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Method Validation of Siemens DCA Vantage Point-of-care (POC) System in Measuring Hemoglobin A_{1c} at Idaho State University Bengal Lab

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Committee Approval

To the Graduate Faculty:

The members of the committee appointed to examine the thesis of Yao Zhang find it satisfactory and recommend that it be accepted.

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I want to dedicate this paper to my parents for their continuous support and encouragement at every stage of my life

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Abstract

Siemens DCA Vantage point-of-care testing (POCT) instruments have fast result turn-around time compared to traditional clinical laboratory testing instruments in measuring hemoglobin A_{1c} (Hb A_{1c}). Utilization of such POCT analyzers facilitates patient-physician interactions which enhances therapy compliance in diabetes monitoring. For laboratories performing only waived testing, there are no CLIA guidelines for putting an instrument in service. In non-CLIA waived laboratories placing the instrument in service requires validation of accuracy and precision and verification of normal range. A study of the Siemens DCA Vantage system was carried out to ensure analytical performance in compliance with CLIA and CAP requirements. Precision of DCA Vantage system was verified with five randomly collected whole blood samples according to manufacturer's instructions and percent coefficient of variation (CV) was reported as 1.36 % for normal values and 2.87 % for abnormal values. A reference range of Hb A_{1c} was verified based on population being tested at Idaho State University Bengal Lab. A Comparison study between DCA Vantage analyzer and Biorad D-10 HPLC reference method instrument showed 1.2 % CV which was within the accepted 3 % correlation coefficient limit. This comparison study verified the accuracy of Hb A_{1c} results measured by DCA Vantage. Limitations of Hb A_{1C} methodology were discussed.

I. Introduction:

1.1 Statement of Purpose

Hemoglobin A_{1c} (Hb A_{1c}) has become an important monitoring test for glycemic control in both type 1 and type 2 diabetic patients (1, 2). The associations of Hb A_{1c} with long-term complications of type 1 diabetes have been well documented in varies papers (2). Hb A_{1c} has been used as an index of mean glycemia and risk assessment tool for development of diabetes (2-4). The rapid result turnaround time of point-of-care (POC) testing instruments has an advantage over traditional clinical laboratory diagnostic tests in measuring Hb A_{1c}. The immediate feedback of Hb A_{1c} results to healthcare providers facilitates decision-making process by developing more intensive treatment plans and monitoring programs to help improve glycemic control (5-7). Not only has it been used as a monitoring test in controlling blood glucose in diabetic patients by many physicians and other healthcare providers, Hb A_{1c} has been included as a diagnostic criterion for diabetes, as well as a recommended test in prediabetes in asymptomatic adults, one recent modification made by the American Diabetes Association (ADA) on its standard of medical care in diabetes 2010 publication (8).

However, little considerations had been given to the analytic performances of either traditional clinical laboratory testing methods or POC testing methods before the standardization of hemoglobin results to a common reference value. Historically, many testing methods have been developed to measure glycolated or glycated hemoglobin or glycohemoglobin, to which Hb A_{1c} belongs. Without standardization of testing methods, different laboratories could report different Hb A_{1c} values based on the testing principle utilized by each individual laboratory (9). Because of the importance of Hb A_{1c} values in

glycemic control monitoring and potential use in diagnosing diabetes, the National Glycohemoglobin Standardization Program (NGSP) initiated the standardization process of Hb A_{1c} to a common reference value in 1996. It was decided to standardize Hb A_{1c} to the values presented in the Diabetes Control and Complications Trial (DCCT) study which linked Hb A_{1c} and long-term complications experienced by type 1 diabetic patients (2). The NGSP certification program and other quality assurance program, such as College of American Pathologists (CAP) survey, ensured the accuracy and analytic performance of traditional clinical instruments in large facilities and hospitals and also POCT instruments in small clinics and doctor's offices.

Some recent in-depth reviews of Hb A_{1c} POCT instruments focus on verification of analytical performance based on Clinical and Laboratory Standards Institute (CLSI) standards, and have pointed out that only a few of POCT instruments meet the requirements in clinical settings (10). Many POCT instruments have imprecisions greater than 3 % coefficient of variance (CV) which is the common accepted % CV in practice (11). This study focused on the verification of analytic performance of Siemens DCA Vantage Hb A_{1c} testing system on parameters such as accuracy and precision. The reference range of Hb A_{1c} measured by DCA Vantage for this project was established with randomly selected samples from healthy individuals representing populations being tested in Bengal Lab at Idaho State University. It was also demonstrated that results delivered by DCA Vantage was comparable to Riorad D-10 which is the reference method used by Portneuf Medical Center (PMC, Pocatello, Idaho), the reference laboratory to Bengal Lab. The goal of this study was to validate utilization of Siemens DCA Vantage Hb A_{1c} testing system to monitor glycemic control in students, staff, as

well as general public around Idaho State University. This procedure is required for CLIA certification of a laboratory and is monitored by Centers for Medicare and Medicaid Services (CMS).

1.2 Working Objectives

1. To verify the accuracy and precision of Hb A_{1c} assay performed on Siemens DCA Vantage system at Bengal Lab in preparation for placing the instrument in service in the Bengal Lab.

