Assessment of Two Diabetes Point-of-Care Analyzers Measuring Hemoglobin A1c in the Peruvian Amazon

by

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Department of Global Health
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Date: February 5, 2016
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Dennis Clements

Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the Department of Global Health
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ABSTRACT

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Abstract

Aims

Measurement of glycated hemoglobin (HbA1c) is an important indicator of glucose control over time. Point-of-care (POC) devices allow for rapid and convenient measurement of HbA1c, greatly facilitating diabetes care. We assessed two POC analyzers in the Peruvian Amazon where laboratory-based HbA1c testing is not available.

Methods

Venous blood samples were collected from 203 individuals from six different Amazonian communities with a wide range of HbA1c, 4.4-9.0% (25-75 mmol/mol). The results of the Afinion AS100 and the DCA Vantage POC analyzers were compared to a central laboratory using the Premier Hb9210 high-performance liquid chromatography (HPLC) method. Imprecision was assessed by performing 14 successive tests of a single blood sample.

Results

The correlation coefficient *r* for POC and HPLC results was 0.92 for the Afinion and 0.93 for the DCA Vantage. The Afinion generated higher HbA1c results than the HPLC (mean difference = +0.56% [+6 mmol/mol]; $p < 0.001$), as did the DCA Vantage (mean difference = +0.32% [4 mmol/mol]). The bias observed between POC and HPLC did not vary by HbA1c level for the DCA Vantage ($p = 0.190$), but it did for the Afinion ($p <$

0.001). Imprecision results were: CV = 1.75% for the Afinion, CV = 4.01% for the DCA Vantage. Sensitivity was 100% for both devices, specificity was 48.3% for the Afinion and 85.1% for the DCA Vantage, positive predictive value (PPV) was 14.4% for the Afinion and 34.9% for the DCA Vantage, and negative predictive value (NPV) for both devices was 100%. The area under the receiver operating characteristic (ROC) curve was 0.966 for the Afinion and 0.982 for the DCA Vantage. Agreement between HPLC and POC in classifying diabetes and prediabetes status was slight for the Afinion (Kappa = 0.12) and significantly different (McNemar's statistic = 89; $p < 0.001$), and moderate for the DCA Vantage (Kappa = 0.45) and significantly different (McNemar's statistic = 28; $p < 0.001$).

Conclusions

Despite significant variation of HbA1c results between the Afinion and DCA Vantage analyzers compared to HPLC, we conclude that both analyzers should be considered in health clinics in the Peruvian Amazon for therapeutic adjustments if healthcare workers are aware of the differences relative to testing in a clinical laboratory. However, imprecision and bias were not low enough to recommend either device for screening purposes, and the local prevalence of anemia and malaria may interfere with diagnostic determinations for a substantial portion of the population.

Dedication

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1. Introduction

Diabetes mellitus is a complex, chronic illness that has emerged as a global health crisis. The term "diabetes" refers to a group of metabolic disorders in which glucose is underutilized and overproduced, resulting in hyperglycemia. The most common classifications of diabetes are type 1 characterized by insufficient production of insulin in the body, type 2 characterized by progressive insulin resistance, and gestational diabetes that appears during pregnancy. Type 2 diabetes comprises 90-95% of worldwide diabetes cases, and it has partially been linked to excess body weight and physical inactivity.[1]

The International Diabetes Federation (IDF) estimates that nearly half of all people with diabetes are undiagnosed.[2] One of the most pressing concerns for halting the rise in diabetes is finding different ways to reach and diagnose the millions of people who unknowingly live with it. One such way may be through the analysis of glycated hemoglobin (HbA1c) to detect people living with diabetes or at high risk for future onset of the disease. Point-of-care (POC) analyzers have been developed that allow healthcare providers to obtain rapid HbA1c results on-site. These results have typically been used to supplement clinical laboratory testing, but there is a great interest to use them for diagnostic tests and therapeutic monitoring purposes.[3] Tests performed in clinical laboratory settings have shown that some POC analyzers perform as well as "gold standard" laboratory-based methods.[4] However, questions remain as to how accurate

they perform in different environments, particularly in remote and rural clinical settings where environmental conditions may be harsher than a clinical laboratory.

1.1 Diabetes in low- and middle-income countries

Over the past few decades, diabetes and other non-communicable diseases have emerged as the dominant sources of global death and disability as part of the epidemiologic transition. The World Health Organization (WHO) estimates 1.5 million deaths were directly caused by diabetes in 2012,[5] and the IDF cites an even higher figure of 5 million deaths caused by diabetes in 2015.[6] Regardless of where in that range the true death toll lies, it is certain that low- and middle-income countries (LMICs) have been disproportionately affected by this disease. LMICs contain 75% of the global diabetes population and 81% of the undiagnosed cases, although these countries only account for 19% of global health expenditures for the disease.[6]

1.2 Indicators of diabetes and prediabetes

Diabetes can be detected three different ways: through an oral glucose tolerance test (OGTT), fasting plasma glucose (FPG), or glycated hemoglobin (HbA1c). An International Expert Committee with members appointed from the American Diabetes Association (ADA), the IDF, and the European Association for the Study of Diabetes convened in 2008 to consider the different tools for diagnosing diabetes.[7] They reached a consensus that HbA1c should be the preferred method for diabetes diagnosis in nonpregnant adults, with the exception of patients with certain hemoglobinopathies or

conditions that influence erythrocyte turnover (such as anemia, chronic malaria, or major blood loss). They recommended that clinicians around the world account for the performance of local HbA1c assays and the prevalence of the aforementioned conditions when considering changing to HbA1c as the main method to diagnose diabetes. If HbA1c testing is not feasible due to high prevalence of those conditions or if costs of providing the assay preclude its routine use, previously recommended methods like OGTT or FPG (performed twice) were deemed acceptable by the committee.

According to the ADA[1] and the WHO,[8] the criterion for the diagnosis of diabetes is an HbA1c level greater than or equal to 6.5%. HbA1c levels between 5.7-6.4% indicate an individual has impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) and is at greater risk for the future development of type 2 diabetes.[7] The ADA uses the term "prediabetes" to describe these individuals.[1] Up to 70% of individuals with prediabetes may develop diabetes, although lifestyle modifications may reduce the risk of conversion by 40-70%.[9]

1.3 HbA1c characteristics

Erythrocytes are the most common type of blood cells and the principal means of delivering oxygen to body tissues. Each erythrocyte contains approximately 200-300 million hemoglobin proteins.[10] If a glucose molecule in the bloodstream is near a hemoglobin molecule, they can covalently bind to each other to create an irreversible complex. This complex is called glycated hemoglobin, also known as HbA1c, and is

considered a proxy for how much glucose is in the blood stream since the rate of formation of HbA1c is directly proportional to the ambient glucose concentration.[11] When instruments measure HbA1c, they are measuring the percentage of overall hemoglobin that is bound to glucose. Since erythrocytes and the hemoglobin in them have a lifespan of about 120 days, HbA1c is a composite measure of glucose exposure with the most recent 30 days contributing about 50% of the measure, the previous 31-90 days contributing about 40%, and the final 10% from the previous 91-120 days.[12]

The main advantage of testing HbA1c is that it indicates glucose control over time. Also, the patient is not required to fast prior to the blood draw, which allows a patient to receive the test at any time. In addition to the convenience of the test, HbA1c shows greater pre-analytical stability, lower biological variability, and less day-to-day perturbations during stress and illness than the glucose tests.[1]

Those who argue that HbA1c should not be the primary method for diagnosing diabetes cite that HbA1c levels can vary for two people with the same glucose levels due to factors that affect interpretation of the test results.[11,13] Glycemia is recognized to change with age, and a positive association between HbA1c levels and age has been identified.[14] Racial and ethnic differences may also have an impact on HbA1c, as African Americans, Hispanics, and Asian/Pacific Islanders have demonstrated higher HbA1c levels compared to non-Hispanic whites.[15] Also, conditions that influence erythrocyte turnover such as anemia or chronic malaria will falsely lower HbA1c test results.[11]

HbA1c may also underestimate glycemic control for diabetic subjects with renal disease.[16]

1.4 Diagnostic procedures for measuring HbA1c

The current gold standard for measuring HbA1c levels is the use of clinical laboratory-based testing methods. The three major laboratory-based testing methods are high-performance liquid chromatography (HPLC), antibody based immunoassay, and enzymatic assay. Use of these methods is often expensive, and the financial and geographic barriers make it difficult and often impossible for health clinics in LMICs to access these tests. Thus, POC analyzers offer a potential solution to overcoming these barriers. Use of a POC analyzer in a clinic or health post allows a medical professional to obtain an HbA1c level in a matter of minutes. This has important implications for patients, as previous studies have shown that obtaining HbA1c levels during the same visit is associated with improvement in glycemic control.[17-19]

