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Validation of the LumiraDx for the measurement of HbA1c for Diabetes diagnosis and monitoring

Lesito, Ofentse Thoriso

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Validation of the LumiraDx for the measurement of HbA1c for Diabetes diagnosis and monitoring.

Ofentse Thoriso Lesito

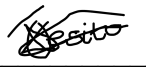
(2088694), MSc (Med) Chemical Pathology

A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science in Medicine (Chemical Pathology).

Johannesburg, 2025

Declaration

I, **Ofentse Thoriso Lesito**, declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science in Medicine (Chemical Pathology) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Candidate Signature)

18th day of July 2025 in Johannesburg

Dedication

Soli Deo Gloria!

To my parents Ramagarasoa and Mpho Lesito, for their love and endless support.

Presentations arising from this research project.

1. Ofentse Lesito, Ngalula Kone, Riffat Munir and Jaya George (December 2023). Evaluation of the LumiraDx point-of-care analyser for the measurement of glycated haemoglobin (HbA1c). Molecular Biosciences Research Thrust (MBRT) Symposium 2023.
2. Ofentse Lesito, Ngalula Kone, Riffat Munir and Jaya George (September 2024). Performance Evaluation of the LumiraDx point-of-care analyser for reliable HbA1c measurement in Diabetes diagnosis and monitoring. The 2024 Faculty of Health Sciences Research Day.
3. Ofentse Lesito, Ngalula Kone, Riffat Munir and Jaya George (November 2024). Performance Evaluation of the LumiraDx point-of-care analyser for reliable HbA1c measurement in Diabetes diagnosis and monitoring. The 4th POCT Africa International Colloquium.

Abstract

Introduction: South Africa has a high number of people living with diabetes, many of whom are either not diagnosed or are poorly controlled. Diabetic control can be monitored using HbA1c. Several studies have shown that point-of-care (POC) testing of HbA1c improves patient management and glycaemic control.

Aim: This study aimed at evaluating the performance of the LumiraDx POC analyser (LumiraDx UK Ltd, London, UK) for the measurement of HbA1c for diabetes diagnosis and monitoring.

Methods: The investigation was conducted in two phases: an initial laboratory evaluation followed by a clinical evaluation. In the laboratory phase, we assessed precision, linearity, accuracy, and lot-to-lot variation. The clinical phase involved method comparison analyses to evaluate the interchangeability of the LumiraDx HbA1c test with a laboratory reference method (Bio-Rad Variant™ II Haemoglobin Testing System (Bio-Rad laboratories Inc., California, United States) and the Premier Hb9210™ (Trinity Biotech Plc., County Wicklow, Ireland)) and another established POC analyser (DCA Vantage® Analyzer (Siemens Healthineers, Erlangen, Germany))

Results: Results showed acceptable precision, with within-run coefficients of variation (CV) of 0.92% and 0.51%, and between-run CVs of 1.07% and 0.66% for control samples. The linearity curve exhibited an R^2 value of 0.99 across the clinically relevant range. The accuracy was confirmed with a concordance correlation coefficient (CCC) of 0.98 (95% CI: 0.97 to 0.99) and a bias of 0.06, indicating strong agreement with the laboratory reference method. Lot-to-lot variation demonstrated correlation coefficients of 0.98, 0.96, and 0.97 for the three different reagent lots compared to the laboratory method. Clinical data analysis revealed CCCs of 0.93 (95% CI: 0.91 to 0.95) and 0.97 (95% CI: 0.97 to 0.98) when compared to Clinic standard of care (SOC) and the Laboratory SOC, respectively.

Conclusion: Collectively, the findings of this study suggest that the LumiraDx HbA1c test is a reliable tool for the measurement of HbA1c, offering a viable alternative to traditional laboratory methods.

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Abbreviations

ADA	American Diabetes Association
ART	Antiretroviral therapy
BMI	Body Mass Index
CDM	Clinical Data Management Software
CE	Capillary Electrophoresis
CLIA	Clinical Laboratory Improvement Amendments
CLSI	Clinical and Laboratory Standards Institute
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CMS	Centers for Medicare & Medicaid Services
CV	Coefficient of Variation
DCCT	Diabetes Control and Complications Trial
DM	Diabetes Mellitus
EDIC	Epidemiology of diabetes intervention and complications
EDTA	Ethylenediaminetetraacetic acid
EFLM	European Federation of Clinical Chemistry and Laboratory Medicine
FDA	Food and Drug Administration
FIND	Foundation for Innovative New Diagnostics
FPG	Fasting plasma glucose test
HbA	Adult haemoglobin
HbA1c	Haemoglobin A1c/ Glycated haemoglobin
HbF	Foetal haemoglobin
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
IDF	International Diabetes Federation
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IVD	In vitro diagnostics
LMICs	Low- and Middle-Income Countries
LTLV	Lot-to-Lot variation
NGSP	National Glycohemoglobin Standardization Program
NHLS	National Health Laboratory Services
NNRTIs	Non-nucleoside reverse transcriptase inhibitors

OGTT	Oral glucose tolerance test
PIs	Protease inhibitors
PLWH	People living with HIV
POC	Point-of-care
QA	Quality Assurance
QC	Quality control
RPG	Random plasma glucose test
SADHS	South African Demographic and Health Survey
SAHPRA	South African Health Products Regulatory Authority
SD	Standard Deviation
SMBG	Self-monitoring of blood glucose
SOC	Standard of care
SSA	Sub-Saharan Africa
TB	Tuberculosis
UKPDS	United Kingdom Prospective Diabetes Study
WHO	World Health Organisation

mmol/L	millimole per litre
µl	microlitre
ml	millilitre
%	Percentage
SD	Standard Deviation
CV	Coefficient variation
CV_a	Analytical coefficient of variation
CVg	Inter-precision variability
CVi	Intra-precision variability
R^2	Coefficient of determination
Pc	Concordance Correlation Coefficient
LoA	Limits of agreement
IQR	Interquartile range
ATE	Allowable total error
TAE	Total Analytical error
CI	Confidence Interval

Chapter 1 Introduction and Literature review

1.1 Diabetes Mellitus

Diabetes mellitus (diabetes) is a metabolic condition resulting from either abnormal insulin production or action, leading to persistent hyperglycaemia. The pathways that contribute to the onset of diabetes are diverse. Some of these pathways lead to insulin deficiency by impairing or destroying the function of pancreatic beta cells (β-cells), while others lead to insulin resistance wherein cells become less receptive to insulin (insulin insensitivity) (1).

Diabetes is classified into multiple types, such as type 1, type 2, gestational and other distinctive forms attributable to different influences like genetic mutations, pancreatic diseases and medication or chemical exposure. The dominant form is type 2 diabetes which stems from an ongoing impairment of β-cell insulin release and the failure of insulin-stimulated cells to properly react to insulin (2). This form is mainly diagnosed in adults but there is a growing prevalence in children and adolescents resulting from increased rates of obesity and an inactive lifestyle (3). On the other hand, type 1 diabetes is marked by an immune-mediated breakdown of pancreatic β-cells responsible for insulin production. It is usually found in children or young adults and results in an absolute undersupply of insulin therefore requiring a lifetime of insulin treatment (4,5). Gestational diabetes develops during pregnancy and is defined by elevated blood glucose levels that normally stabilize after childbirth; nonetheless, it poses danger for both mother and child to acquire type 2 diabetes later (1,6).

1.2 Global prevalence of Diabetes

In 2021, the "International Diabetes Federation (IDF)" projected that 537 million individuals from age 20 to 79 globally had diabetes, signifying 10.5% of the population. By 2030, the prevalence is anticipated to climb to 643 million (11.3%) and will likely get to 783 million (12.2%) by 2045. Moreover, fewer than one-third of those affected manage to achieve good control of their condition (7).

It is further projected that globally, 240 million individuals live with undiagnosed diabetes (**Figure 1.1**) (7). This underscores the compelling need to enhance the capacity to diagnose diabetes, especially among those who are unaware of their condition, and to ensure that suitable and well-timed care is provided to all individuals with diabetes promptly.

1.3 Diabetes Prevalence in Sub-Saharan Africa and South Africa

In 2021 it was predicted that 24 million people aged 20 to 79 years in the IDF African Region had diabetes, yielding a regional prevalence rate of 4.5% (8). This is projected to expand by 2045 to 55 million (129% increase). Furthermore, 54% of individuals with diabetes in this region are undiagnosed, the greatest percentage of all the IDF Regions (**Figure 1.1**) (7,8).