2. To complete the correlation studies of Siemens DCA Vantage Hb A_{1c} analyzer by comparing test results measured at Idaho State University Bengal Lab and Portneuf Medical Center.

3. To verify the appropriate use of Hb A_{1c} reference range established by manufacture with the population being tested at Idaho State University Bengal Lab.

4. To establish a Point-Of-Care Testing quality assurance program to monitor the accuracy and precision of Hb A_{1c} testing at Idaho State University Bengal Lab.

1.3 Significance of Hb A_{1c} test in monitoring and diagnosing/screening for diabetes mellitus

According to the National Diabetes Statistics Report by Centers for Disease Control and Prevention (CDC) in 2014, there are 29.1 million people with diabetes. Of those, 8.1 million people go undiagnosed, which is almost 30 percent of all reported numbers. Worldwide, approximately 250 million people are projected to have diabetes by 2025. (12, 13). Over the last twenty years, the number of people with diagnosed diabetes has increased from 7.8 million in 1994, to 22 million in 2013. Diabetes is associated not only with heart diseases and stroke, it can also cause multi-organ complications such as

end-stage renal disease, diabetic neuropathy, and diabetic retinopathy (3, 14-17). These serious complications lower individuals' standards of living and are associated with reduced life-expectancy. American Diabetes Association (ADA) reported total of 245 billion dollars direct cost associated with diagnosed diabetes in 2012. The medical expenditure in people with diagnosed diabetes was two times higher than what the medical expenditure would be without diabetes (18).

Of the population who are diagnosed with diabetes, 90 percent have type 2 diabetes. About 86 million people have prediabetes, or as categorized as at-risk of progression to type 2 diabetes (13, 19). Women who have gestational diabetes (GDM), which develops during pregnancy, have increased risk of developing type 2 diabetes later in life (20). People with diagnosed type 2 diabetes, and who are at high risk of developing type 2 diabetes, can participate in diabetes prevention programs to delay the progress of disease or to prevent the development of disease. Patients at high risk who participated in intensive therapy, such as sulfonylurea, insulin or metformin, and/or lifestyle intervention reduced the risk of developing type 2 diabetes (3, 21). Diabetes associated complications can be monitored by patients at their convenient environments or by healthcare providers at doctors' visits by utilizing POCT instruments.

2010 ADA Diabetes Guidelines included Hb A_{1c} levels as one of the criteria for diagnosis of diabetes. Before the publication of this recommendation, diagnosis of diabetes has been based on three blood glucose tests which include fasting blood glucose greater than or equal to 126 mg/dL or two-hour oral glucose tolerance test (OGTT) greater than or equal to 200 mg/dL or random blood glucose test greater than or equal to 200 mg/dL in symptomatic patients (22). However, limitations such as fasting for at least

eight hours before sample collection, patient preparation for OGTT, and low sensitivity for random blood glucose tests have made Hb A_{1c} testing more appealing to be considered as a diagnosing test for diabetes. Hb A_{1c} testing can be done at patients' convenience without the requirement of 8-hour fasting and it measures the blood glucose over the time of two or three months instead of single measurement of glucose exposure (23, 24). Not only is Hb A_{1c} used as a diagnostic tool, but it was also used as a test to measure the outcome of glycemic control and to monitor treatment compliance in diagnosed patients (25). Including Hb A_{1c} consultation as part of patient-physician interactions reduced Hb A_{1c} levels in at-risk patients (25).

1.4 Siemens DCA Vantage System

Hb A_{1c} Point-of-Care testing (POCT) instruments have rapid results turn-around time and great accessibility. POC testing has allowed healthcare providers the opportunity to consult and educate patients on their most current blood glucose level. Patients participated in personalized monitoring program inclusive of individual's current Hb A_{1c} status and goals demonstrated Hb A_{1c} reduction among people who have greater than or equal to 7.0 % Hb A_{1c} (25). Most recent ADA recommendations included Hb A_{1c} as a diagnosing criterion for diabetes which used 6.5 % glycohemoglobin as a fixed cutoff point for diagnosis (22). In order to be used in treatment monitoring and disease diagnosis, reproducibility of Hb A_{1c} needs to meet current regulatory requirements. Thus it is critical to ensure analytical performances which measured by accuracy, precision, percent coefficient of variation, and percent bias from reference method of POCT instruments. Lenters-Westra and Slingerland reported only two out of eight Hb A_{1c} POCT instruments met the analytical performances criteria based on CLSI EP10, EP5, and EP9

protocols (10). Bruns and Boyd also drew the attention to analytical performances of Hb A_{1c} POCT instruments because of the practical use of Hb A_{1c} diabetes monitoring, diagnosis, and evaluation of estimated average blood glucose (26).