Although use of POC analyzers can provide the patient with results in an expeditious manner, the evidence is mixed regarding whether they are accurate enough to aid in clinical decision making. Guidelines published by an expert committee on diabetes in 2011 that were reviewed and accepted by the National Academy of Clinical Biochemistry (NACB) and approved by the ADA recommends "point-of-care Hb A1c assays are not sufficiently accurate to use for the diagnosis of diabetes."[20] The quality of this evidence for this recommendation was rated moderate, meaning "further research is

likely to have an important impact on our confidence in the estimate of effect and may change the estimate and the recommendation."[20] In 2014, Lenters-Westra and Slingerland demonstrated that a few POC devices produced excellent results that were even better than some laboratory-based methods.[4] Nevertheless, in 2015 the ADA still did not recommend POC analyzers for the diagnosis of diabetes due to the lack of mandatory proficiency testing (PT) for laboratories performing the test.[1] Although PT is required for laboratory-based methods like HPLC, it is not required for most POC analyzers since they are waived by the Clinical Laboratory Improvements Amendments.[21]

1.5 Standardized testing for HbA1c instruments

The ADA recommends that healthcare facilities only use HbA1c methods that are National Glycohemoglobin Standardization Program (NGSP) certified and standardized to the Diabetes Control and Complications Trial (DCCT).[1] To receive NGSP certification, a device must pass an annual test under optimal conditions that involves the analysis of 40 blood samples at the device's manufacturing site using a single lot of reagents and one instrument. To pass the test, 37 of the 40 HbA1c values must be within 6% of the results from an NGSP secondary reference laboratory.[22]

In addition to the NGSP testing, the College of American Pathologists (CAP) conducts regular testing of HbA1c analyzers. The CAP has two different programs for HbA1c testing. The first program (GH2) involves sending three samples of fresh whole

blood to multiple clinical laboratories twice a year in order to measure bias (difference between the POC analyzer and the known value) and imprecision through the coefficient of variation (CV).[23] The second program produces the same outcomes, but instead with five blood samples that are sent out three times a year. In contrast with the NGSP certification, the CAP values reflect aggregate performance across numerous laboratories, with multiple analyzers from the same model, and typically different reagent lots. However, CAP data may not be representative of most users of POC analyzers since participating sites are more likely to be associated with a CAP-accredited laboratory and may have more experienced testing personnel.[21] Therefore, additional research is needed to determine the accuracy of POC analyzers in field-settings, particularly amongst vulnerable and underserved populations.

1.6 Afinion and DCA Vantage POC analyzers

The Afinion™ AS100 Analyzer (Alere Technologies) is a POC device that utilizes borate affinity chromatography to measure the total percentage of glycation using a 1.5μL sample of blood.[24] It has a reported HbA1c range of 4.0-15.0% and produces results in three minutes. Operating conditions are between 15-32°C (59-89°F) with 10-90% humidity for the analyzer,[25] and 18-30°C (64-86°F) for the test cartridge.[24] The base station costs approximately $3,500 and a package of 15 single-use testing kits costs $120.[3]

The DCA Vantage™ Analyzer (Siemens Medical Solutions Diagnostics) uses an immunoassay based on antibodies binding to glycated hemoglobin tetrapeptide or

hexapeptide molecules using a 1.0μL sample of blood.[3] It has a reported HbA1c range of 2.5%-14.0% and produces results in six minutes.[26] Operating conditions are between 15-32°C (61-88°F) with 15-90% relative humidity. The base station costs $2,100-3,600 and a package of 10 single-use testing kits costs $75.[3] Both instruments utilize reagent cartridges that are used to collect samples of either capillary or venous blood and are inserted in the device for analysis.

1.7 Study aims

The Afinion and DCA Vantage have shown promising results from NGSP and CAP testing.[23,27] However, there is limited research on how these analyzers perform in field-settings, particularly in an LMIC where operating conditions may be relatively harsh. To our knowledge, no studies have investigated the performance of HbA1c devices in South America. With a suspected increase of non-communicable diseases in this area and the logistical difficulties that make obtaining laboratory-confirmed HbA1c values a near-impossibility for many of the communities, this area is an ideal location to research and potentially implement POC analyzers. The aim of this study was to evaluate the performance of the Afinion and DCA Vantage analyzers to test HbA1c in the Amazonian region of Peru and compare the results to an NGSP certified HPLC analyzer.

2. Materials and Methods

A cross-sectional, prospective study was performed in communities surrounding the Amarakaeri Communal Reserve in the Peruvian Amazon rainforest. This study was part of a larger demographic health survey that sought to capture baseline health information for communities in this region.

2.1 Setting

Encompassing 60% of Peru's total land area with only 8% of the country's population living there, the Peruvian Amazon rainforest is a vast territory with high biological biodiversity and high rates of poverty.[28] Madre de Dios (MDD) is a department located in the Southern Amazon basin. It is home to over 137,000 people, with several indigenous tribes disbursed throughout the department.[29] The economy is based heavily on natural products and raw materials such as cotton, coffee, sugarcane, and Brazil nuts. Gold mining and logging are other large industries in the region, and are often performed illegally. Leading causes of morbidity and mortality are respiratory disease, trauma, cancers, malaria, cardiovascular disease, and diabetes, but other common diseases like dengue, rabies, leptospirosis, leishmaniasis among others have been emerging.[29] The recent construction of the Interoceanic Highway, land conflicts from agricultural expansion, Brazil nut and logging concessions, expansion of gold mining, and a rapidly growing and increasingly urbanized population are some unique challenges faced in this region. The isolated nature of the geography in MDD, along

with shortages in healthcare workers in the region makes providing healthcare services to the people there very difficult. Medical care in this region is typically provided at government-run health posts that have variable quality and supplies to provide basic medical care. Numerous studies have shown that there are large health disparities that persist in this part of the country.[30,31]

A major challenge for healthcare workers in the Amazon region is combating the rising incidence of non-communicable diseases, and in particular, diabetes. Diabetes mellitus was the tenth leading cause of death in the Madre de Dios (MDD) department in 2012, with an estimated 2.4% of all reported deaths attributed to the disease.[29] Mortality from diabetes mellitus was especially high for women in the MDD region, with 5.5% of all female deaths attributed to the disease in 2012.[29] These numbers may be underestimated, as no hospital or clinic in the region currently has the technology capable of testing HbA1c levels. The current standard of testing in this region is to measure fasting blood glucose levels. However, the variability of glucose levels in blood calls into question the reliability of these tests. Therefore, there is a critical need in the region to incorporate POC tests that can accurately detect and manage HbA1c levels in an inexpensive and timely manner.

For this study, the following six communities were identified to participate: Huepetuhe, Quebrada Nueva, Caychihue, Setapo, Puquiri, and Quince Mil (Fig 1). The majority of people living in these communities are of Spanish and American Indian

descent or have migrated from the Andean Highlands and are involved in occupations such as agriculture, fishing, logging, Brazil nut harvesting, and artisanal gold mining. These communities were selected for this study based on their proximity to the city of Mazuco, the central location where all of the POC testing was performed, and also they were suspected to have the highest rates of diabetes in the region due to large populations of gold miners there.