Type 2 diabetes has been increasingly prevalent in Sub-Saharan Africa (SSA) in recent years, coinciding with an epidemiological transition. Key factors driving this transition include rapid urbanization, urban poverty, and globalization. These changes have led to a shift from a physically active and healthy lifestyle to a more sedentary one, in conjunction with a rise in the intake of unhealthy, high-fat fast foods (3). Although the frequency remains low in some rural groups, it has been noted to be high in metropolitan regions. Type 1 diabetes among children under the age of 19 increased fivefold in the Region between 2011 and 2021, from four cases per 1000 children to almost 20 cases per 1000 children (8).

Diabetes is the second leading cause of mortality in South Africa and is expected to continue rising. However, there is insufficient epidemiological studies in the country that utilize biomarkers to gage the prevalence of diabetes (3). The “South African Demographic and Health Survey (SADHS) 2016” found that a considerable proportion of women (64%) and men (66%) are pre-diabetic and at risk of acquiring diabetes (3). Furthermore, according to IDF Diabetes Atlas 2021, South Africa was in the top five nations in Africa with high prevalence of diabetes (4.2 million people, 20 – 79 years) in 2021 and 45.4% South Africans remain undiagnosed (**Figure 1.1**) (7,8).

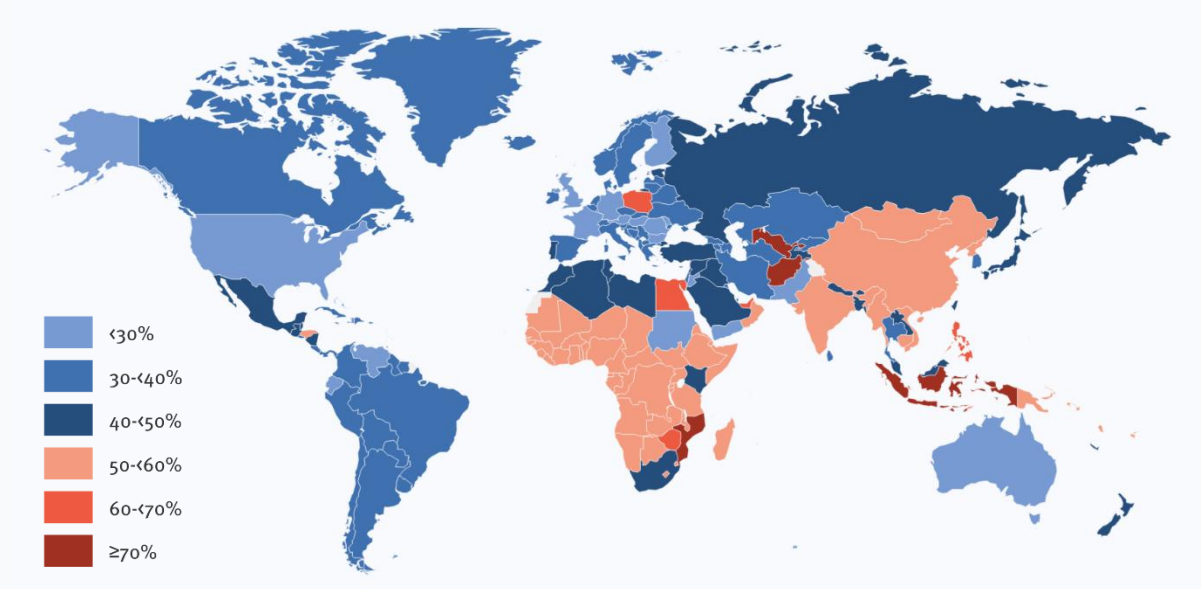


Figure 1.1: The percentage of individuals with undiagnosed diabetes according to the IDF Diabetes Atlas 2021 (7).

1.4 Diabetes among HIV-infected and TB-infected individuals

The occurrence of diabetes in individuals with HIV and TB differs across geographic areas, demographic groups, and levels of healthcare access. Patients with both HIV and TB may experience a higher prevalence of diabetes, which significantly affects their management and health outcomes (9). To tackle the challenges associated with these overlapping conditions, integrated healthcare approaches are crucial. Consistent screening and monitoring for diabetes in populations affected by HIV and TB can enhance patient care and improve health results (10,11).

The diabetes challenge in SSA, particularly in South Africa, is made worse by the persistent HIV epidemic. Due to the widespread use of antiretroviral therapy (ART), the lifespan of people living with HIV (PLWH) has risen substantially, resulting in an older HIV-positive population that is more frequently facing comorbidities like diabetes, often at younger ages compared to the general population (12,13). According to studies, alongside demographic and lifestyle variables, long-term use of ART – particularly 'HIV protease inhibitors (PIs)' and 'non-nucleoside reverse transcriptase inhibitors (NNRTIs)'– raises the susceptibility of acquiring diabetes (14–16). Moreover, a significant proportion of PLWH also suffer from undiagnosed diabetes. For example, a study conducted in Limpopo, South Africa, revealed that 58.6% of PLWH were not aware of their diabetes status (17). Additionally, individuals infected with HIV frequently exhibit poor glycaemic control. Research by Henry et al.

demonstrated that only 22% of HIV-infected individuals with diabetes achieved adequate glycaemic regulation, while a study by Roerink et al. indicated that only 54% of HIV-infected patients with diabetes maintained HbA1c levels below 7% (18,19). Another study conducted in western Kenya examined 454 adult TB patients co-infected with HIV, using point-of-care HbA1c testing to evaluate the prevalence of diabetes and pre-diabetes. The findings revealed a significant occurrence of pre-diabetes at 37.5% and diabetes at 5.1% among these patients. Additionally, only 7 out of 23 patients (30%) with diabetes knew their prior diabetes status (20). These studies emphasize the vital importance of screening HIV- and TB-infected individuals for diabetes.

1.5 Diagnosis of Diabetes

Each form of diabetes is diagnosed using a specific set of tests to detect blood glucose levels. When symptoms like extreme thirst, frequent micturition, and unexplained weight loss appear, type 1 diabetes is frequently diagnosed, often in emergency settings or at routine checkups (21). A blood glucose test is usually required for confirmation of type 1 diabetes and can sometimes be further confirmed by the presence of autoantibodies (5). Type 2 diabetes, on the contrary, is typically detected during routine screenings, especially for people over 45 or those who have risk factors including obesity or a family history of the disease. Hyperglycaemia is the basis for the diagnosis of type 2 diabetes (22).

Diagnosing diabetes involves several key laboratory tests. Table 1.1 shows four tests and different definitions of diabetes, prediabetes and normal using the different tests. The fasting blood glucose (FPG) test requires fasting overnight (minimal 8 hours) before measuring glucose levels. A measurement of 7.0 mmol/L or above signifies diabetes. The oral glucose tolerance test (OGTT) also requires fasting, then a glucose solution is consumed. The glucose levels are tested prior to consumption and 2 hours after. A measurement of 11.1 mmol/L or above confirms diabetes. The OGTT is recommended only when the results of fasting, or random blood glucose are equivocal: FPG 5.5–6.9; RPG 5.5–11.0 mmol/L. The random blood glucose (RPG) test can be performed at any time without fasting. A measurement of 11.1 mmol/L or above, along with indicators of diabetes, confirms diabetes diagnosis. Lastly, the HbA1c test shows the “weighted” average blood glucose levels within the last 2 to 3 months, with levels of ≥6.5% signifying diabetes. This test does not necessitate fasting (23,24).

Table 1.1: The classifications of Prediabetes and Diabetes based on different tests * (25)

	Fasting Plasma Glucose	Oral Glucose Tolerance Test [a]	Random Plasma Glucose	Haemoglobin A1c
Diabetes	≥7.0 mmol/L	≥11.1 mmol/L	≥11.1 mmol/L with polyuria and polydipsia	≥6.5%
Prediabetes	5.6–6.9 mmol/L	7.8–11.1 mmol/L		5.7%–6.4%
Normal	≤5.5 mmol/L	≤7.7 mmol/L		<5.7%

[*] All-laboratory values should be confirmed with repeat testing.

[a] Test performed only when result for FPG is 5.5–6.9 mmol/L and RPG is 5.5–11.0 mmol/L.