Siemens DCA Vantage system is one of the two Hb A_{1c} POCT instruments that meet the analytical performance criteria (10). According to the manufacturer package insert, the precision of Siemens DCA Vantage was evaluated and the within-run percent CV were 2.2 % to 3.7 % imprecision where between-run percent CV were 0.9 % to 4.3 % imprecision. The accuracy of Siemens DCA Vantage was examined by comparing Hb A_{1c} results with results measured by high pressure liquid chromatography (HPLC) reference method at the Glycohemoglobin Reference Laboratory at the University of Missouri Medical Center, which gave percent CV 2.9 % to 5.4 % imprecision. The Hb A_{1c} testing method of Siemens DCA Vantage system is certified by National Glycohemoglobin Standardization Program (NGSP) and the results are traceable to International Federation of Clinical Chemistry (IFCC) reference materials and methods (27).

1.5 Significance of method validation and quality assurance program

Before placing a new test system into use, method validation is to be completed to ensure the instrument has been installed properly and the analytical performance is in accordance with manufacturer's package inserts, and in compliance with all regulatory requirements by accrediting agencies such as Joint Commission for Accreditation of Healthcare Organizations (JCAHO) and CAP. Method validation and/verification process differs by the complexity of test principles and procedures. According to Clinical Laboratory Improvement Amendments of 1988 (CLIA'88), laboratory diagnostic tests

can be classified as moderate to high complexity tests and waived tests. Method validation for moderate/high complexity tests must include accuracy, precision, and reportable range studies, as well as establishment or verification of reference ranges provided by manufacturers (28). For laboratories perform only waived tests, manufacturer's instructions must be followed to report patient results. JCAHO has more strict requirements than CLIA for waived test which also include verification of reportable range, and reference range at the individual laboratory (29). CAP states that all tests including waived test need to follow requirements for moderate/high complexity for method validation studies (30).

Instrument performance also needs to be monitored by quality assurance program which is an on-going process to ensure results delivered is reliable. Testing personnel needs to be deemed competent by laboratory supervisors or evaluators by performing test samples following individual laboratory's standard operating procedures (SOPs) at initial hire, six months and twelve months after hire, and annually after the first year of hire. Quality control frequency and calibration are set according to manufacturer's requirements and laboratory SOPs. Laboratories also participate in proficiency programs such as the CAP survey and other certified programs to compare instrument performance among laboratories and to resolve bias of test systems. Participating in proficiency programs is also a good way to trouble shoot instrument malfunctions and perform maintenance by technical service.

II. Literature review

2.1 Diagnosing tests of diabetes

Before the publication of ADA recommendation to include Hb A_{1c} as one of the diagnosing criteria of diabetes, diabetes has been diagnosed by blood glucose tests, fasting plasma glucose (FPG) or 2-hour plasma glucose (2HPG) during oral glucose tolerance test (OGTT). Currently, two-hour PG of greater than or equal to 200 mg/dL, and FPG of greater than or equal to 126 mg/dL, or Hb A_{1c} of greater than or equal to 6.5 % are diagnostic for diabetes. In symptomatic patients, diabetes can be easily diagnosed by random PG of greater than or equal to 200 mg/dL (8, 31). Although most diabetes are type 1 or type 2 diabetes, gestational diabetes (GDM) affects both the baby and the mother for having higher risk of developing type 2 diabetes later in life (20). GDM is either diagnosed by one-step approach of 2-hour 75g OGTT, or two-step approach of 1-hour 50g OGTT followed by 3-hour 100g OGTT.

Glucose level measured by FPG and 2HPG were thought to follow a unimodal normally distributed curve, thus diagnosis of diabetes was based on statistical parameter of greater than two standard deviation from the mean glucose concentration (32). Later it was discovered by Bennett and others that glucose concentration in Pima Indians and other high-risk populations follow bimodal distribution (33). This discovery had led to National Diabetes Data Group (NDDG) to publish the classification and diagnosis criteria of diabetes based on glucose distribution in 1979 (34). Beginning of 1997, ADA began to establish diagnosing criteria based on the relationship of glycemia and risk of retinopathy, a common complication experienced by many diabetic patients (9). FPG

greater than or equal to 126 mg/dL had been set so that either FPG or 2HPG would make diagnosis that resulted in similar prevalence of diabetes in population (9).

Several limitations of FPG and 2HPG tests have urged the exploration of other potential diagnostic tests for diabetes. FPG and 2HPG tests are not concordant, which means one person may be diagnosed with diabetes based on one test but may not have been diagnosed by the other test. This discrepancy demonstrated by several epidemiological studies as well as the International Expert Committee Report in 1997 (9, 35). Results measured by FPG and 2HPG tests reflect the distribution of glucose in patient blood at the time of testing can be manipulated by short-term lifestyle changes and dieting (36). Many different instruments and glucose testing methods showed bias from reference method (37). The high intra-individual variance of FPG, low sensitivity compared with 2HPG, and instability at room temperature has made it less than optimal in diagnosing diabetes (38, 39). Although 2HPG has been used as the gold standard in diagnosing diabetes, it also has some drawbacks which limit the use of this test in high-risk patients. The major disadvantage is the lengthy pre-analytical process. Glucose levels are measured before, one hour, and two hours after glucose challenge; some patients experience nausea and discomfort from the glucose drink where others refuse to undergo this time-consuming procedure repeatedly (36, 40-41). Both FPG and 2HPG tests require patients to fast at least eight hours before the test and may include dietary restrictions prior to the test. Thus it has become a routine practice to schedule early morning visits which have made it very inconvenient for most of patients unless other physical screening tests that required fasting were also scheduled at the same time.

