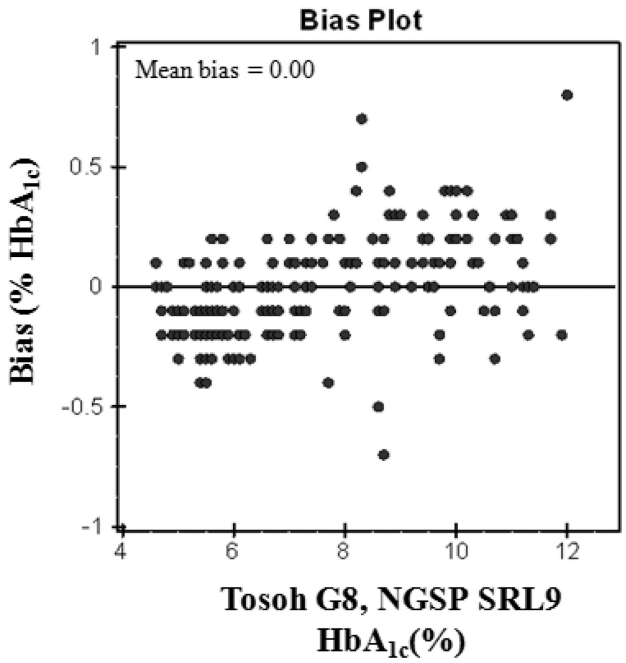
A





B





C

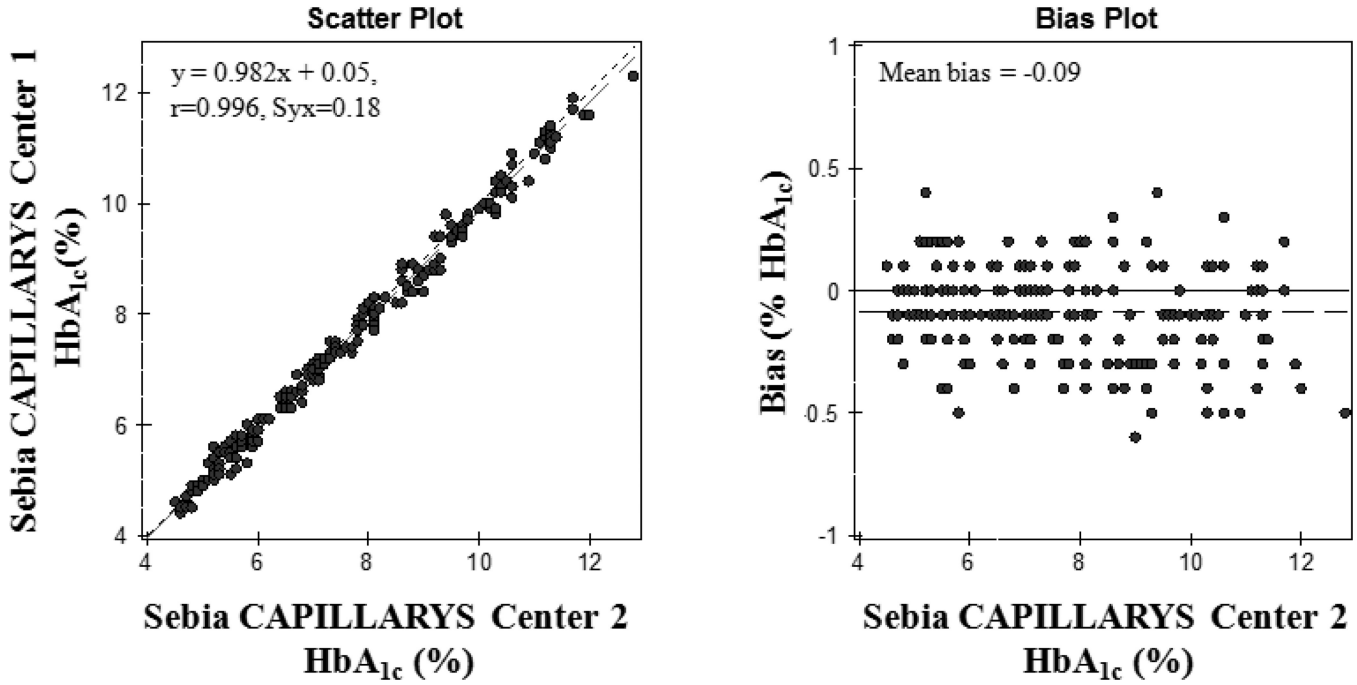


Figure 1.