1.6 Monitoring of Diabetes

People living with diabetes are faced with a higher disease stress attributable to the possible occurrence of macrovascular and microvascular secondary conditions. These secondary conditions can be avoided or their occurrence delayed by achieving optimal glycaemic control (26,27). Therefore, because maintaining optimal glycaemic control is top priority, routine monitoring of glycaemic levels is crucial in the management of diabetes. HbA1c is a key parameter for monitoring long-term glycaemic control and is useful in informing the need for modifications in patient treatment plans and to assist in educating the patient on the disease (28). Studies like the "Diabetes Control and Complications Trial (DCCT)/Epidemiology of diabetes intervention and complications (EDIC)" and the "United Kingdom Prospective Diabetes Study (UKPDS)" have set the specific HbA1c goals which patients should strive to meet to reduce the development of secondary conditions when diabetes is diagnosed at the HbA1c cut-off point of ≥6.5%. These multicentre randomized trials investigated the effects of intensive therapy and standard therapy on the vascular and neurological comorbidities in individuals with type 1 diabetes (DCCT/EDIC) and newly diagnosed type 2 diabetic patients (UKPDS) (26,27). They emphasized the significance of HbA1c in controlling diabetes by illustrating that improved glucose control, as shown by the decrease in HbA1c levels, reduces the incidence of diabetic complications (26,27,29).

An HbA1c goal of under 7% is recommended to help avert both microvascular and macrovascular complications, especially when aggressive treatment begins early in the disease (26,27). Targeting a HbA1c level below 6.5% in newly diagnosed healthy individuals with a reasonable life expectancy can be beneficial for lowering microvascular risks, if done appropriately. In contrast, for individuals who are weak, very dependent, have various health difficulties, significant cardiac or vascular issues, advanced kidney disease, a short life expectancy, or are unaware of low blood sugar; a HbA1c range of 7.1% to 8.5% may be more appropriate. In palliative care settings, the primary goal would be to prevent symptomatic hyperglycaemia. In individuals who have good control and are reaching their target goals, HbA1c is monitored every six months. On the other hand, HbA1c is monitored at three month intervals in individuals not meeting the desired goal and whose treatment has been modified or strengthened (30).

Based on HbA1c guidelines from the "American Diabetes Association (ADA)", patients with an HbA1c level above 7% are classified as having poorly-controlled diabetes, while those with an HbA1c level of 7% or lower are considered to have well-controlled diabetes (31).

1.7 The Biochemistry of HbA1c

In healthy adults, haemoglobin is made up of around 0.5% foetal haemoglobin (HbF), 2.5 % HbA2 and 97% adult haemoglobin (HbA). About 6% is glycated (HbA1), whereas 94% of HbA is non-glycated (HbA0). HbA1 is made up of HbA1a and HbA1b, which jointly account for about 1%, and 5% is HbA1c, the major constituent (32) (**Figure 1.2A**).

HbA1c is generated via a non-enzymatic reaction, as shown in **Figure 1.2B**, that occurs when normal HbA is subjected to constantly elevated glucose levels, such as those found in diabetic individuals. The excess glucose attaches itself non-enzymatically to the N-terminal valine of haemoglobin's β-chain (with other possible binding sites including β-Lysine-66 and α-Lysine-6), forming a Schiff base. Initially, this Schiff base is unstable and reversible. However, it eventually undergoes an Amadori rearrangement, resulting in the formation of an irreversible and stable ketoamine, which is the HbA1c compound. The reaction occurs slowly and is solely dependent on concentration, therefore, the magnitude of HbA1c formed is related only to the concentration of HbA and glucose present (28,32).

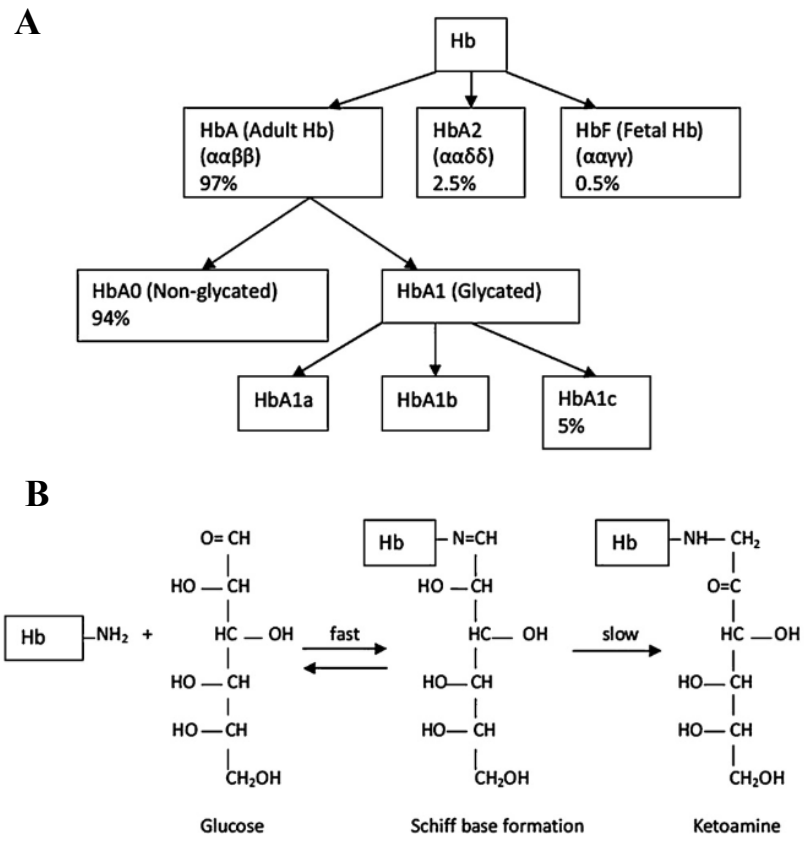


Figure 1.2: The types of haemoglobin and the biochemistry of glycated haemoglobin (HbA1c) **(A)** Haemoglobin forms in healthy adults. **(B)** The two-step nonenzymatic reaction that produces HbA1c. The arrows indicate the direction of the reaction. Obtained from (32).

1.8 Limitations and Rationale of HbA1c testing

HbA1c levels are impacted by the life expectancy and turnover rate of red blood cells. Thus in conditions where red blood cell turnover is impaired, such as in pregnancy, or instances of anaemia, blood loss, or renal failure, as well as when there is evidence of haemoglobin disorders, HbA1c may not truthfully reflect current glycaemic levels (33–35).When discrepancies arise between HbA1c results and self-monitoring of blood glucose (SMBG) readings, clinicians should consider these factors and may need to conduct laboratory tests to screen for potential hemoglobinopathies. Additionally, HbA1c does not provide information regarding the frequency or severity of hypoglycaemic events or glycaemic variability; therefore, results are to be explained jointly with SMBG data and the individual's clinical history (33).

Despite certain drawbacks, HbA1c remains an essential biomarker for diabetes, influencing glucose monitoring and treatment decisions. As a result, both the "World Health Organisation (WHO)" and the ADA suggest adopting HbA1c measurements to diagnose and monitor diabetic individuals, based on the result outcomes of the DCCT/ EDIC and the UKPDS trials (26,27,36). This support from the WHO and ADA is credited to the international standardization of the HbA1c test, which has improved its analytical accuracy and has led to greater patient satisfaction (37,38).

The HbA1c test has evolved into a reliable and standardized technique for guiding treatment decisions and anticipating patient outcomes. Nonetheless, the IDF underlines that HbA1c testing must use a procedure recognized by the National Glycohemoglobin Standardization Program (NGSP) and according to the DCCT guidelines (7).

1.9 Methods used for measuring HbA1c

HbA1c is measured using two fundamental concepts that distinguish between glycated and non-glycated haemoglobin: 1) charge differences and 2) structural differences. The first concept is utilized in ion-exchange chromatography techniques like high-performance liquid chromatography (HPLC), as well as electrophoresis-based tests. The second concept applies to immunoassays and tests that use boronate affinity chromatography.

1.9.1 Methods dependent on charge differences

Cation-exchange HPLC is a technique that divides a mixture depending on the positive charge characteristics of its components. A blood sample is passed through a column containing negatively charged resin beads, where different charged haemoglobin fractions (including HbA1c) bind to the column based on their net positive charge. HbA1c has a slightly different net positive charge compared to other haemoglobin fractions which allows it to bind to the negatively charged resin bead on the column with a distinct affinity. The gradient of increasing ionic strength in the buffer flowing through the column causes selective elution of the different haemoglobin fractions from the column, with HbA1c eluting at a specific retention time. The HbA1c is detected by measuring absorbance of the different haemoglobin fractions (usually at 415nm) as they pass through a flow cell, generating a distinct peak on the chromatogram based on its unique retention time (39).