Hb A_{1c}, a glycoprotein was first separated and characterized by two different groups ten years apart (42, 43). The association of increasing Hb A_{1c} in diabetic patients was demonstrated by Rahbar in 1968 (43). Hb A_{1c} predicts mean glucose level in blood over two to three months and it measures chronic glucose exposure (23, 44). The International Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997 examined FPG, 2HPG, and Hb A_{1c} and the association with retinopathy that showed after a certain glucose level the risk of developing retinopathy and glycemia behaved in apparent linear fashion (9). Compared to FPG, Hb A_{1c} has stronger and more consistent association with long-term microvascular complications and is stable at room temperature after sample collection (45). Different from FPG and 2HPG tests, glycemia measured by Hb A_{1c} does not fluctuate due to short-term modification of diet or lifestyle changes (39). In 1993, the Diabetes Control and Complications Trial (DCCT) published the results based on large-number randomized cohort studies which demonstrated the relationship between glycohemoglobin and the development and progression of diabetes-associated long-term complications such as retinopathy, nephropathy, neuropathy, and cardiovascular diseases in type 1 diabetic patients (2). Shortly after the publication of this study, ADA recommended patients targeted blood glucose control at the level suggested in DCCT study as treatment goals in diabetes (7). Another similar study conducted by the United Kingdom Prospective Diabetes Study (UKPDS) also demonstrated results comparable to the DCCT study in type 2 diabetes (3). Because of these reasons, the 1997 Expert Committee considered Hb A_{1c} as the best diagnostic test for diabetes but did not recommend its utilization mainly because of results variance due to lack of standardization of testing methods. Before the national effort of standardizing

glycohemoglobin values independent from testing methods lead by National Glycohemoglobin Standardization Program (NGSP), intra-laboratory coefficient of variation (CV) ranged from 0.2 % to 28.7 % imprecision where inter-laboratory CV was as high as 22 % (9).

2.2 Concerns of Hb A_{1c} testing methods

Several reasons have contributed to the variation of Hb A_{1c} values. Different testing methods exist in measuring different forms of glycohemoglobin. Testing methods such as ion-change chromatography and electrophoresis separate glycated hemoglobin from non-glycated hemoglobin based on charge difference. Other methods such as boronate affinity chromatography and immunoassay separate glycated from non-glycated hemoglobin based on structure differences (46). Hb A_{1c} is a form of glycohemoglobin, as defined by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), is the "stable adduct of glucose and the N-terminal amino group of the β-chain of hemoglobin A_0 [N-(deoxyfructosyl) hemoglobin]. A 1993 CAP proficiency survey reported that only 50 % of laboratories participated in the survey reported Hb A_{1c} results. Twenty-nine percent of CAP survey participating laboratories reported results in hemoglobin A_1 where the remaining 21 % of laboratories reported results in total glycated hemoglobin (47).

Interferences for specific testing methods lead to generation of faulty results. Patients with hemoglobinopathy, hemoglobin variants, can have falsely increased or decreased Hb A_{1c} results due the charge difference of these different hemoglobin forms and Hb A_{1c}. Hb A_{1c} measured by ion exchange chromatography and electrophoresis are known to be affected by these hemoglobin variants (48). Several studies also pointed out

the possibility that Hb A_{1c} increases with age and are affected by racial disparities (49). Hemoglobinopathies or diseases affects red cell turnaround time (hemolytic anemia, acute or chronic blood loss, chronic kidney and liver diseases) and hemoglobin variants such as hemoglobin S or C may give erroneous results depending on the resting methods that have been used (50).

2.3 Standardization of Hb A_{1c} testing methods to improve analytic performance

In 1996, NGSP started a national effort to decrease variance and imprecision of Hb A_{1c} results by standardizing Hb A_{1c} to DCCT reference value. Different manufacturers of Hb A_{1c} testing instruments along with secondary reference laboratories worked together to calibrate and compare their Hb A_{1c} results to DCCT reference values. Certification of traceability to DCCT reference value had been granted by NGSP to different Hb A_{1c} instrument manufacturers. Laboratories performing Hb A_{1c} testing are recommended by ADA to participate in CAP proficiency-testing surveys to ensure analytical performance and to reduce bias and imprecisions in Hb A_{1c} test reporting (51).