Scatter and bias plots showing HbA_{1c} results obtained in 2 centers (A, center 1; B, Center 2) compared to those obtained in an NGSP SRL (Tosoh G8) at Center 1. C, comparison of HbA_{1c} values obtained with Capillarys 2 Flex Piercing instruments at the 2 centers. In the scatter plots, the lines represent $x = y$ (dotted line) and the Deming regression (dashed line). In the bias plots, the dashed line represents the mean bias.

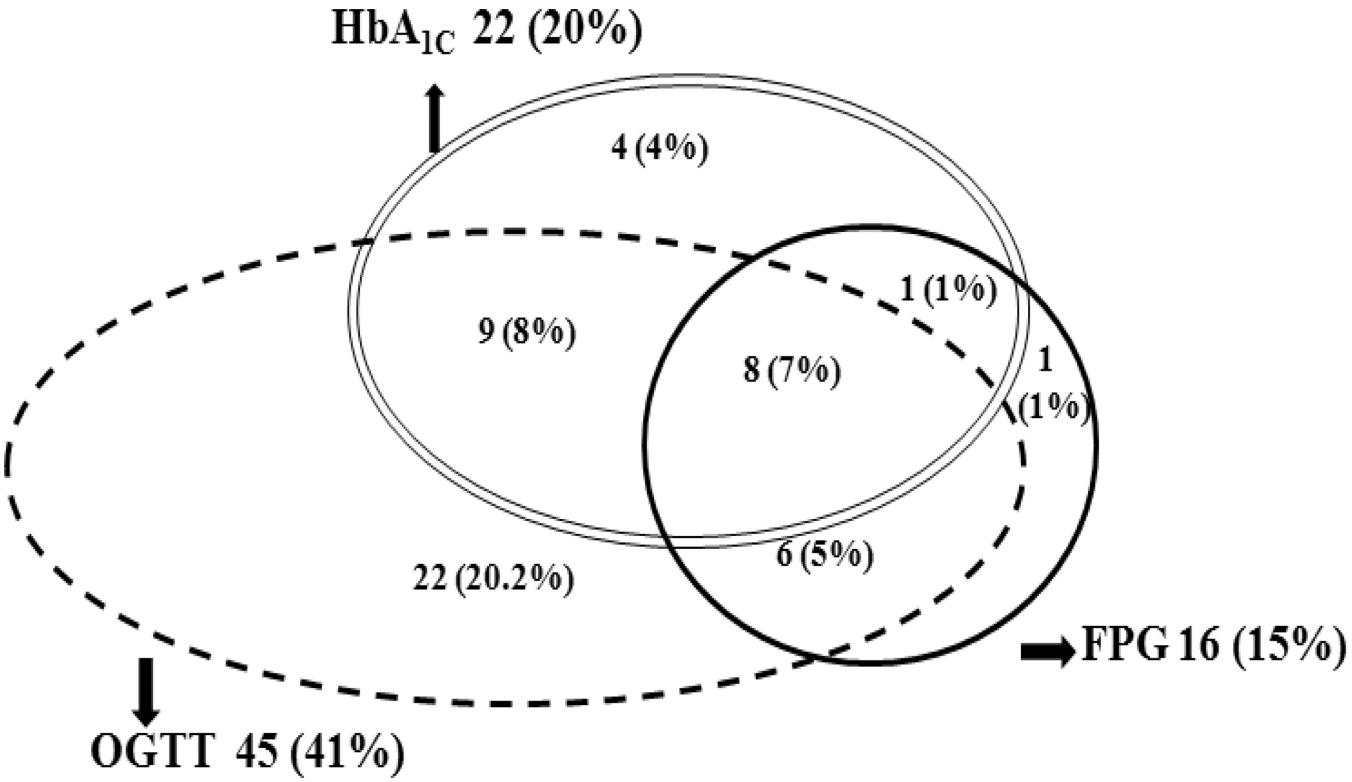


Figure 2.