Generally, the main benefit of cation-exchange HPLCs is a small analytical coefficient of variation (CVa). Majority of HPLC technologies can attain a CVa of less than 2.0% (as per

DCCT values), while modern HPLC technologies can attain a CVa of less than 1.0% (as per DCCT values), making these technologies preferable to alternative methods for measuring HbA1c (40). The HPLC method has great precision, accuracy, and the capability to detect and separate distinct haemoglobin variants, which renders it the gold-standard method for HbA1c measurement in healthcare settings (41,42). However, one significant downside of this method is its sensitivity to disturbance from particular haemoglobin variants, which might result in false low or high HbA1c values (43). To reduce mistakes caused by haemoglobin variant interference, each chromatogram should be examined for irregularities, either manually or by automated computer systems. This method necessitates specialized abilities from the professionals operating the device.

1.9.2 Methods dependent on structural differences

To measure HbA1c, immunoassays use antibodies designed specifically to recognize and bind to specific amino acids, as well as the glucose moiety on the N-terminal of the haemoglobin's β-chain (44). The bound antibodies are then detected using various approaches such as enzyme-linked immunosorbent assay (ELISA), fluorescence-based, turbidimetric inhibition and latex agglutination inhibition. The amount of HbA1c is quantified by the determination of the percentage of HbA1c relative to total haemoglobin which is typically measured using a bichromatic approach (**Figure 1.3B**) (45,46).

A notable benefit to immunoassays is that they are generally quick and can be automated, making them suitable for high-throughput clinical settings that require the analysis of a larger number of samples daily (47). However, a notable disadvantage when compared to most of the cation-exchange HPLCs systems, is the relatively high CVa associated with immunoassays. External quality assessment programs indicate that only the newly developed cation-exchange HPLCs can achieve a between-laboratory CV of less than 2.0% (as per DCCT values) (48).

Affinity separation relies on the molecular binding of the cis diols present in glucose within HbA1c to a boronate matrix (**Figure 1.3A**). This method quantifies the "total" glycation. HbA elutes from the column without binding to the boronate matrix, while HbA1c is released upon buffer changes. The resulting chromatogram displays two distinct peaks: one corresponding to non-glycated haemoglobin and the other to glycated haemoglobin (49).

The fundamental benefit of affinity chromatography lies in its non-interference from haemoglobin variants or derivatives, which has established it as the "reference" standard for

individuals having haemoglobin variants over a prolonged length of time (43). However, Rolfing et al. found that interference occurs when HbF levels approach 20% because HbF lacks β-chains, resulting in disproportionately low glycation (50).

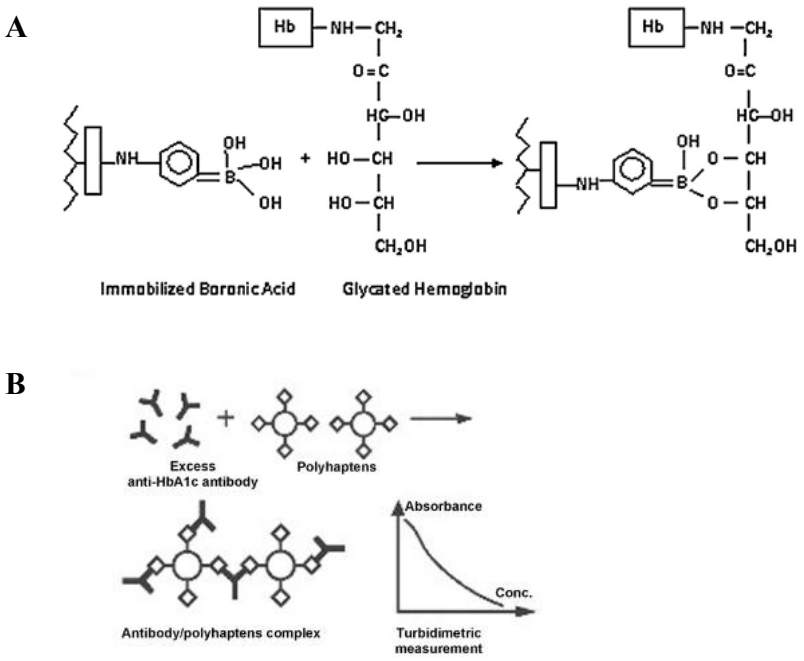


Figure 1.3: Two methods for measuring HbA1c dependent on structural differences. **(A)** Principle of affinity separation chromatography. **(B)** Principle of immunoturbidimetry. Obtained from (32).

1.10 Point-of-care testing

Point-of-care (POC) testing can be described as diagnostic testing conducted in proximity to or at the patient care site, with the results potentially influencing the patient's management (51). The methodologies used by most POC instruments in the quantification of HbA1c are immunoassay, boronate affinity or enzymatic based (51). Immunoassay and boronate affinity have been previously touched on. The enzymatic process involves two distinct stages: 1) the proteolytic cleavage of HbA1c to liberate glycated peptides and 2) a consequent reaction catalysed by an enzyme, such as an oxidase or dehydrogenase, that demonstrates selectivity for the glycated peptides (52).

POC testing has been used for a number of years, mostly in healthcare settings such as clinics, hospitals, and emergency rooms, as well as for self-monitoring at home (51). In the 1950s, the idea of “near-patient” or “on-site testing” for analysis of blood was first

investigated in England and was said to be "near-patient testing"(53). Dr. Gerald J. Kost introduced the phrase "point-of-care testing" in the early 1980s, following considerable exploration on the use of biosensors to monitor ionized calcium levels in whole blood (54). The phrase "point-of-care testing" was afterwards associated with the description of "testing at or near the site of patient care" (55).

The early 1970s and late 1960s saw the invention of a portable glucometer, which is widely regarded as the first POC device (56,57). POC tests used currently encompass urine analysers, blood gas analysers, glucose meters, pregnancy tests and HIV rapid testing. POC testing was formerly limited to clinical settings; however, its use has subsequently spread to local pharmacies, medical practices, and mobile healthcare units (51). POC tests may be used by laboratory-trained staff, non-laboratory healthcare practitioners, or even laypeople, depending on regulatory requirements (51).

The initial "ASSURED" conditions outlined the fundamental qualities of first-generation POC tests, which were characterized as “affordable, sensitive, specific, user-friendly, rapid, robust, equipment-free, and deliverable” (**Table 1.2**) (58). POC tests are classified according to their complexity into three categories: 1) waived tests, which are simple and often used in home settings; 2) moderate and 3) high complexity tests, which necessitate specialized training for proper administration (59). All POC testing is subject to the regulations outlined in the “Clinical Laboratory Improvement Amendments (CLIA)”. This legislation, enacted in 1988, is enforced by two federal agencies: the “Food and Drug Administration (FDA) and the Centers for Medicare & Medicaid Services (CMS)” (59). In South Africa, the “South African Health Products Regulatory Authority (SAHPRA)” regulates POC testing.

Table 1.2: The ASSURED specifications defining the fundamental features for all POC testing devices (58).

• **A**ffordable – Accessible to persons at risk of disease
• **S**ensitive – Minimal occurrence of false negatives
• **S**pecific – Low occurrence of false positives
• **U**ser-friendly – Simple approaches for performing tests
• **R**apid & **R**obust – Fast results without the need for refrigeration
• **E**quipment-free – There is no need for complex system
• **D**elivered – Readily available to end users

Initially, central laboratories viewed POC testing as a competitive option that provided supplementary and inferior testing. However, this viewpoint is rapidly changing as POC technology advances. The primary driver for the development of POC testing in diabetes is to monitor patient glycaemic control, educate the patient and the adjustment of treatment if needed (60). Furthermore, high rates of patient attrition and poor patient adherence in patient care have encouraged the search for alternatives to centralized testing. The ADA currently strongly advocates for POC HbA1c testing since it allows for more prompt treatment adjustments (36).