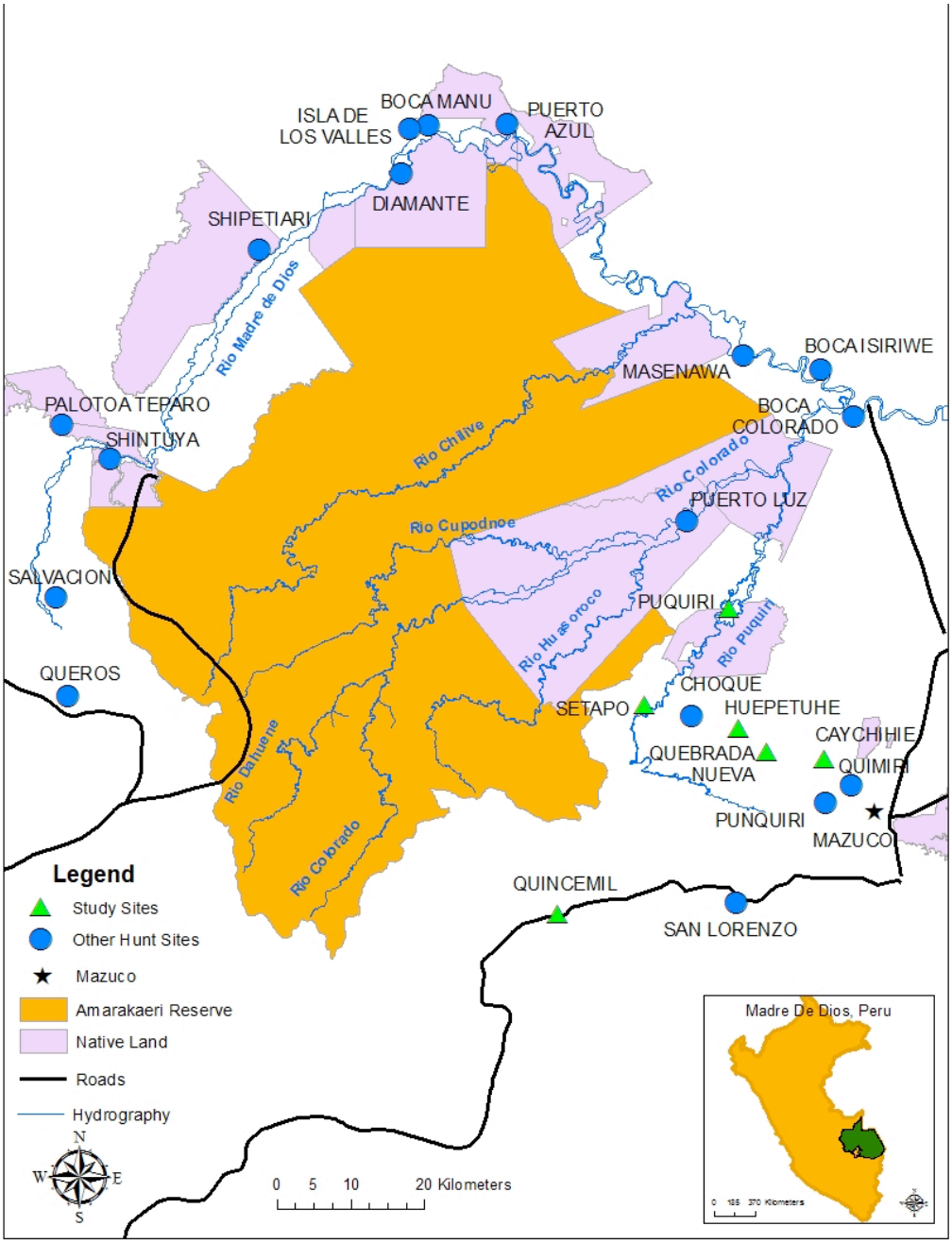

Figure 1: Map of study sites and location where HbA1c values were obtained using point-of-care analyzers.

Green triangles represent the location of the six communities selected for this study, the black star represents the site of HbA1c analysis using the point-of-care analyzers, and the blue circles are locations of other communities that were a part of the Hunt Demographic Health Survey but not selected for this study.

2.2 Ethical considerations

Approval for this study was obtained from the Institutional Review Board of the Universidad Peruana Cayetano Heredia. All adult participants and children age 12 and older provided informed consent and were advised on the research aims and objectives, rationale, expected benefits and rights of participants.

2.3 Sampling strategy

All households with at least one woman of child-bearing age (WCBA, 15-49 years) were eligible for selection in this study. For the communities with less than 75 households, each household was approached to participate in the study. For communities with more than 75 households, community maps were obtained or created to draw a random sample. Selected households that met the eligibility criterion were introduced to the study by trained fieldworkers and family members were asked if they would like to participate. If consent was obtained, surveys were administered and biomarker samples were collected from the mother of the sentinel family unit, her spouse, and any children aged 12 and under.

2.4 Data collection

Trained field teams collected survey information and biomarkers. Field teams consisted of one interviewer to administer surveys and one nurse to collect biometric samples. All fieldworkers were trained in human subjects research, project goals, survey administration, biomarker collection, and sample storage.

Venous blood samples were drawn from consenting participants and collected in K2 collection tubes containing edetic acid (EDTA) as a preservative. The tubes were labeled with the patient's name and unique identifying number and placed in a Credo Cube™ cold box to store the samples between 2-8°C. Capillary blood samples were also drawn and anemia testing was performed with a HemoCue® POC device.

Per manufacturer recommendations, each analyzer was verified to be in proper working condition by testing controls supplied by the manufacturer before any tests were performed on participant samples. Batches of blood samples were delivered approximately twice per week to the town of Mazuco. Since HbA1c measurement can be affected by high temperature, the heat-sensitive color pads on the front of each box of reagents was checked to ensure that the maximum temperature limit of 40°C was not exceeded.

Blood samples were tested with each POC analyzer. In addition to recording data on the HbA1c level of each sample, data was collected on the temperature, humidity, and barometric pressure of the room at the time of testing using the Ambient Weather WS-110 Wireless Weather Station. To test the imprecision of each POC analyzer, one blood sample was selected due to its HbA1c level near the 6.5% (48mmol/mol) diagnostic threshold for diabetes and measured 14 consecutive times with each device.

After obtaining HbA1c levels from the POC analyzers, the samples were frozen and shipped to the Medlab Laboratory in Lima, Peru. There, HbA1c levels were obtained for each sample using HPLC via the Premier Hb9210™ Analyzer (Trinity Biotech). Control samples with HbA1c values of 5.5% and 11.5% were tested daily on the Premier Hb9210 to ensure proper calibration of the analyzer.

2.5 Analytic strategy

Calculations for imprecision were performed by use of Microsoft® Excel 2011 (Microsoft Corp.). Statistical analysis for everything else was performed with STATA v13.1 (StataCorp LP). Reported *p*-values were two sided and considered statistically significant at $\alpha = 0.05$. HbA1c was reported in DCCT aligned units (%) and converted to IFCC units (mmol HbA1c per mol unglycated hemoglobin) using the following master equation from Hoelzel et al.:[32]

$$IFCC = (NGSP - 2.15) / 0.0915$$

2.5.1 Imprecision

To validate that the POC analyzers were suitable for use with different reagent cartridges, the imprecision of each device was evaluated. There is some variation in the terminology used, but for the purposes of this study, within-run imprecision, also known as repeatability or consistency, is defined by Chesher[33] as, "The closeness of agreement between results of successive measurements obtained under identical conditions." As Chesher notes, while the term precision describes variation around a

central value, what is really measured is imprecision. The standard deviation (SD) or variance (SD^2) are the typical measurements of imprecision for a normal distribution. If the population mean (μ) and SD (σ) are not known, the coefficient of variation (CV) can be used to express the imprecision of measurement of a particular assay by estimating μ and σ from the sample standard deviation (S) and the mean of the duplicates ($\bar{x}$).[33] The CV is commonly used in biochemistry studies and is often cited in HbA1c POC studies such as the CAP surveys.[23,34] For the duplicate samples in this study, the CV was calculated using the following formula:

$$\mathrm{CV} = \frac{s}{\bar{x}}$$

CVs were only calculated from DCCT units, but it should be noted that CVs would be higher in IFCC units (mmol/mol) due to the conversion equation.

2.5.2 Accuracy

Univariate regression analysis was performed and Pearson's correlation coefficient (*r*) was calculated for the paired samples to assess the strength of relationship between each POC analyzer and the gold standard HPLC method. To check that the data met the assumptions of regression, statistical tests were performed to identify outliers (through analysis of residuals, leverage, Cook's D and DFITS) confirm the linearity of the data (scatter plot with a lowess smoother), approximate normal distribution (kernel density plot, P-P normal probability plot, Q-Q normal probability

plot, inter-quartile range, Shapiro-Wilk W test), and homoscedasticity (White's test, Breusch-Pagan test) (Appendix A).

Bias is the average difference between an estimator and the true value. For this study, bias was calculated as the mean difference between each POC analyzer and the HPLC method. Paired *t* tests were performed to analyze the statistical significance of the biases. Univariate linear regression was performed to test whether the bias was constant across the range of HbA1c concentrations.

Mean relative difference was calculated as the difference between the mean of each POC analyzer and the HPLC method divided by the mean of the HPLC method (using DCCT units). The NGSP uses ±6.0% as the criterion for certification of HbA1c analyzers.[22] The number of samples that fell outside that range were summarized for each POC analyzer.

Bland-Altman plots depict individual subject differences between two tests plotted against each individual's mean HbA1c value.[34] Examination of these plots gives a rough indication of systematic bias and random error by examining the direction and magnitude of the scatter around the zero line, respectively. Limits of agreement (LOA) are calculated by multiplying the standard deviation of the differences between the two tests by 1.96, then adding and subtracting that value from the mean difference to obtain a 95% confidence interval. Bland-Altman plots were generated for this study to visually

represent bias and the LOA between each POC analyzer and the gold standard HPLC method.