Meanwhile, IFCC began to develop higher-order reference methods to analyze Hb A_{1c} in 1997. In 2002, an approved IFCC reference method was published (52). In the HPLC/ electrospray mass spectrometry (ESI/MS) reference method, hemoglobin solutions were made from human erythrocytes hemolysates, which were then treated with endoproteinase Glu-C to cleave hemoglobin into glycated hemoglobin marked as Hb A_{1c} and non-glycated hemoglobin marked as Hb A_0. The percentage of Hb A_{1c} was expressed as the ratio of glycated hemoglobin Hb A_{1c} to Hb A_0. Hb A_{1c} and HbA_0 mixture was first separated by reversed phase HPLC and the resulting Hb A_{1c} and Hb A_0 detected by ESI/MS were measured and the ratio of Hb A_{1c} to Hb A_0 was determined. In the

HPLC/capillary electrophoresis (HPLC-CE) method, Hb A_{1c} and HbA_0 mixture was separated by a reversed phase HPLC with C18 column, a different column used as in ESI/MS method. The C18 fraction was further separated by capillary electrophoresis in a H_3PO_4/NaH_2PO_4 buffer and the hemoglobins separated were detected by UV light. The areas containing Hb A_{1c} and Hb A_0 were measured and the ratio of Hb A_{1c} to Hb A_0 was determined. IFCC reference methods included removal of heat labile Schiff base that was formed during an intermediate reaction during Hb A_{1c} formation. This step had partially contributed to small results variance reported by the 4^{th} Comparison Study of the Network for Hb A_{1c} Reference Laboratories. Hb A_{1c} measured by HPLC-CE method reported identical results with carbamylated and acetlylated samples which eliminated the interference from carbamylation and acetlylation of hemoglobin observed in ion exchange chromatography. Hb A_{1c} measured by either HPLC-ESI/MS or HPLC-EC test demonstrated increased specificity and showed no interferences from Hb S, Hb C or Hb A_2.

IFCC network offers participating laboratories and manufacturers monitoring program with samples that have IFCC assigned values to be traceable to IFCC reference methods thus to maintain the accuracy of Hb A_{1c} testing results. However, there is no certification program for participating laboratories or specific standards of how much of variations individual laboratories can deviate from IFCC reference methods (53).

Imprecisions and variations of Hb A_{1c} tests have been lowered substantially since the worldwide standardization process (54). Compared with 12 – 15 % of the day-to-day intra-individual CV of FPG test, the percent CV for Hb A_{1c} was less than 2 % (55, 56). In the 2009 International Expert Committee report on role of A_{1c} testing in diagnosing

diabetes, Hb A_{1c} was first recommended to be used as diagnostic test (40). Soon ADA adapted this recommendation to be included in its diabetes guidelines in 2010 (8).

2.4 Screening for at-risk individuals

The 1997 International Expert Committee introduced the concept of "impaired fasting glucese" (IFG) and "impaired glucose tolerance" (IGT) as the intermediate states between diabetes and non-diabetes (9). Since then, these two terms have been used to identify high-risk individuals as well as to follow more intensive treatment interventions to delay the development and progression of diabetes (40). ADA listed risk factors for diabetes and recommended Hb A_{1c}, along with FPG and 2HPG testing frequency to screen "pre-diabetes" or high-risk category (57). However, the low sensitivity of 51 % of 2HPG in identifying IGT individuals is somewhat a problem since about 50 % those who later developed type 2 diabetes would not have been identified relying solely on 2HPG for screening (58). It is now acknowledged that diabetes, especially development of type 2 diabetes, is a gradual process without clear cut-off points or values to differentiate diabetics from non-diabetics. And Hb A_{1c} test which reflects the chronic glucose exposure in patient whole blood samples is thought to evaluate a continuous risk of developing diabetes (59, 60). The diabetes risk assessment is carried out to include all patients that are at risk rather than excluding people whose Hb A_{1c} value is below the 6.5 % cutoff value (9).

III. Study Design:

3.1 Sample collection, handling, and storage

Twenty whole blood samples were randomly collected from apparently healthy individuals (faculty and students) at Idaho State University in September 2015. Of these twenty samples, ten samples were analyzed. Specimens were collected in EDTA anticoagulant tubes by venipuncture and stored in refrigerators at 4 ˚C for no longer than two weeks until ready to be tested on Siemens DCA Vantage analyzer. Samples with normal, low or high Hb A_{1c} values, obtained from the reference laboratory at Portneuf Medical Center (PMC) were used to carry out comparison study between DCA Vantage and Rio-Rad D-10. These samples were transported on ice to Idaho State University Bengal Lab within thirty minutes and immediately stored in refrigerators at 4 ˚C until ready to be tested.

3.2 Instrumentation, test kits, and supplies

Siemens DCA Vantage analyzer, reagent kit, glass capillary holders, and quality control (QC) materials were purchased from Labsco (Louisville KY). Each reagent kit includes individually packaged reagent cartridges and a calibration card with encoded calibration parameters specific to the lot of reagent kit in use. The calibration card has normal and abnormal calibration curves on either side of the card is used to calibrate the instrument every day patient testing is performed. An optical test cartridge is provided by the manufacturer to perform optical checks on the electronic and mechanical systems of the instrument at time of installation. System configurations such as date, time, printer, and QC settings are adjusted following prompt on instrument monitor.