1.11 Advantages of POC HbA1c testing

1.11.1 Impact on patient glycaemic control

The year 1999 saw the first randomized trial comparing HbA1c POC testing with laboratory testing. The study included an immediate assay group (POC testing) and a control group (laboratory testing) of diabetic patients (61). The study concluded that patient glycaemic control significantly improved when POC testing was used compared to laboratory testing. Further studies from then have been done using similar approaches and some using different populations which have similarly concluded that immediate HbA1c results leads to a significant decrease in HbA1c and improved patient glycaemic control (62–65). A study done in a community health centre in Zandspruit, South Africa showed improved patient glycaemic control relating to POC testing of patients. The results of this study revealed a decrease in the mean HbA1c levels of the diabetic patients from the first POC test measurement to the most recent (15month gap/interval). Furthermore, the number of individuals who achieved glycaemic control rose by 125% whereas the number of individuals

with poor glycaemic control declined by half (66). Another study by Pillay et al. revealed that patients who had POC testing at baseline had a substantially better glycaemic control after 3 months (67).

1.11.2 Timely diagnosis

A major limitation of conventional HbA1c laboratory testing is the delay in receiving results, which are usually not available during the patient's clinical visit due to the time needed for processing and reporting. This delay requires healthcare providers to communicate test results and any necessary treatment adjustments later, potentially delaying therapy changes and negatively impacting patient adherence to treatment plans (68). Furthermore, pre-visit HbA1c testing, which might take place a few days before the clinic appointment, causes additional inconveniences and costs for patients because it requires more time and expenses associated with follow-up appointments. It is also typical for patients to arrive at their appointments without having completed the required laboratory tests (69). In contrast, POC HbA1c testing is intended to provide results quickly, usually within 3 to 15 minutes. It eliminates the need for additional visits to draw blood prior to consultations and having to communicate the results on another visit (**Figure 1.4**). This testing approach can be especially beneficial for underserved populations who have limited access to healthcare, as it improves their ability to obtain timely diagnoses and effective management of diabetes (70).

A research study done by a practising pharmacist in the United States revealed that POC HbA1c testing effectively and efficiently facilitated the timely identification of pre-diabetes (71). Another study done in a dental setting found that POC testing may be a possible technique for identifying of individuals with probable type 2 diabetes (72). Furthermore, a study done to assess the performance of POC HbA1c testing in diagnosing undiagnosed diabetes in a resource-limited population in China, suggested that POC HbA1c testing can present great capacity for diagnosing diabetes (73).

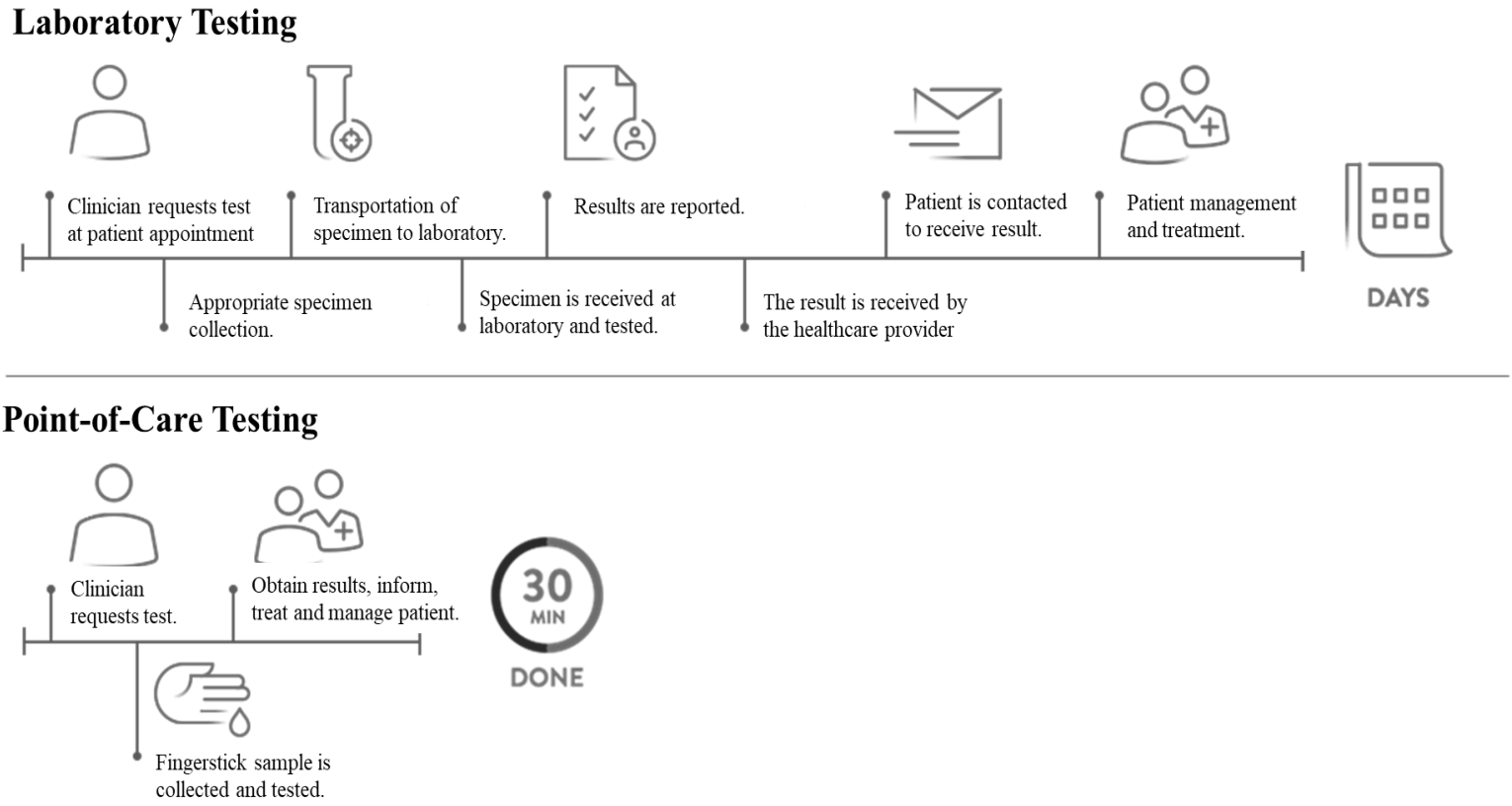


Figure 1.4: A comparative analysis of the time costs associated with POC testing versus laboratory testing**.** Adapted from (74).

1.11.3 Convenient blood collection method

Laboratory testing usually requires a venous blood sample taken by a trained phlebotomist, but POC HbA1c testing simply requires a single drop of blood acquired via fingerstick. This approach is less intrusive and can be used in situations where a phlebotomist is unavailable. Additionally, teaching workers to do fingerstick blood collection is easier than training for venipuncture. Furthermore, with POC testing, the capillary blood samples are measured immediately, whereas venous samples must be moved to a disposable cartridge or suitable material for testing, typically handled by a technician in a laboratory setting utilizing a pipette. According to studies, most patients prefer fingerstick collection over venipuncture, even if having to do it more than once (for multiple POC tests) (75,76).

1.12 Limitations for POC for diagnosis

1.12.1 POC HbA1c testing may be less accurate than laboratory testing

POC HbA1c analysers generally, have poorer analytical performance than laboratory-based tests resulting in significant analytical coefficient of variation (CVa) and bias. Several studies have assessed the performance of POC HbA1c measurement methods in research laboratories, comparing their results to those obtained using authorized laboratory tests. In general, a few POC HbA1c analysers performed similarly to specific laboratory techniques in a study setting, showing high precision and accuracy. However, many of these analysers showed subpar performance (77,78). Criel, Jonckheere and Langlois (77) found that one out of three POC HbA1c, while Lenters-Westra and Slingerland (78) found that three out of seven POC HbA1c methods had failed to meet widely accepted analytical performance standards.

A meta-analysis and systematic review published in 2017 looked at 61 studies that compared 13 POC HbA1c analysers against laboratory assays. Each of the 13 devices had some level of bias, with mean biases ranging from −0.96 to 0.67. Nine of the analysers yielded negative mean bias which was significant for three of the analysers. This indicates that lower HbA1c results were observed compared to laboratory measurements. Overall, the precision of these analysers was poor, as indicated by CVs above 2% at low HbA1c levels, with the exception of one device (79).

While POC analysers offer certain advantages in specific settings, they must meet the same standards as laboratory-based HbA1c methods, as both serve the same purpose. Therefore, careful selection and evaluation of POC devices is essential (**Table 1.3**) (77).