2.5.3 Sensitivity, specificity, PPV, NPV

To evaluate the clinical effectiveness to diagnose diabetes and identify prediabetes, we calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each POC analyzer based on an HPLC HbA1c ≥ 5.7% (39 mmol/mol). Receiver operating characteristic (ROC) curves were generated to evaluate the accuracy of POC HbA1c level as a predictor of prediabetes or diabetes.

McNemar's test was performed to test whether the categorization of patients as prediabetic or diabetic (HbA1c ≥ 5.7% [39 mmol/mol]) differs by use of POC or HPLC methods. The kappa statistic was also calculated to assess the agreement between each POC analyzer and the HPLC results for categorizing patients as prediabetic, diabetic, or normal.

2.5.4 Effect of ambient testing conditions

To test whether ambient conditions had an affect on the HbA1c results, a multiple regression was run to predict HPLC HbA1c from each POC measurement in addition to temperature, humidity, and barometric pressure. Full models were fit with complete data, and variables with the least significant parameter estimate were removed one at a time until only statistically significant variables remained in the final model.

3. Results

HbA1c results using the Afinion and DCA Vantage POC analyzers and the Premier Hb9210 HPLC analyzer were obtained for 203 individuals. 137 participants were female (72%) and the average age was 36.6 years, with an age range of 12-75 years. Capillary blood samples were analyzed for 196 participants, and 38 individuals (19.3%) had hemoglobin concentrations indicating anemia. Anemia was more common in women (21.4%) than men (13.7%). The overall prevalence was of "mild" public health significance according to WHO thresholds.[35] 198 participants responded to the survey question on malaria, and 29 individuals (14.6%) self-reported a previous case. Two individuals (1.0%) reported being diagnosed with malaria within the previous four months.

The HbA1c values ranged from 4.4% (25 mmol/mol) to 9.0% (75 mmol/mol) per the HPLC Premier Hb9210 analyzer (Table 1). Mean values for HbA1c concentration were 5.8% (39 mmol/mol) for the Afinion, 5.5% (37 mmol/mol) for the DCA Vantage, and 5.2% (33 mmol/mol) for the Premier Hb9210. 16 of the 203 samples tested by the Afinion reported an error code indicating that the blood sample had hemolyzed to a level that would interfere with analysis. No error codes were seen with the DCA Vantage or the Premier Hb9210. Hemolysis is not likely to be an issue for samples that are tested immediately in a clinical setting.

Table 1: Average HbA1c concentration (DCCT units)

	HbA1c (%)		
	Afinion	**DCA Vantage**	**Premier Hb9210**
N	187	203	203
Mean	5.8	5.5	5.2
Median	5.7	5.4	5.1
Range	5.0-9.6	4.7-9.1	4.4-9.0

3.1 Imprecision analysis

A single sample of intravenous blood was tested 14 consecutive times with different reagent cartridges for the Afinion and the DCA Vantage. The mean HbA1c value for the Afinion sample was 6.7% (50 mmol/mol), and 6.4% (46 mmol/mol) for the DCA Vantage. The sample standard deviation (S) was 0.12% for the Afinion, and 0.26% for the DCA Vantage. The corresponding CV was lower for the Afinion (1.75%) than the DCA Vantage (4.01%).

3.2 Accuracy analysis

The residuals for each POC device demonstrated an approximate normal distribution. Results from the univariate regression analysis and Pearson's correlation coefficient (r) indicate a strong positive correlation between the HbA1c values of the HPLC analyzer to the Afinion (Fig 2; $r = 0.92$; $r^2 = 0.84$; $p < 0.001$) and the DCA Vantage (Fig 3; $r = 0.93$; $r^2 = 0.87$; $p < 0.001$).

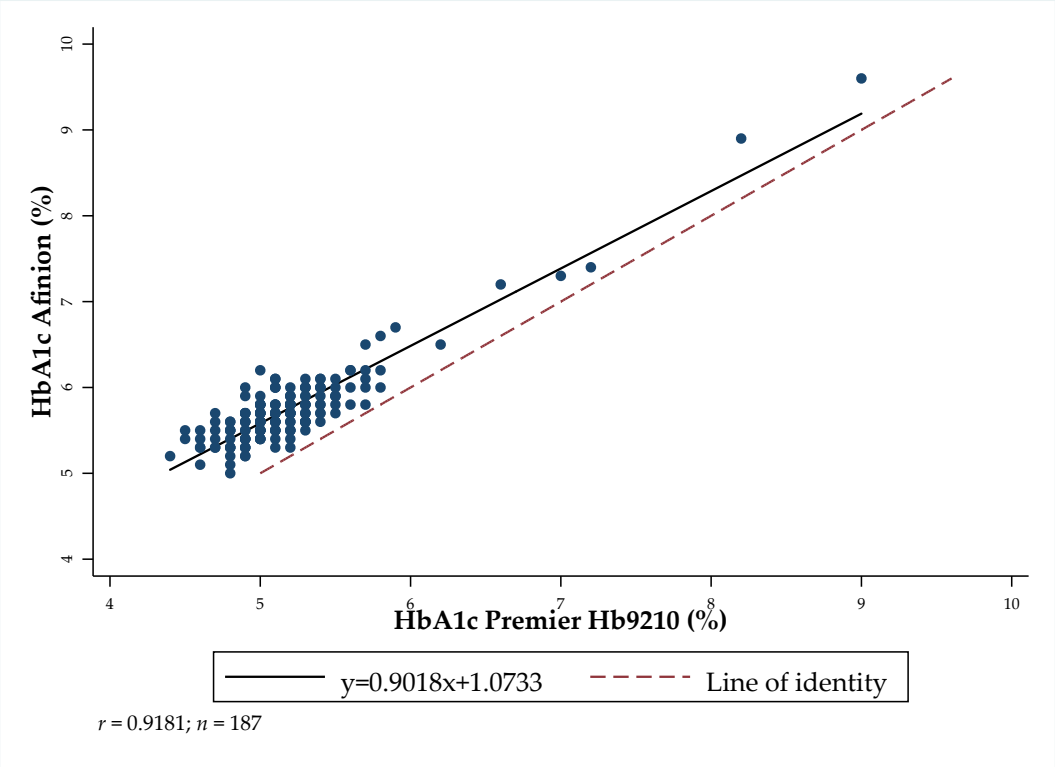

Figure 2: Correlation between the Afinion analyzer to the Premier Hb9210 for measuring HbA1c (DCCT units).

Blue dots represent individual samples, the dotted red line represents the line of identity x = y, and the solid black line is the regression line. Abbreviations include: DCCT, Diabetes Control and Complications Trial.

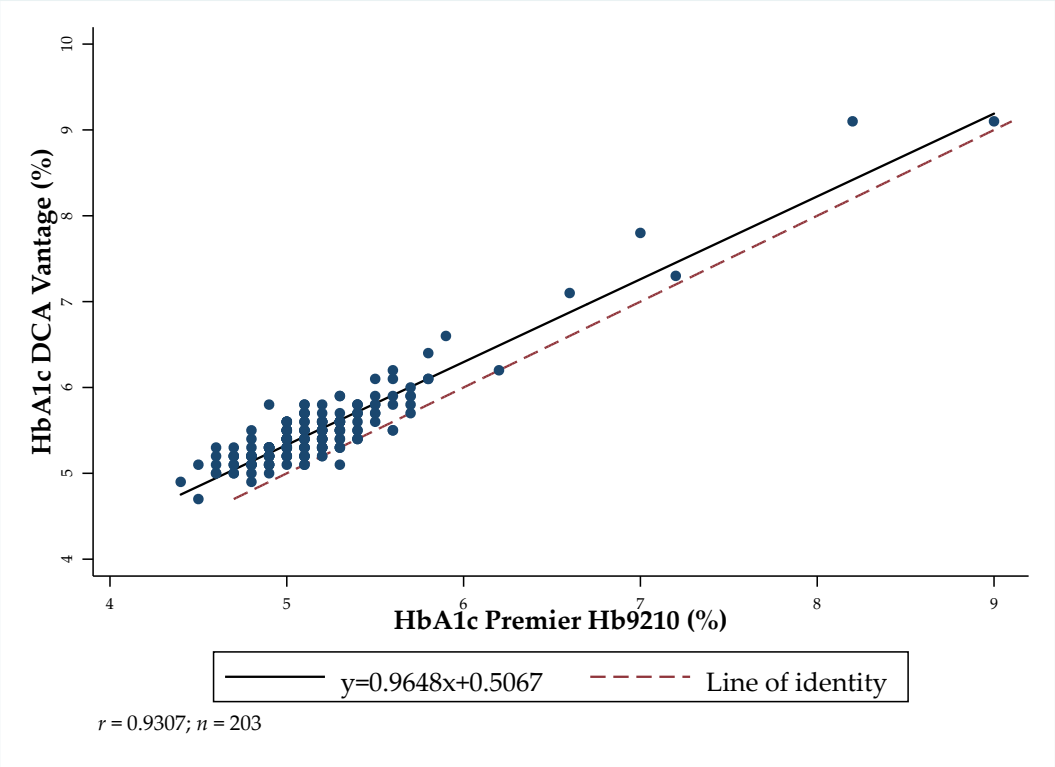

Figure 3: Correlation between the DCA Vantage analyzer to the Premier Hb9210 for measuring HbA1c (DCCT units).