3.3 Siemens DCA Vantage Hb A_{1c} testing principle

Siemens DCA Vantage system measures percent Hb A_{1c} expressed as the ratio of Hb A_{1c} concentration and total hemoglobin in patient whole blood, multiplied by 100. Total hemoglobin is measured based on spectrophotometry. Hemoglobin from patient whole blood sample is oxidized to methemoglobin by an oxidant potassium ferricyanide. Methemoglobin is then react with thiocyanate in a buffer solution to form a colored species thiocyan-methemoglobin. The light absorbance of thiocyan-methemoglobin developed at 531 nm wavelength is proportional to the concentration of total hemoglobin in patient samples following Beer's Law. Hb A_{1c} concentration is determined by inhibition of latex agglutination reaction. Agglutination reaction between the agglutinator (synthetic polymer of immunoreactive portion of Hb A_{1c}) and Hb A_{1c} mouse specific monoclonal antibody coated latex increased the scattering of light which is measured by increase in absorbance at 531 nm. Inhibition of agglutination of Hb A_{1c} with the addition of patient whole blood samples decreased the light scattering which is also measured at 531 nm. The change of absorbance corresponded to Hb A_{1c} concentration in patient sample, which is determined based on a calibration curve of absorbance and concentration.

3.4 Point-of-care operating procedures on study samples

Specimens for reference range verification and correlation studies were treated the same as patient samples and procedures were followed as such. Before running an Hb A_{1c} test, contents of reagent cartridge package were inspected for any damaged, loose or missing pull-tabs, missing desiccant or loose desiccant particles. Reagent cartridge package was allowed to warm to room temperature for 10 minutes. The capillary holder

was inspected for missing and/or damaged parts before use. Whole blood sample was well mixed and the stopper/cap of EDTA collecting tube was removed so that a small amount of blood remains on the stopper. A glass capillary holder was then filled with 1 µl of specimen and was inserted into the reagent cartridge after cleaning with a lint-free tissue. The barcode on the reagent cartridge was swiped and inserted into the instrument for testing. Percent Hb A_{1c} is available in approximately 7 minutes.

3.5 Statistical analysis

Excel worksheets for reference range verification and correlation studies were created by modifying the Method Validation Policy at Portneuf Medical Center. Twenty whole blood samples obtained from apparently healthy individuals were used to verify reference range established by manufacture. Ten samples out of these twenty were analyzed. Reference range was reported as total population mean ± 2 standard deviation. Whole blood samples were provided by Portneuf Medical Center, the reference laboratory used by this study for patient and instrument correlation studies. Statistical values reported in correlation studies include the following:

1. Mean, standard deviation, and coefficient of variation of two different methods performed in this study.
2. Linear regression analysis of Hb A_{1c} values obtained by DCA Vantage Analyzer and reference method performed at Portneuf Medical Center.
3. Percent difference (bias) of two different methods performed.

3.6 Quality control/quality assurance program

Siemens DCA Vantage system automatically performs self-checks of the optical measurement system to monitor the testing procedures and patient results. Electronic,

mechanical, reagent checks, and calibration verification were performed each time a patient sample or a quality control was run. The system automatically reports an error message to remind the operator to resolve any problems before reporting a patient result. Liquid quality control materials were purchased from Siemens and QC materials were prepared according to manufacturer's insert. Unconstituted QC materials were stored till the expiration date specified by manufacture at 1 – 6 ˚C. Reconstituted QC materials were stored under the same condition up to 3 months. Quality control was run each day patient samples were ran. At Bengal Lab, liquid quality control will be run every day before patient testing, with new shipment of reagents, with new lot of reagents, with new calibration, training a new testing personnel, and performing necessary trouble-shooting for the analyzer.

Maintenance of Siemens DCA Vantage system ensures the analytical performance of the analyzer accurately delivering of testing results. Maintenance will be performed weekly, quarterly, and/or as needed. The following is the maintenance schedule recommended by manufacture:

Weekly	Cleaning the Onboard Barcode Reader Window Cleaning the Exterior
Quarterly	Removing and cleaning the Cartridge Spring and Cartridge Area Changing the Air Filter Performing Optical Test
As Needed	Cleaning the Exterior Changing the Air Filter Cleaning the Onboard Barcode Reader Window Removing and cleaning the Cartridge Spring and Cartridge Area Performing Optical Test Calibrating the Touchscreen Replacing the Fuse

IV. Results:

Hemoglobin A_{1c} testing on Siemens DCA Vantage system is certified as CLIA-waived test. Specifications of testing method performance are not required to be verified or calibrated according to CLIA standards at time of instrument installation with the exception of reference range verification. However, for the purpose of this study, we want to verify the precision and reference range as well as to compare DCA Vantage method with a reference method to ensure Hb A_{1c} results delivered at Bengal Lab are accurate and the instrument is performed according to manufacturer's instructions.

The precision of Hb A_{1c} measured by DCA Vantage system was verified with two levels of patient samples provided by Portneuf Medical Center. Each level of sample was run with five replicates. And the sample average, standard deviation, and coefficient of variation were calculated as summarized in Table 1.

Table 1. Verification of precision of Hb A_{1c} on DCA Vantage system.

	Level 1	Level 2
Sample mean	10.0	5.2
Standard deviation (SD)	0.2881	0.0707
Coefficient of variation (% CV)	2.87	1.36

To obtain reference range of Hb A_{1c} measured by DCA Vantage system, a total of 10 study samples were randomly selected from healthy participants of both males and females and various ages. Sample mean and standard deviation (SD) were used to calculate reference range as the mean ± 2SD shown in Table 2.