Table 1.3: Overall advantages and disadvantages of POC testing

Advantages	Disadvantages
• Rapid: result accessible during the patient's healthcare visit. • Only one visit needed: test done at location where patient receives care. • Convenient: capillary blood sample. • Has the ability to reach resource-limited populations. • Improved patient outcomes and overall care. • Simple to use equipment.	• Testing costs potentially higher than those for laboratory measurement. • Maintenance of Quality Control (QA) and Quality Assurance (QA) is challenging. • Accuracy concerns.

1.12.2 Tendency for variability between different lots in POC HbA1c testing

The laboratory's ability to consistently deliver reliable results over time is limited by lot-to-lot variation (LTLV), which impacts calibrators and reagents. Clinically significant LTLV has been shown to influence patient management decisions. For instance, a study by Thaler et al. demonstrated that a change in the reagent lot number for an HbA1c assay led to an average increase of 0.5% in HbA1c levels, potentially causing more patients to be misdiagnosed with diabetes (80). Lot variation in reagents for POC testing is a recognized challenge, and the Clinical and Laboratory Standards Institute (CLSI) generally recommends validating new reagent lots (81,82). POC testing may be particularly vulnerable to LTLV due to the more frequent changes in reagent lots that occur in this setting. One study by Lenters-Westra and Slingerland noted that they frequently found significant differences among reagent lots for the HbA1c POC tests they examined (83). Similarly, another study by Lenters-Westra and Slingerland evaluating performance of HbA1c POC tests reported that the results of some of the tests were lot number-dependent (84).

1.13 Landscape of HbA1c POC technologies

There are two types of POC analysers used to measure HbA1c, handheld meters and benchtop instruments (85). Both types use strips or cartridges containing all essential reagents

for the assay (51). Certain analysers can be calibrated by the user using calibration cartridges supplied by the manufacturer, whereas others are calibrated by the manufacturer and do not need human interaction (86). Novel HbA1c devices use capillary blood obtained via fingerstick, as opposed to traditional venous blood collection, with some instruments offering both collection methods. These POC analysers can provide results within minutes and are designed for ease of use; however, the intricacy of the testing procedure may vary among instruments (86).

Of the nearly 330 HbA1c techniques that have obtained NGSP certification, more than 80 are designated as POC. The NGSP certifies POC HbA1c analysers according to the same standards as techniques used in authorized laboratories (87). The Foundation for Innovative New Diagnostics (FIND), a global diagnostics alliance focused on diagnostics, has compiled an overview for in vitro diagnostic (IVD) POC analysers used in HbA1c testing. This initiative is designed to help healthcare practitioners choose suitable analysers for diagnosing and managing diabetes in primary healthcare, particularly in low- and middle-income countries (LMICs) (88). **Appendix 1** has a summary table for several of these analysers.

1.13.1 The LumiraDx POC analyser

The LumiraDx Platform (LumiraDx UK Ltd, London, United Kingdom (UK)) is a multipurpose POC medical device for in vitro diagnostic testing. It comprises a portable benchtop LumiraDx instrument and a LumiraDx test strip, with results stored on-board and accessible through a connectivity solution. The LumiraDx offers a range of diagnostic assays for various conditions, including infectious diseases, cardiovascular diseases, coagulation disorders, and diabetes. The supplier is also focused on expanding the platform's molecular diagnostic capabilities by developing an innovative and rapid POC molecular TB test. This broad range of tests is made possible by combining various technologies—such as immunoassays, molecular assays, enzyme assays, and metabolic assays—for different specimen types, including nasal samples and blood samples, all within a single device that uses multiple assay-specific test strips. The NGSP-certified LumiraDx HbA1c test measures HbA1c in whole blood capillary or venous samples by using the fluorescence-based immunoassay principle (89,90). The test was created to evaluate long-term glycaemic control in diabetics and to aid in screening for persons at risk of developing the condition. The LumiraDx platform is an excellent tool for integrating many approaches into a single POC

platform with a unified workflow. This method may improve testing efficiency and reduce costs.

The LumiraDx HbA1c test employs a fluorescence microfluidic immunoassay to assess HbA1c levels in human whole blood. The test strip contains microchannels coated with antibodies that specifically capture the HbA1c molecule. As the blood sample passes through these channels, the antibodies bind to the HbA1c molecules (91–93). Once captured, a secondary antibody attached to a fluorescent label is introduced, binding to the HbA1c molecule to form a sandwich complex (91,94). The fluorescence emitted by the labelled secondary antibodies is detected using a fluorescence detection system, and the intensity of this fluorescence, measured within the Test Strip's designated area, is directly related to the HbA1c concentration.

1.13.2 The DCA Vantage Analyser

The DCA Vantage® Analyzer (Siemens Healthineers, Erlangen, Germany) uses the test principle of inhibition of latex agglutination to measure HbA1c. A synthetic polymer with numerous copies of the immunoreactive part of HbA1c acts as an agglutinator to trigger the agglutination of latex particles covered with HbA1c-specific mouse monoclonal antibodies. This agglutination reaction leads to an amplified scattering of light, which is measured as an increase in absorbance at 531 nm. However, when HbA1c is present in the whole blood specimen, it contends for the restricted number of antibody-latex binding sites, triggering an inhibition of the agglutination process. This decreased agglutination results in a reduced scattering of light, which is quantified as a decline in absorbance at 531 nm. The concentration of HbA1c is then determined using a calibration curve of absorbance versus HbA1c concentration (95).

1.14 Aim and Objectives of the study

The aim of this study was to assess the analytical performance of the LumiraDx POC analyser in both laboratory and clinical environments for measuring HbA1c levels in the monitoring and diagnosis of diabetes. Additionally, the study aimed to determine if the results are affected by test kit lot-to-lot variation.

Specific objectives included:

1. To determine the accuracy, precision, and linearity of the LumiraDx platform in the measurement of HbA1c.
2. To assess if and how lot-to-lot variation affects patient results.
3. To evaluate the clinical performance of the LumiraDx HbA1c assay in comparison to another point-of-care HbA1c testing method and the laboratory standard HbA1c testing method.
4. To assess whether HIV or TB influences the concordance between the different testing methods.

1.15 Justification of the study

The need for faster diabetes diagnosis is critical, as it facilitates timely intervention and improvement of patient glycaemic control, ultimately reducing the risk of diabetes-related complications (96). Utilizing POC testing presents an opportunity to enhance the efficiency of diabetes diagnosis and management (97). However, given the limitations associated with POCs such as variability in analytical performance and LTLV, identifying reliable POCs is vital. It is important to evaluate POC analysers based on their performance in real-world settings, even if they are NGSP-certified. The NGSP certification reflects performance under optimal conditions using a single reagent lot, which does not translate to clinical environments where multiple lots are used concurrently, leading to inconsistent results (81). Consequently, NGSP certification does not guarantee accuracy, as some certified analysers may fail performance evaluations in practical studies (83).

The following evaluation of the LumiraDx HbA1c test will help inform its application in monitoring glycaemic control in diabetic patients, screening at-risk individuals, and diagnosing diabetes in the South African healthcare context. To date, no published studies have specifically evaluated the LumiraDx analyser for HbA1c measurement.

This dissertation is organized into several key sections. After this introductory section, Chapter 2 gives a full overview of the methods employed in this study, highlighting the research design and the specific procedures carried out during Phase 1 (Laboratory Evaluation) and Phase 2 (Clinical Evaluation). Chapter 3 presents the results of the analysis, including key findings represented in tables and graphs. Finally, Chapter 4 offers a discussion of these results, exploring their implications, limits, and possible directions for future research.

Chapter 2 Methodology

2.1 Study design

The performance evaluation of the LumiraDx for the measurement of HbA1c was a comparative observational study conducted in two distinct phases: an initial laboratory evaluation followed by a clinical evaluation (**Figure 2.1**). The laboratory evaluation phase aimed to establish the precision, linearity and accuracy of the test against the standard reference laboratory method. LTLV was performed alongside the laboratory validation. This foundational evaluation was critical for determining the test's reliability in controlled conditions. Subsequently, the clinical evaluation phase sought to assess the interchangeability of the LumiraDx HbA1c test with established reference laboratory method (Bio-Rad Variant™ II Haemoglobin Testing System and the Premier Hb9210™) and POC (DCA Vantage® Analyzer) HbA1c test in a real-world setting involving patients. This study was performed using CLSI protocols (EP-15, EP-06, EP-09 and EP-26) as guidelines. Many regulatory bodies and accreditation agencies, recognize and recommend CLSI guidelines for such studies, making them a preferred choice (98).