Blue dots represent individual samples, the dotted red line represents the line of identity x = y, and the solid black line is the regression line. Abbreviations include: DCCT, Diabetes Control and Complications Trial.

The bias (mean difference) between the Afinion and the HPLC analyzer was +0.56% (+6 mmol/mol) (95% CI 0.53% to 0.59% [6 mmol/mol]), p-value < 0.001 by paired t test. The bias between the DCA Vantage and the HPLC analyzer was +0.32% (+4 mmol/mol) (95% CI 0.30% to 0.35% [3-4 mmol/mol]), p-value < 0.001. The bias observed was constant across the range of HbA1c concentrations for the DCA Vantage ($p = 0.190$). The bias was not constant across the range of HbA1c concentrations for the Afinion ($p < 0.001$), and it increased as HbA1c levels decreased.

The mean relative difference in HbA1c values (in DCCT units) comparing the POC analyzers relative to the HPLC method was 11.1% for the Afinion and 6.3% for the DCA Vantage, both of which fail NGSP criteria of 6.0%. There was an HbA1c difference greater than 6.0% observed in 157 of the 187 samples tested by the Afinion (84%) and 86 of the 203 samples tested by the DCA Vantage (42%).

The Bland-Altman limits of agreement between the Afinion and the HPLC analyzer were 0.16% to 0.97% (2 to 11 mmol/mol) (Fig 4). Therefore, for a new individual from the studied population, it would be expected with an approximate 95% probability that the difference between the Afinion and the HPLC analyzer should lie within 0.16% to 0.97% (2 to 11 mmol/mol). The limits of agreement between the DCA Vantage and the HPLC analyzer were -0.05% to 0.70% (-1 to 8 mmol/mol) (Fig 5). Thus, for a new individual from the studied population it would be expected that the difference between

the DCA Vantage and the HPLC analyzer should lie within -0.05% to 0.70% (-1 to 8 mmol/mol).

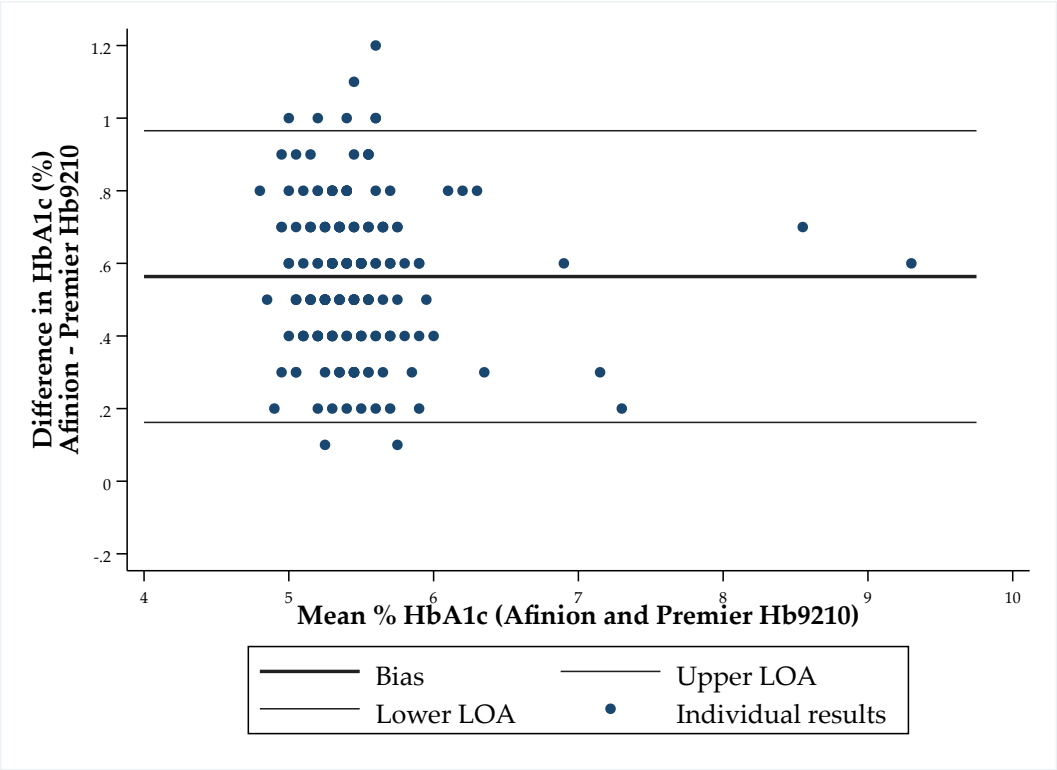

Figure 4: Bland-Altman plot of the differences in HbA1c measurement (using DCCT units) between the Afinion and Premier Hb9210 by mean HbA1c level (*n* = 187).

Blue dots represent individual samples. The horizontal black lines represent the bias (mean difference between the Afinion and the Premier Hb9210) and its limits of agreement. Abbreviations include: LOA, limits of agreement; DCCT, Diabetes Control and Complications Trial.

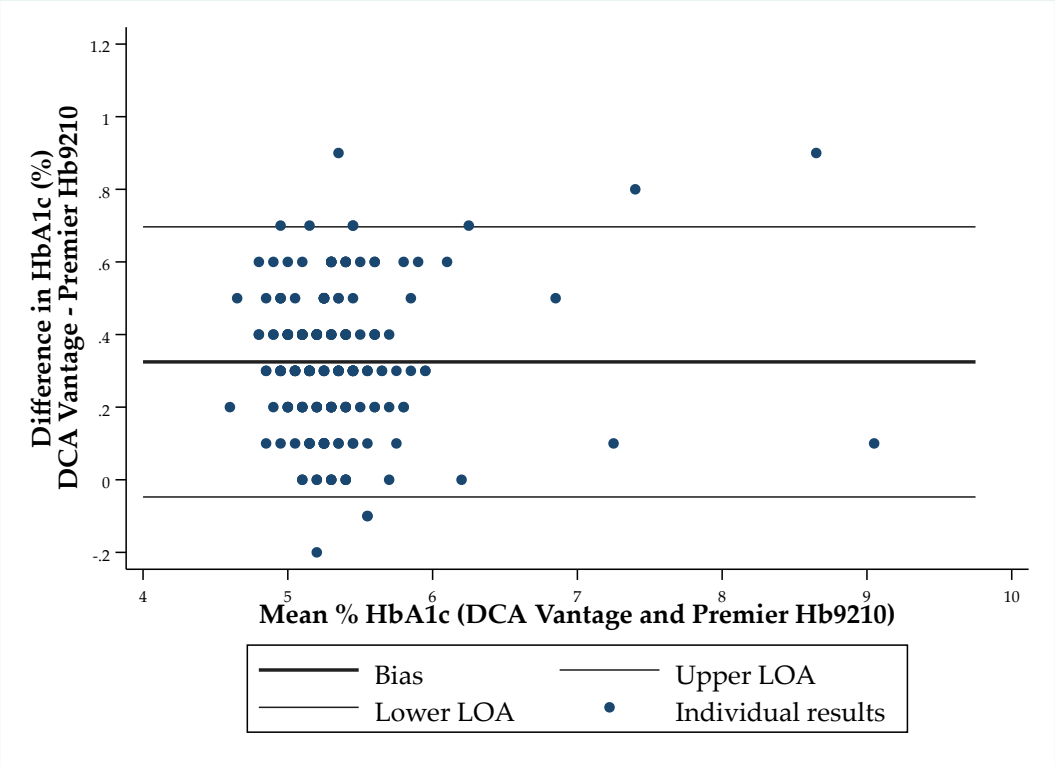

Figure 5: Bland-Altman plot of the differences in HbA1c measurement (using DCCT units) between the DCA Vantage and Premier Hb9210 by mean HbA1c level (*n* = 203).