The number of true positive, false positive and false negative cases of abnormal glucose tolerance by FPG or HbA_{1c} in 109 African immigrants. Dashed line, abnormal glucose tolerance defined by 2h glucose ≥ 140 mg/dl (7.8 mmol/l). True positive and false positive identified by HbA_{1c} ≥ 5.7% (39 mmol/mol) (double lines) and FPG ≥ 100 mg/dl (5.6 mmol/l) (solid line). The numbers (percentage) of participants are indicated for each category.

Table 1

Imprecision of Capillarys 2 Flex Piercing HbA_{1c} assay.

NGSP units							
% HbA_{1c} Mean	Imprecision (CV, %)						
	Center 1			Center 2			Inter-laboratory
	Within-run	Between-run	Total	Within-run	Between-run	Total	Total
4.7	2.2	0.0	2.3	2.0	0.0	2.0	2.1
6.3	1.2	0.6	1.4	1.4	0.4	1.5	1.4
7.4	1.2	0.7	1.4	1.3	0.0	1.3	1.3
11.1	1.1	0.5	1.2	0.9	0.4	1.0	1.1

SI units							
HbA_{1c} Mean (mmol/mol)	Imprecision (CV, %)						
	Center 1			Center 2			Inter-laboratory
	Within-run	Between-run	Total	Within-run	Between-run	Total	Total
28	3.7	0.0	3.8	3.4	0.9	3.5	3.7
45	1.9	0.7	2.0	2.1	0.7	2.2	2.1
57	1.7	0.9	2.0	1.9	0.0	1.9	1.9
98	1.3	0.5	1.4	1.0	0.5	1.2	1.3

Table 2

Metabolic and demographic characteristics[†]