The following section of the dissertation provides details of the laboratory evaluation phase and the clinical evaluation phase which includes participant recruitment, sample collection protocols, analysers used, and statistical analyses employed throughout the study.

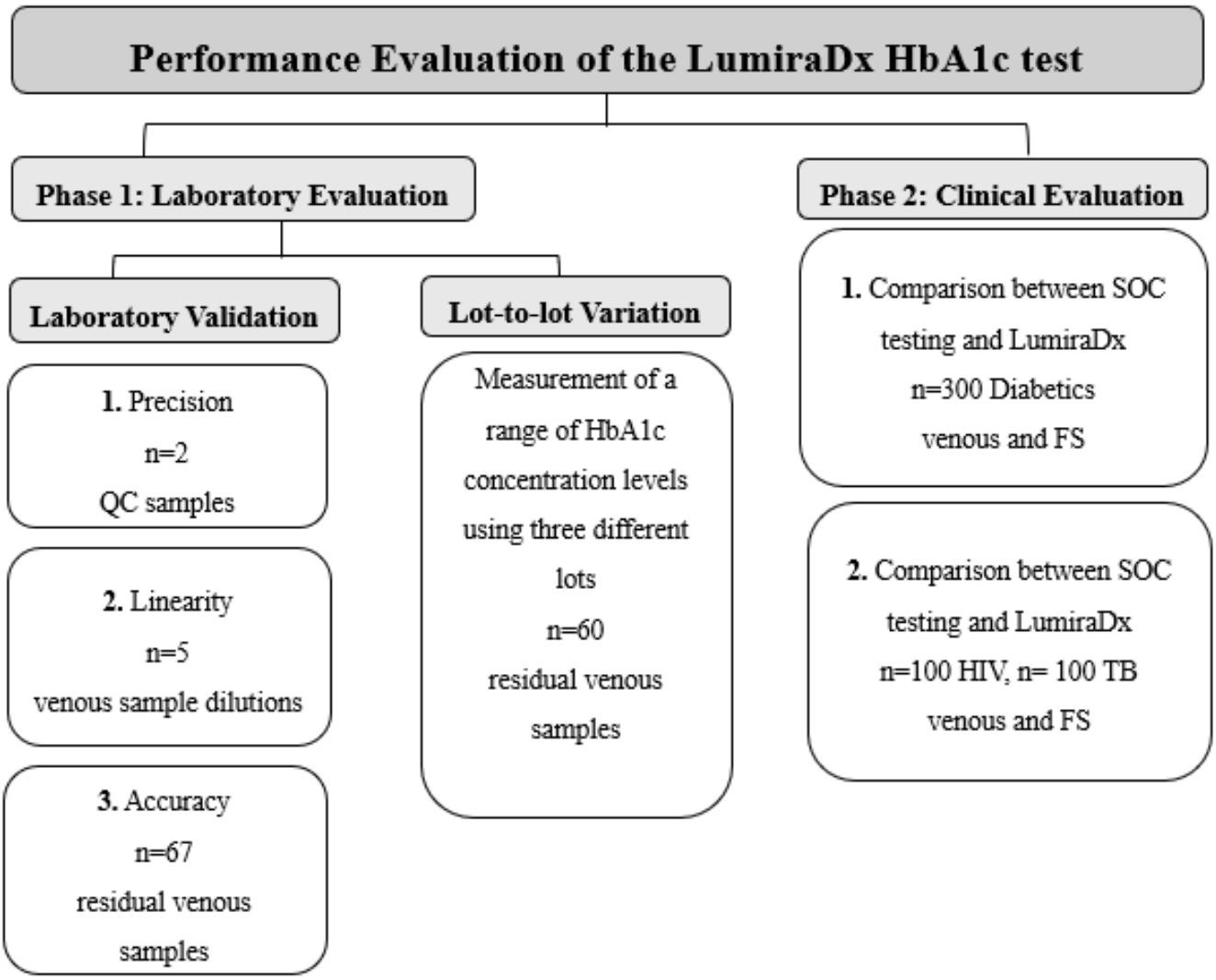


Figure 2.1: An overview of the research study workflow. QC- quality control, SOC- standard of care, FS- fingerstick.

Phase 1: Laboratory evaluation

The laboratory validation of the LumiraDx HbA1c test was conducted in the National Health Laboratory Services (NHLS) Chemical Pathology Laboratory at the University of the Witwatersrand, Johannesburg. The precision, linearity and accuracy of the LumiraDx HbA1c test was evaluated using the CLSI EP-15, 6 and 9 guidelines as references. These were the selected parameters as they are fundamental indicators of a method's performance. They afford a thorough assessment of the method's dependability and appropriateness for its intended purpose (99). To assess these parameters, a combination of LumiraDx HbA1c quality controls and residual clinical venous samples from the NHLS were utilized.

2.2 Precision

Precision was assessed in this validation of LumiraDx because it is critical to ensure that the analyser can provide readings that are reproducible and reliable. To ensure this, two assessments of precision were conducted. The first assessment focused on intra- and inter-run variability (within-run and between-run), while the second assessed intra- and inter-instrument variability.

To determine run variability, QC samples (a low (5.8%) and a high (10.6%)) from LumiraDx were tested in five replicates each. This was done daily over the course of five consecutive days. The testing was carried out using a single instrument and a single operator to minimize variability from factors such as temperature, humidity, and operational settings while ensuring consistent technique and reducing variability from differing methods or expertise among operators (100,101).

To determine instrument variability, five residual venous samples (collected within the past 24hrs) stored at temperature of 2-8℃ were obtained from the laboratory. These samples varied in HbA1c concentration. They were each tested in triplicates across three different LumiraDx instruments within a single day. A single operator was used.

2.3 Linearity

Testing for linearity of laboratory results involves evaluating whether the test method provides results that are equivalent to the quantity of HbA1c over the expected range of values. To determine the capability of the LumiraDx HbA1c test to give results that are directly proportional to the quantity of HbA1c being measured, six samples with known HbA1c concentrations spanning the measuring range of the test (4.0% to 14%) were tested on the LumiraDx. These samples were prepared by combining high and low HbA1c residual venous samples in different ratios (**Table 2.1***)*.

Table 2.1:The percentages and volumes of low and high HbA1c samples in a mixture of 1000 µl (1 ml).

Sample	Percentage of [HIGH] sample (Volume)	Percentage of [LOW] sample (Volume)
1 [LOW]	0	100% (1000 µl)
2	10% (100 µl)	90% (900 µl)
3	25% (250 µl)	75% (750 µl)
4	50% (500 µl)	50% (500 µl)
5	75% (750 µl)	25% (250 µl)
6 [HIGH]	100% (1000 µl)	0

2.4 Accuracy

To determine the degree to which the measured values of HbA1c corresponds to the actual value of HbA1c in the sample, comparison between the results of the LumiraDx HbA1c test and an NGSP-certified laboratory method was performed. The HbA1c levels of 67 residual ethylenediaminetetraacetic acid (EDTA) venous samples were measured on the LumiraDx and on the laboratory SOC (Bio-Rad Variant™ II Haemoglobin Testing System). The results were used to determine agreement and bias in HbA1c measurement of the two methods. An overall analysis was performed from which the results were stratified to include two sub-groups: non-diabetic (<6.5%) and diabetic (≥6.5%). These subgroups were included to assess whether performance was different for non-diabetics and diabetics.

2.5 Lot-to-lot variation.

To assess the variability in measurements when using different reagent lots, a total of 60 residual venous blood samples were measured using three LumiraDx HbA1c kits with distinct lot numbers (6001152, 6001151 and 6001128). Each sample was tested on a single LumiraDx instrument three times using each of the three lots on the same day. This was done after the sample had been tested on the reference laboratory method once. The set of results from each lot was compared separately to those of the reference laboratory method to determine if results were different and if the lots compared differently to the reference method and to each other.

2.6 Bridging study for the changeover of strips

While conducting the study, the manufacturers of the LumiraDx HbA1c assay improved the assay, leading to changes in the kit and the discontinuation of the old test strips. To continue the study, we had to switch to the new test strips. To validate this transition, we conducted a bridging study comparing the old assay which took 7 minutes to complete to the new assay that provided results in 5 minutes. The intention of this bridging study was to ensure that the use of the new test strips would not impact the study's findings or effectiveness. Twenty-four venous samples were tested both using the old assay and the new assay and results were compared to determine if they are interchangeable.