Blue dots represent individual samples. The horizontal black lines represent the bias (mean difference between the Afinion and the Premier Hb9210) and its limits of agreement. Abbreviations include: LOA, limits of agreement; DCCT, Diabetes Control and Complications Trial.

3.3 Sensitivity, specificity, PPV, NPV

HbA1c results were categorized based on current practice guidelines from the ADA[1] (Tables 2 & 3). Per the laboratory HbA1c results from the HPLC analyzer, the prevalence of diabetes was 2.5% (5 of 203) and 4.9% for prediabetes (10 of 203).

Table 2: Categorization of HbA1c results within specific ranges for the Afinion point-of-care analyzer compared to HPLC test results

	HPLC Results			
Afinion Test	**Diabetes[a]**	**Prediabetes[a]**	**Normal[a]**	**Total**
Diabetes	5	4	0	**9**
Prediabetes	0	6	89	**95**
Normal	0	0	83	**83**
Total	**5**	**10**	**172**	**187**

[a] Ranges based off of current practice guidelines from the American Diabetes Association.[1] HbA1c for diabetes ≥ 6.5%, prediabetes = 5.7-6.4%, normal ≤ 5.6%. Abbreviations include: HPLC, high performance liquid chromatography.

Table 3: Categorization of HbA1c results within specific ranges for the DCA Vantage point-of-care analyzer compared to HPLC test results

	HPLC Results			
DCA Test (%)	**Diabetes[a]**	**Prediabetes[a]**	**Normal[a]**	**Total**
Diabetes	5	1	0	**6**
Prediabetes	0	9	28	**37**
Normal	0	0	160	**160**
Total	**5**	**10**	**188**	**203**

[a] Ranges based off of current practice guidelines from the American Diabetes Association.[1] HbA1c for diabetes ≥ 6.5%, prediabetes = 5.7-6.4%, normal ≤ 5.6%. Abbreviations include: HPLC, high performance liquid chromatography.

For detecting individuals with prediabetes or diabetes (HbA1c ≥ 5.7%, 39 mmol/mol), the Afinion and the DCA Vantage both had a sensitivity of 100% compared to the gold standard HPLC. Therefore, all true positive cases were correctly categorized by both POC devices. The specificity was 48.3% for the Afinion and 85.1% for the DCA Vantage. Thus, 48.3% of true negative cases were correctly classified by the Afinion and 85.1% of true negative cases were correctly classified by the DCA Vantage. The PPV was 14.4% for the Afinion and 34.9% for the DCA Vantage. Therefore, only 14.4% of subjects with a positive test from the Afinion actually had prediabetes or diabetes, and only 34.9% of individuals with a positive test from the DCA Vantage were actually positive. The NPV for both the Afinion and the DCA Vantage was 100%, so all subjects identified as negative by the POC devices were correctly classified. The area under the ROC curve was 0.966 for the Afinion (Fig 6), and 0.982 for the DCA Vantage (Fig 7), both of which were excellent.

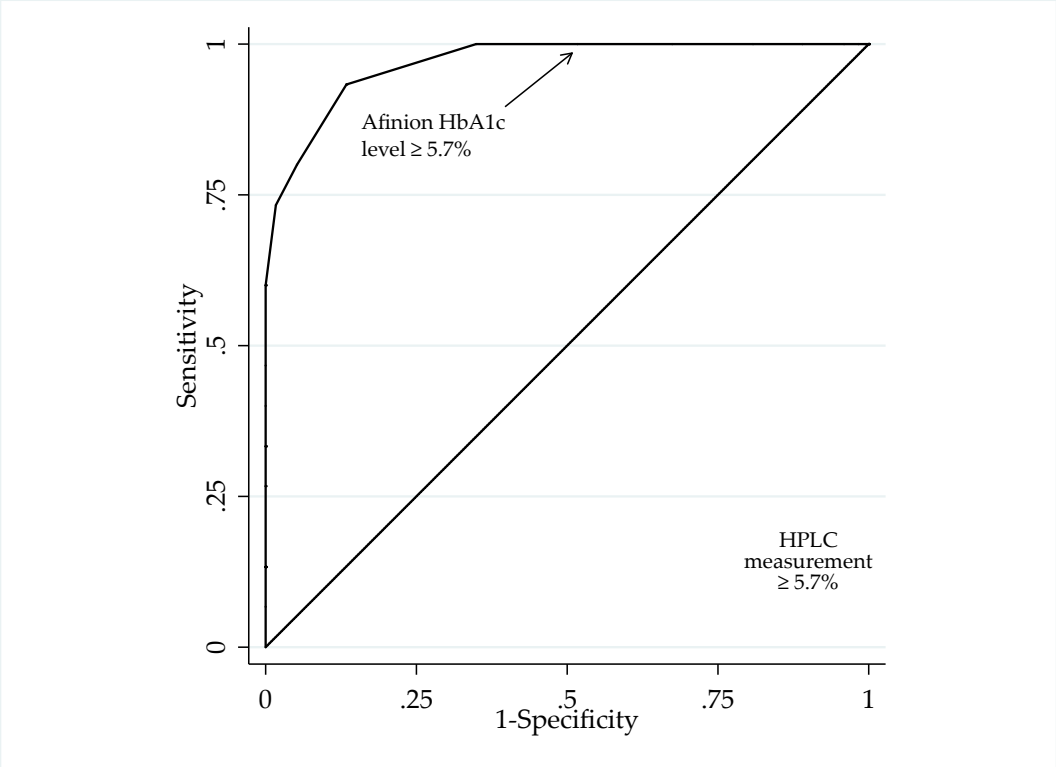

Figure 6: ROC curve for diagnosing and screening for prediabetes or diabetes using the Afinion POC analyzer compared to HPLC HbA1c ≥ 5.7% (39 mmol/mol) as the gold standard.

Abbreviations include: ROC, receiver operating characteristic; POC, point-of-care; HPLC, high-performance liquid chromatography.

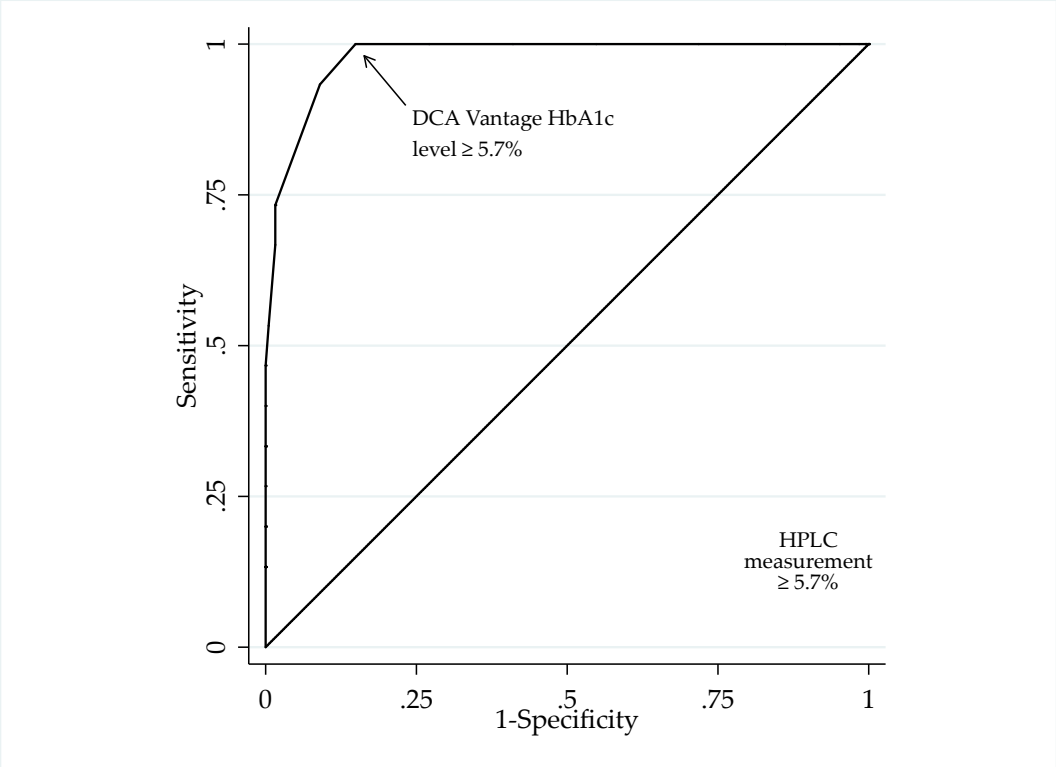

Figure 7: ROC curve for diagnosing and screening for prediabetes or diabetes using the DCA Vantage POC analyzer compared to HPLC HbA1c ≥ 5.7% (39 mmol/mol) as the gold standard.