Table 2. Verification of reference range study of Hb A_{1c} on DCA Vantage system.

Sample mean	5.2
Standard deviation	0.2
Coefficient of variation (% CV)	3.85
Reference range	4.8, 5.6

Five study samples were run on both Biorad D-10 and DCA Vantage. The Riorad D-10 HPLC was the reference method used for the DCA Vantage system. Figure 1 compares Hb A_{1c} results measured by these two different test systems.

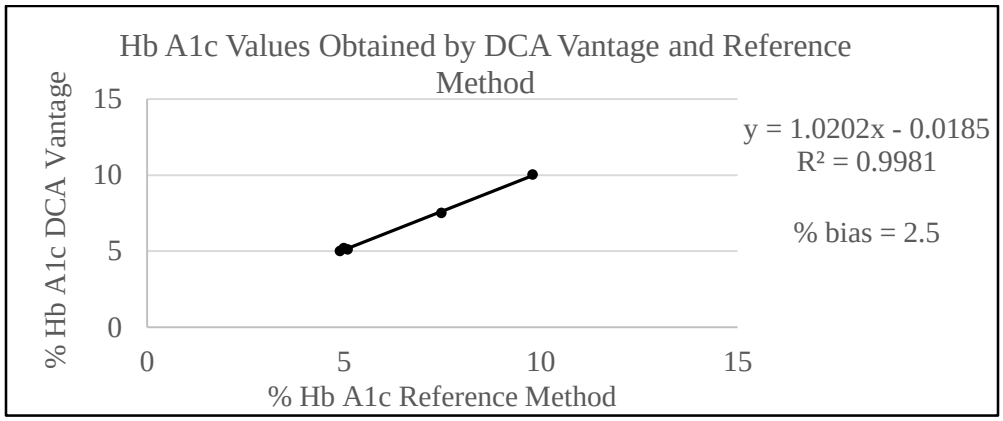

Fig. 1 Linear regression of method comparison of Hb A_{1c}.

V. Discussions

According to the mean Hb A_{1c} value and % CV reported in Table 1, this study presented within-run mean Hb A_{1c} of 5.2 at normal level (level 1) and 10.0 at abnormal level (level 2). The SD and % CV of within-run mean Hb A_{1c} at normal level were 0.0707 and 1.36% respectively. The SD and % CV of within-run mean Hb A_{1c} at abnormal level were 0.2881 and 2.87% respectively. These values compared favorably with the precision study performed by Siemens at three different sites. The SD of within-run mean Hb A_{1c} at normal level was reported by Siemens ranging from 0.11 to 0.16 which was bigger than the SD reported in this study. SD of within-run mean Hb A_{1c} at abnormal level was reported by Siemens ranging from 0.33 to 0.44 which was smaller than what was reported in this study but a larger sample size was used. SD measures the dispersion of data around mean. A small SD indicates that data is scattered around the mean very closely while a large SD indicates that data is spread out. The % CV reported in this study were both less than 3% which is the % CV accepted in clinical laboratories. The small % CV reported demonstrated the consistency of Hb A_{1c} values measured by DCA Vantage system. The reference range was verified by using samples randomly selected from Idaho State University faculties and
staff. The reference range of Hb A_{1c} was 4.8 % to 5.6 % (Table 2) compared with the reference range of 4.3 % to 5.7 % reported by Siemens. Five samples ranging from low to high Hb A_{1c} values were measured by Siemens DCA Vantage and Biorad D-10, the reference method used by PMC. The % bias between these two methods was 2.5 % imprecision.

One major limitation of the experimental design of this study was that only 5 samples were compared for the correlation study between Siemens DCA Vantage and Biorad D-10. These 5 samples were only ran once. One of those five values was unavailable which made the comparison study less reliable. Ideally at least duplicate samples should be ran and mean, SD, and % CV of the reference method should be included in the comparison study. Another limitation of this study was that precision of Siemens DCA Vantage included only within-run data from single day of testing. Further study of precision should include between-run sampling for 20 days to examine the day-to-day variation of testing performance. However, if the QC recommendations are followed, those data will be available after the instrument is put in service and the daily QC samples are run. This is customary in the laboratory. With POC testing this is not specified as a requirement. The reason for this abbreviated procedure is primarily cost. The cost of each run on this instrument such as reagents and controls which are considered consumables exceeds $20 per test. An in-depth method validation study should also include the detailed cost evaluation consisting of instrument purchase, instrument maintenance and technical support contract with the vender, reagent cost, personnel training and competency, participation in national proficiency surveys. Testing volume should also be considered when making purchase and selecting instruments. The use of a POC instrument in a clinical laboratory setting is rarely cost effective.