Parameter	Total Group n=109	Normal Hb n=83	Variant Hb n=26	P value[*]
Male (%)	72	72	69	NS
Age (y)	40±10	40±10	41±10	NS
BMI (kg/m^2)	28.1±4.5	28.1±4.7	28.0±3.7	NS
WC (cm)	92±12	92±12	92±11	NS
Hemoglobin, g/dl (g/l)	14.1±1.3 (141±13)	14.1±1.3 (141±13)	14.3±1.1 (143±11)	NS
Hematocrit (%)	41.9±3.3	42.1±3.3	41.2±3.2	NS
MCV (fl)	84.8±5.7	85.2±5.5	80.7±4.9	<0.001
Reticulocytes (%)	1.27±1.18	1.27±0.52	1.27±0.37	NS
Bilirubin Total, mg/dl (μmol/l)	0.53±0.22 (9.0±3.8)	0.51±0.22 (8.7±3.8)	0.57±0.23 (9.7±3.9)	NS
Bilirubin Direct, mg/dl (μmol/l)	0.15±0.06 (2.6±1.0)	0.14±0.06 (2.4±1.0)	0.16±0.06 (2.7±1.0)	NS
ALT (U/l)	34±17	33±18	35±16	NS
AST (U/l)	24±11	25±12	23±6	NS
BUN, mg/dl (mmol/l)	12±3 (4.3±1.1)	12±3 (4.3±1.1)	12±2 (4.3±0.7)	NS
Creatinine, mg/dl (μmol/l)	0.89±0.17 (79±15)	0.89±0.17 (79±15)	0.92±0.16 (81±14)	NS
eGFR (mL/min/1.73 m^2)[‡]	110±20	111±21	104±15	NS
Iron, μg/dl (μmol/l)	83.5±27.1 (14±5)	82.5±28.5 (14±5)	86.8±22.1 (15±4)	NS
Transferrin, mg/dl (g/l)	246±35 (2.46±0.35)	244±32 (2.44±0.32)	244±32 (2.44±0.32)	NS
Transferrin saturation (%)	24.8±8.7	24.5±9.0	25.9±7.7	NS
Ferritin μg/l (pmol/l)	113±90 (253±202)	110±92 (247±206)	121±86 (271±193)	NS
Vitamin B12, pg/mL (pmol/l)	613±335 (452±247)	601±347 (443±256)	652±297 (481±219)	NS
Folate, ng/mL (nmol/l)	12.6±4.7 (28.6±10.7)	12.4±4.7 (28.1±10.7)	13.5±4.8 (30.6±10.9)	NS
HbA_{1C}, % (mmol/mol)	5.4±0.8 (35±8)	5.4±0.8 (35±9)	5.4±0.3 (36±4)	NS
Abnormal glucose tolerance[#] (%)	41% (45/109)	40% (33/83)	46% (12/26)	NS
Diabetes[&] (%)	10% (11/109)	10% (8/83)	12% (3/26)	NS
Pre-Diabetes[//] (%)	31% (34/109)	30% (25/83)	35% (9/26)	NS
FPG, mg/dl (mmol/l)	92±17 (5.1±0.9)	93±18 (5.2±1.0)	90±10 (5.0±0.6)	NS
2h glucose, mg/dl (mmol/l)	142±45 (7.9±2.5)	142±48 (7.9±2.7)	140±45 (7.8±2.5)	NS
S_I ((mU/l)$^{-1}$ min^{-1})	3.27±1.79	3.41±1.80	2.89±1.76	NS
AIRg (mUL^{-1} min)	747±526	711±390	851±801	NS

[†] Data represent mean ± SD

[*] To compare normal and variant hemoglobin subgroups, the student t-test and chi-square test were applied for continuous variables and categorical variables, respectively.

[‡] eGFR calculated based on the Modification of Diet in Renal Disease (MDRD) Equation

[#] Defined as 2h glucose≥7.8 mmol/l

&Defined as 2h glucose≥11.1 mmol/l

//Defined as 2h glucose≥7.8 mmol/l and <11.1 mmol/l

Abbreviations: BMI, body mass index; MCV, mean corpuscular volume; eGFR, estimated glomerular filtration rate; HbA_{1C}, hemoglobin A1C; FPG, fasting plasma glucose; visceral adipose tissue; WC, waist circumference; SI, insulin sensitivity index; AIRg, acute insulin response to glucose.

Table 3

Detection of abnormal glucose tolerance*

Group	Sensitivity#			Specificity#		
	FPG	HbA_{1c}	Combined	FPG	HbA_{1c}	Combined
Whole Cohort (n=109)	31% (14/45)	38% (17/45)	51% (23/45)	97% (62/64)	92% (59/64)	91% (58/64)
Normal Hemoglobin (n=83)	33% (11/33)	36% (12/33)	52% (17/33)	96% (48/50)	94% (47/50)	92% (46/50)
Hemoglobin Variant (n=26)	25% (3/12)	42% (5/12)	50% (6/12)	100% (14/14)	86% (12/14)	86% (12/14)

*Abnormal glucose tolerance defined by 2h glucose ≥140 mg/dl (7.8 mmol/l)

#Sensitivity and specificity to detect abnormal glucose tolerance (diabetes and prediabetes) were calculated based on the ADA criteria [1] i.e., FPG (FPG ≥ 100 mg/dl (5.6 mmol/l)), HbA_{1C} (HbA_{1C} ≥ 5.7% (39 mmol/mol)) or combined (either FPG ≥ 100 mg/dl (5.6 mmol/l) or HbA_{1C} (HbA_{1C} ≥ 5.7% (39 mmol/mol)).