Abbreviations include: ROC, receiver operating characteristic; POC, point-of-care; HPLC, high-performance liquid chromatography.

McNemar's test was performed to test whether the dichotomous classification of patients as either not having (HbA1c < 5.7%, 39 mmol/mol) or having prediabetes/diabetes (HbA1c ≥ 5.7%, 39 mmol/mol) differs by use of POC or HPLC methods. There was a statistically significant difference in categorization for both the Afinion (McNemar's statistic = 89; $p < 0.001$) and the DCA Vantage (McNemar's statistic = 28; $p < 0.001$), indicating poor agreement.

Cohen's kappa statistic for the agreement between the Afinion and the HPLC method for the trichotomous categorization of patients as diabetic, prediabetic, or normal using ADA cutoffs[1] was 0.12 (95% CI 0.06-0.19), indicating slight agreement. The kappa statistic for the DCA Vantage was 0.45 (95% CI 0.29-0.60), indicating moderate agreement.

3.4 Effect of ambient conditions

Results from the multiple regression analyses indicate that temperature (°F) and humidity (%) were not significantly related to the HbA1c results from either POC device. Barometric pressure (inHg) was not significantly related to the DCA Vantage results, but was associated with the Afinion HbA1c. Each unit increase in barometric pressure was associated with a 0.342% (DCCT units) increase in HbA1c (95% CI 0.050-0.634%, $p = 0.022$). The final model with Afinion HbA1c and barometric pressure

statistically significantly predicted HPLC HbA1c, $F(2,184) = 511$, $p < 0.001$, $r^2 = 0.85$, with the regression equation:

HPLC = (Afinion * 0.938) + (barometric pressure * 0.342) – 9.954

4. Discussion

In this study, we compared the accuracy and imprecision of the Afinion and DCA Vantage POC analyzers with the Premier Hb9210 HPLC analyzer to measure HbA1c in six Amazonian communities. Although the correlation between the POC analyzers and the laboratory-based HPLC method was excellent ($r > .9$), we observed a significant positive bias ($p < 0.001$) in HbA1c for both POC analyzers. The bias did not vary meaningfully across HbA1c levels for the DCA Vantage, but it was found to be higher at lower HbA1c levels for the Afinion, which may lead to unnecessary medicalization and create unsustainable burdens for the healthcare system. The mean relative difference for both the Afinion and the DCA Vantage exceeded the NGSP criterion of 6.0%, although the DCA Vantage was close to meeting this target. In this study, a target difference of 6.0% may be too strict, particularly since the mean HbA1c for this population was very low.

Peterson et al.[36] have also reported significant biases for the Afinion and the DCA Vantage, and Malkani et al.[37] also found a significant bias for the DCA Vantage. However, after programming the slope and intercept from their regression analyses into the DCA Vantage analyzer, they were able to reduce the average difference to less than 0.2% HbA1c, and 0.0000% HbA1c, respectively. If POC testing is implemented, this could be a useful feature for health posts to incorporate to harmonize the results between the DCA Vantage and laboratory-based instruments.

The generally accepted performance criteria for imprecision is an intra-laboratory CV <2% in DCCT units (<3% in SI units).[4] We found that the CV for the Afinion (1.75% in DCCT units) met these standards, but the DCA Vantage (4.01%) did not. In practice, a low CV is important for clinicians to determine whether changes in HbA1c results over time reflect clinically significant changes in a patient's glycemic status. A 0.5% (5 mmol/mol) difference in HbA1c is commonly used as an indication to adjust therapeutic options, although ultimately a statistically significant change in the health status of the patient should depend on the device's CV and the within-person biological variation for HbA1c.[4]

A review of the literature reveals that the CV we found for the Afinion is consistent with values reported in other studies, which range from 0.5%[36] to 3.1%.[3] The CV that we found for the DCA Vantage is slightly higher than values previously published, which range from 1.55%[38] to 3.74%.[39] The direction and magnitude of the bias for these POC analyzers varies. The bias reported in the literature ranges from -1.1%[40] to 0.8%[36] (DCCT units) for Afinion and -0.53%[37] to 0.9%[36] for the DCA Vantage. Some studies have found that the Afinion produces lower HbA1c measurements than HPLC methods,[3,4,40-43] while others report higher HbA1c measurements.[4,36,40,43-46] Eight studies have demonstrated lower results from the DCA vantage compared to HPLC methods,[3,4,37,38,43,45,47,48] and five have reported higher values.[4,36,43,46,47] The only published

study to test the Afinion or DCA Vantage in an LMIC or tropical environment was performed by Wan Mohd Zin et al. in Malaysia.[44]

Both the Afinion and the DCA Vantage showed excellent sensitivity and NPV and poor specificity and PPV due to each device overestimating the HbA1c for nearly every participant. However, the unexpectedly low number of patients identified with prediabetes (10) or diabetes (5) in this study may limit the impact of these results.

Communities in the Peruvian Amazon are rapidly urbanizing, yet access to laboratory diagnostics for clinical care remains lacking. Point-of-care testing represents an opportunity for health posts in this region to obtain clinical information that would otherwise be unavailable for patients due to financial and transportation constraints. Information to help diagnose and manage treatment for patients with diabetes will be particularly important in South and Central America, where it is projected that the number of people with the disease will increase 65% by 2040.[6]

While we were able to demonstrate the accuracy and imprecision of two POC analyzers compared to an NGSP-certified HPLC method, we acknowledge there were a few limitations with our study. First, interference from hemoglobin variants was not evaluated. The Afinion and DCA Vantage are unaffected by most common variants (HbS, HbC, HbE, HbD heterozygotes) but HbF levels greater than 10-15% can interfere with results.[21] Also, because this study used samples from a community screening, most of the samples had low HbA1c levels and there were a limited number of samples

around the 6.5% HbA1c diagnostic cutoff. Finally, since all reagent cartridges were from a single lot for each analyzer, we were unable to test potential lot-to-lot variability, which can be an additional source of error in clinical use.

Although we observed a significant positive bias among the POC analyzers evaluated, we conclude that the Afinion and the DCA Vantage analyzers can be used for therapeutic adjustments if healthcare workers are aware of the differences relative to laboratory-based methods. The Afinion demonstrated superior precision and the DCA Vantage demonstrated superior accuracy in our study, and both analyzers should be considered in health clinics in the Peruvian Amazon with limited access to laboratory facilities. However, the imprecision and bias were not low enough to recommend the use of either POC device for screening purposes. There were not enough patients around the 6.5% HbA1c diagnostic threshold to provide a definitive recommendation for or against the use of these POC devices for diagnostic purposes, but the high levels of bias that were consistently obtained for patients mainly at lower HbA1c levels indicate that the POC devices may not be appropriate for diagnostic purposes in this population. Overall, we found that nearly 1 in 5 participants in this study were anemic and 1 in 7 self-reported a previous case of malaria, which means that a large portion of the population in this region would produce HbA1c results that were unreliable for diagnostic determinations using any kind of assay. Additional limitations such as the possibility of other interferences (hemoglobinopathies like sickle cell disease,

thalassemia, HbF) and potential reagent lot-to-lot variability add to the uncertainty when using a POC analyzer. If HbA1c testing is introduced in the Peruvian Amazon, we recommend the integration of routine proficiency testing amongst clinics using the analyzers to ensure consistency and enhance reliability of the results.

Appendix A

Two samples were found to have high residuals (>±3) and high leverage for both the Afinion and the DCA Vantage POC analyzers. Although both samples were candidates to be removed from analysis as outliers, they were ultimately not removed from the statistical analysis because of their large effect on the regression equation, substantially lower Pearson's correlation coefficients, and because those two represented nearly half of all diabetic patients.

References

1. American Diabetes Association. *Classification and diagnosis of diabetes. Sec. 2. In* Standards of Medical Care in Diabetes, 2015. *Diabetes care* 2015; **38 Suppl. 1**: S8-S16.

2. Beagley J, Guariguata L, Weil C, Motala AA. Global estimates of undiagnosed diabetes in adults. *Diabetes research and clinical practice* 2014; **103**(2): 150-60.

3. Whitley HP, Yong EV, Rasinen C. Selecting an A1C Point-of-Care Instrument. *Diabetes spectrum : a publication of the American Diabetes Association* 2015; **28**(3): 201-8.

4. Lenters-Westra E, Slingerland RJ. Three of 7 hemoglobin A1c point-of-care instruments do not meet generally accepted analytical performance criteria. *Clinical chemistry* 2014; **60**(8): 1062-72.