VI. Conclusions

Hb A_{1c} value measured by Siemens DCA Vantage system has an imprecision of less than 3 % CV which meets the general requirement of analytical performance criteria in clinical laboratories. Hb A_{1c} values measured by Siemens DCA Vantage were comparable to what was reported by Biorad D-10 HPLC reference method at Portneuf Medical Center with % bias of 2.5 which is also within the recommended allowances for accuracy. This study verified the reference range to be 4.8 % to 5.6 % at Bengal Lab with the reference range reported by Siemens. A quality control schedule was proposed for the laboratory which will assure that the precision and accuracy will be continuously monitored. Therefore, it was decided that the Bengal Lab has demonstrated that this POC instrument, the Siemens DCA Vantage can be put in service with no further validation.

VII. References

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Appendices

Appendix 1: Figures

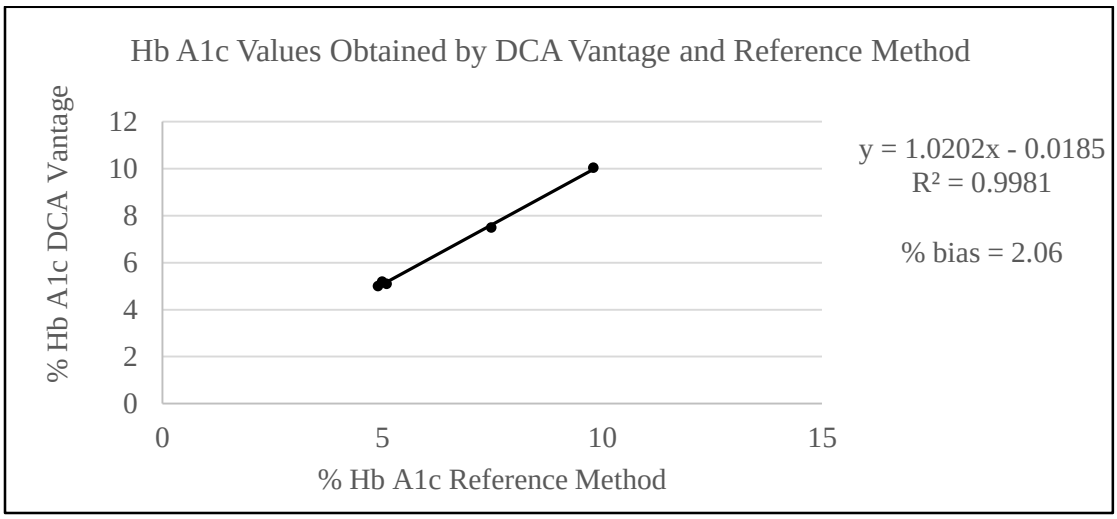

Fig. 1 Linear regression of method comparison of Hb A_{1c} .

Appendix 2: Tables

Table 1. Verification of precision of Hb A_{1c} on DCA Vantage system.

	Level 1	Level 2
Sample mean	10.0	5.2
Standard deviation (SD)	0.2881	0.0707
Coefficient of variation (% CV)	2.87	1.36

Table 2. Verification of reference range study of Hb A_{1c} on DCA Vantage system

Sample mean	5.2
Standard deviation	0.2
Coefficient of variation (% CV)	3.85
Reference range	4.8, 5.6

Appendix 3: Raw data of verification of precision and formulas used in statistical analysis

Number of replicates	Level 1	Level 2
1	10.5	5.1
2	9.8	5.3
3	9.8	5.2
4	10.1	5.2
5	10.0	5.2

Sample mean was calculated based on the following formula:

Sample mean at Level 1 = $\frac{sum\ of\ Hb\ A1c\ at\ Level\ 1}{number\ of\ replicates}$, n = 5

Sample mean at Level 2 = $\frac{sum\ of\ Hb\ A1c\ at\ Level\ 2}{number\ of\ replicates}$, n = 5

Standard deviation of sample was calculated with Excel function STDEV S, available in Excel 2013 version.

Percent coefficient of variation was calculated based on the following formula:

% coefficient of variation (% CV) = $\frac{\text{standard deviation of sample}}{sample\ mean} * 100\ \%$

Appendix 4: Raw data of verification of reference range and formulas used in statistical analysis

Sample number	Hb A1c values
1	5.0
2	5.2
3	5.4
4	5.1
5	5.0
6	5.3
7	5.1
8	5.3
9	5.0
10	5.6

Sample mean was calculated based on the following formula:

Sample mean = $\frac{sum\ of\ Hb\ A1c\ values}{number\ of\ samples\ tested}$, n = 10

Standard deviation of sample was calculated with Excel function STDEV S, available in Excel 2013 version.

Percent coefficient of variation was calculated based on the following formula:

% coefficient of variation (% CV) = $\frac{\text{standard deviation of sample}}{sample\ mean} * 100\ \%$

Reference range was calculated based on the following formula:

Reference range = sample mean ± 2* standard deviation

Appendix 5: Raw data of correlation study and formulas used in statistical analysis

	Hb A_{1c} values measured at Bengal Lab	Hb A_{1c} values measured at PMC	(Difference)
	9.80	9.90	0.1
	5.00	5.00	0.0
	4.90	4.90	0.0
	5.10	5.10	0.0
% Bias (Difference)			2.5

Percent bias was calculated as the difference between Hb A_{1c} values measured at Bengal Lab and the Hb A_{1c} values measured at PMC, multiplied by 100 %.