5. World Health Organization. Global Health Estimates: Deaths by Cause, Age, Sex and Country, 2000-2012. Geneva, WHO; 2014.

6. International Diabetes Federation. IDF Diabetes Atlas, Seventh Edition 2015. 2015. http://www.diabetesatlas.org (accessed Dec. 25, 2015.

7. International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes care* 2009; **32**(7): 1327-34.

8. World Health Organization. WHO Guidelines Approved by the Guidelines Review Committee. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation. Geneva: World Health Organization
Copyright (c) World Health Organization 2011.; 2011.

9. Tabak AG, Herder C, Rathmann W, Brunner EJ, Kivimaki M. Prediabetes: a high-risk state for diabetes development. *Lancet (London, England)* 2012; **379**(9833): 2279-90.

10. Duncan MacDougall DS. The Physiology of Training for High Performance. Oxford, United Kingdom: Oxford University Press; 2014.

11. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM. Tests of glycemia in diabetes. *Diabetes care* 1995; **18**(6): 896-909.

12. Lenters-Westra E, Schindhelm RK, Bilo HJ, Slingerland RJ. Haemoglobin A1c: Historical overview and current concepts. *Diabetes research and clinical practice* 2013; **99**(2): 75-84.

13. Bonora E, Tuomilehto J. The pros and cons of diagnosing diabetes with A1C. *Diabetes care* 2011; **34 Suppl 2**: S184-90.

14. Pani LN, Korenda L, Meigs JB, et al. Effect of aging on A1C levels in individuals without diabetes: evidence from the Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001-2004. *Diabetes care* 2008; **31**(10): 1991-6.

15. Herman WH. Do race and ethnicity impact hemoglobin A1c independent of glycemia? *Journal of diabetes science and technology* 2009; **3**(4): 656-60.

16. Freedman BI, Shihabi ZK, Andries L, et al. Relationship between assays of glycemia in diabetic subjects with advanced chronic kidney disease. *American journal of nephrology* 2010; **31**(5): 375-9.

17. Cagliero E, Levina EV, Nathan DM. Immediate feedback of HbA1c levels improves glycemic control in type 1 and insulin-treated type 2 diabetic patients. *Diabetes care* 1999; **22**(11): 1785-9.

18. Ferenczi A, Reddy K, Lorber DL. Effect of immediate hemoglobin A1c results on treatment decisions in office practice. *Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists* 2001; **7**(2): 85-8.

19. Miller CD, Barnes CS, Phillips LS, et al. Rapid A1c availability improves clinical decision-making in an urban primary care clinic. *Diabetes care* 2003; **26**(4): 1158-63.

20. Sacks DB, Arnold M, Bakris GL, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Clinical chemistry* 2011; **57**(6): e1-e47.

21. Little RR. Analysis of the accuracy and precision of the Axis-Shield Afinion hemoglobin A1c measurement device. *Journal of diabetes science and technology* 2012; **6**(2): 387-8.

22. National Glycohemoglobin Standardization Program. Summary of NGSP Criteria 2015. http://www.ngsp.org/critsumm.asp (accessed Dec. 25, 2015.

23. College of American Pathologists. College of American Pathologists (CAP) GH5 Survey Data: (updated 8/15) http://www.ngsp.org/CAP/CAP15b.pdf (accessed Dec. 25, 2015.

24. Alere Technologies AS. Afinion package insert. 2015. http://www.cliawaived.com/web/items/pdf/ABBT-06L13-10insert.pdf (accessed Dec. 25, 2015.

25. Alere Technologies AS. Alere Afinion AS 100 Analyzer User Manual. 2014.

26. Siemens Medical Solutions USA I. DCA Vantage Analyzer Technical Specifications. 2015. http://usa.healthcare.siemens.com/point-of-care/diabetes/dca-vantage-analyzer/technical-specifications (accessed Dec. 25, 2015.

27. NGSP. List of NGSP Certified Methods, updated 12/15. 2015. http://www.ngsp.org/docs/methods.pdf (accessed Dec. 25, 2015.

28. Cossio R, Menton M, Cronkleton P, Larson A. Community forest management in the Peruvian Amazon: A literature review. Working Paper 136. Bogor, Indonesia: Center for International Forestry Research; 2014.

29. Ministerio de Salud de Peru (MINSA). Informacion Estadistica. 2015. http://www.minsa.gob.pe/index.asp?op=6 (accessed Dec. 26, 2015.

30. Huicho L, Trelles M, Gonzales F, Mendoza W, Miranda J. Mortality profiles in a country facing epidemiological transition: an analysis of registered data. *BMC public health* 2009; **9**: 47.

31. Gyorkos TW, Joseph SA, Casapia M. Progress towards the Millennium Development Goals in a community of extreme poverty: local vs. national disparities in Peru. *Tropical medicine & international health : TM & IH* 2009; **14**(6): 645-52.

32. Hoelzel W, Weykamp C, Jeppsson JO, et al. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. *Clinical chemistry* 2004; **50**(1): 166-74.

33. Chesher D. Evaluating assay precision. *The Clinical biochemist Reviews / Australian Association of Clinical Biochemists* 2008; **29 Suppl 1**: S23-6.

34. Atkinson G, Nevill AM. Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports medicine (Auckland, NZ)* 1998; **26**(4): 217-38.

35. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 2011. http://www.who.int/vmnis/indicators/haemoglobin.pdf (accessed Jan. 12, 2016.

36. Petersen JR, Omoruyi FO, Mohammad AA, Shea TJ, Okorodudu AO, Ju H. Hemoglobin A1c: assessment of three POC analyzers relative to a central laboratory method. *Clinica chimica acta; international journal of clinical chemistry* 2010; **411**(23-24): 2062-6.

37. Malkani S, Korpi-Steiner N, Rao LV. Reducing analytical variation between point-of-care and laboratory HbA1c testing. *Journal of diabetes* 2013; **5**(2): 192-6.

38. Szymezak J, Leroy N, Lavalard E, Gillery P. Evaluation of the DCA Vantage analyzer for HbA 1c assay. *Clinical chemistry and laboratory medicine* 2008; **46**(8): 1195-8.

39. Torregrosa ME, Molina J, Argente CR, Ena J. Accuracy of three hemoglobin A1c point-of-care systems for glucose monitoring in patients with diabetes mellitus. *Endocrinologia y nutricion : organo de la Sociedad Espanola de Endocrinologia y Nutricion* 2015; **62**(10): 478-84.

40. Font MT, Brichs MC, Alvarez MC, Olivella JM, Turo JS, Fernandez MP. [Capillary HbA1c determination on type 2 diabetes patients in a primary health centre]. *Atencion primaria / Sociedad Espanola de Medicina de Familia y Comunitaria* 2011; **43**(10): 536-43.

41. Lenters-Westra E, Slingerland RJ. Six of eight hemoglobin A1c point-of-care instruments do not meet the general accepted analytical performance criteria. *Clinical chemistry* 2010; **56**(1): 44-52.

42. Lee JY, Hong KS, Cho SE. [Comparison of HbA1c analyzers: D-10, Variant II Turbo, Cobas Integra 800, and Afinion AS100]. *The Korean journal of laboratory medicine* 2010; **30**(4): 345-50.

43. Solvik UO, Roraas T, Christensen NG, Sandberg S. Diagnosing diabetes mellitus: performance of hemoglobin A1c point-of-care instruments in general practice offices. *Clinical chemistry* 2013; **59**(12): 1790-801.

44. Wan Mohd Zin RM, Ahmad Kamil ZI, Tuan Soh TR, Embong M, Wan Mohamud WN. Haemoglobin A1c: comparing performance of two point of care devices with laboratory analyser. *BMC research notes* 2013; **6**: 540.

45. Wood JR, Kaminski BM, Kollman C, et al. Accuracy and precision of the Axis-Shield Afinion hemoglobin A1c measurement device. *Journal of diabetes science and technology* 2012; **6**(2): 380-6.

46. Sanchez-Mora C, M SR-O, Fernandez-Riejos P, et al. Evaluation of two HbA1c point-of-care analyzers. *Clinical chemistry and laboratory medicine* 2011; **49**(4): 653-7.

47. Lenters-Westra E, Slingerland RJ. Evaluation of the Quo-Test hemoglobin A1c point-of-care instrument: second chance. *Clinical chemistry* 2010; **56**(7): 1191-3.

48. El Arabi H, Willems D, Melot C, Dorchy H. [Evaluation of DCA vantage for rapid in-clinic measurement of HbA1c on capillary blood in young type 1 diabetic patients]. *Revue medicale de Bruxelles* 2013; **34**(2): 87-9.