2.7 HbA1c analysers used in Phase 1

The HbA1c measurement instruments used during phase 1 of the study include the analyser of interest in this study, the LumiraDx, and the SOC instrument used for routine testing at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) hospital NHLS laboratory which was the Bio-Rad Variant™ II Haemoglobin Testing System (Bio-Rad laboratories Inc., California, United States).

2.7.1 The LumiraDx HbA1c testing procedure

The instrument underwent a process of onboard quality control checks before it was ready for testing. From then, the user was required to login with their credentials. When ready for testing, the foil pouch of the HbA1c Test Strip was opened just before use, and the Test Strip was inserted into the LumiraDx instrument. The instrument indicated when it was ready for the sample to be added which occurs once the Test Strip was warmed up. The Test Strip was used immediately after being removed from the foil pouch, and it was not used if the foil pouch showed any noticeable evidence of damage, such as tears or holes (**Figure 2.2**).

To test a sample, 15µl was pipetted from the EDTA venous blood using a calibrated pipette and then placed inside the tube containing lysis buffer and the transfer loop placed inside and rolled at least 10 times between the thumb and index finger to lyse the blood. The opening of the buffer tube was then directed away from the face, and the transfer loop stem was snapped at the break point by pressing it against the inside of the buffer tube *(***Figure 2.2A**).

The upper part of the stem of the transfer loop was disposed of, while the loop and the remaining stem stayed in the tube with the hemolysate sample. The dropper lid was then

placed on the tube to close it. The sample was tested as promptly as possible; however, it was noted that the sample remained stable at room temperature in the buffer solution for up to 5 hours. If, for any reason, the sample could not be tested immediately, the tube was placed in a holder until the test could be conducted, with a maximum delay of one hour.

The hemolysate sample was applied by positioning the dropper of the lysis device and squeezing it to generate a single, large drop (20µl) on the sample application area of the inserted Test Strip. The hemolysate drop was allowed to touch the Sample Application Area of the Test Strip. The sample was then taken up into the Test Strip by capillary effect, and once it was detected, the instrument alerted, and a validation message appeared on the screen. The Lysis Device, lancet/pipette tip and Test Strip were disposed of in the appropriate waste bin (sharps container and biohazardous waste bin).

For Quality Control (QC) testing, the LumiraDx QC kit was used. This contains two vials with different levels of HbA1c with ranges assigned based on the LumiraDx HbA1c Test Strip. These vials contain liquid human whole blood with stabilisers and are supplied ready to use. When transferring the QC solution from the vial to the Test Strip, a calibrated laboratory pipette capable of accurately dispensing 20µl of solution was used. The QC solution was applied directly from the vial to the sample application area of the Test Strip by the pipette being held over sample application area of the Test Strip. Then instrument testing proceeded. The pipette tip was disposed in the appropriate clinical waste bin and the QC vials were closed properly ensuring that the rubber stopper was replaced immediately. No lysis buffer step was required to run a LumiraDx HbA1c QC test. The QC samples were tested during the precision analyses and once when a new lot of the HbA1c kit was used during the laboratory phase.

While sample testing was in progress, the door of the instrument was kept closed. The test progress was indicated on the touchscreen. The results appeared on the instrument touchscreen within approximately 5 or 7minutes of adding the sample and running the test. For QC testing, the instrument screen displayed the test result, the acceptable range, and a PASS or FAIL status. While for patient testing, the results were displayed as test value in mmol/mol and % HbA1c units of measurement (**Figure 2.2A**). If a test had to be repeated due to instrument error or test failure, a new Test Strip, pipette tip and Lysis Device (the buffer tube with the dropper lid) was used. Additionally for patient testing using capillary blood, a new lancet and a different finger was used.



Figure 2.2: The LumiraDx test procedure and HbA1c Test Strip **(A)** The LumiraDx test procedure for HbA1c measurement from a capillary blood sample, displaying the testing duration. 1) Test Strip is placed into instrument, 2) Capillary sample collection using transfer loop, 3) Sample placed into lysis buffer and lysed by rolling transfer loop between fingers, 4) A large drop of hemolysate (~20µl) is added over the sample application area of the inserted Test Strip and 5) Sample is processed by instrument and results are displayed on the touchscreen. **(B)** The LumiraDx HbA1c Test Strip showing the microchannels and the sample application area.

2.7.2 The Bio-Rad Variant II Haemoglobin A1c testing system

The Bio-Rad Variant™ II Haemoglobin Testing System (Bio-Rad laboratories Inc., California, United States) is an automated laboratory analyser for the quantification of HbA1c in whole blood. The system eliminates the need for sample preparation and minimizes the operator's involvement. Testing on this instrument was done by a designated laboratory technician at the CMJAH NHLS chemical pathology laboratory.

To initiate the system, several systematic steps were performed. First, the instrument was controlled via a connected computer, where it was powered on and allowed to undergo a warm-up phase. Following this, the flow rate was set to 1.7 ml/min, which took approximately 10 minutes. After establishing the flow rate, the system was flushed, and the sampler was washed. To ensure the absence of air in the system, deionized water was injected into the system, and the absence of any air was indicated by a squeaking sound. Proper functionality of both the sampling station and chromatographic station was ensured, and QC was run.

Once the system was prepared, patient samples were run. To run patient samples, the labelled and barcoded (sample identifier) EDTA tubes containing venous blood were placed inside racks and placed on the sampling station of the instrument. To commence sample analysis, the computer interface was used to select "return to ready," followed by "run," and then "start." The sampling station then moves the racks into position and scans the barcode. Thereafter, the system extracted the samples from the sealed tube, diluted and injected the sample into the chromatographic station for analysis. The Clinical Data Management Software (CDM) from Bio-Rad handled the results. It was linked to the laboratory information system for immediate data transfer and enhanced data handling. Results are displayed in a chromatogram with the % HbA1c result.

Phase 2: Clinical evaluation

2.8 Participant Recruitment

This section outlines the sampling strategy, patient participation, participant eligibility criteria and the ethics for phase 2 of the study.

2.8.1 Sampling strategy

The targeted sample sizes shown in the flow chart above **(Figure 2.1**) were decided and informed several key factors, including the CLSI EP-09 guideline, which recommends a minimum of 40 samples. Additionally, the decision was informed by sample size calculation (**Equation 2.1**) and insights from previous clinical studies such as Nathan et al., which evaluated the performance of a POC analyser using venous blood from 300 patients and fingerstick samples from 402 patients (102–104).

$$n = \frac{z^2 \times p \times (1-p)}{e^2}$$

$$= \frac{1.96^2 \times 0.5 \times (1-0.5)}{0.05^2}$$

$$= 385$$

Equation 2.1: Sample size calculation using Cochran's Formula. n- sample size, z- Z-score, p – estimated population proportion, e- margin of error.

2.8.2 Study site and population.

The clinical evaluation phase of the study included participants tested at two public healthcare facilities in Johannesburg, Gauteng (CMJAH and Hillbrow Community Health Centre (Hillbrow clinic)). Participants were enrolled from the Diabetes clinic and the Infectious Diseases clinics (HIV/TB) run at these healthcare facilities.

2.8.3 Patient participation

Participants were provided with an information sheet to review, along with a brief verbal explanation of the study and the requirements for participation (**Appendix 2**). Those who volunteered to take part were asked to sign a consent form and answer a few optional questions, time permitting (**Appendix 3** and **Appendix 4**). The data collected included their age, sex, Body Mass Index (BMI), height, weight, and information on the presence or absence of any known blood disorders, as well as their HIV and TB status.

2.8.4 Eligibility criteria

The inclusion criteria were as follows:

i) Participants of age ≥18 years as this is the legal age in which a person can give consent.
ii) Visiting the diabetes or infectious diseases clinic for assessment of condition, diagnosis or monitoring of HbA1c levels as these patients are easily more accessible.
iii) Participants willing and able to sign written consent form as well as fill in questionnaire (**Appendix 3** and **Appendix 4**) after having read the information sheet (**Appendix 2**).

The exclusion criteria were as follows:

i) Patients <18 years (children) as they are considered a vulnerable population group.
ii) HbA1c levels <4.0 and >14% as this is outside of the measurement range of the LumiraDx instrument which is 4% to 14%.
iii) Patients with conditions known to preclude accurate HbA1c measurement such as pregnancy and hemoglobinopathies as this would affect the reliability of the study.

2.8.5 Ethics

This research was performed according to the Helsinki Declaration. The Wits Human Research Ethics Committee (Medical) approved this study (Certificate number: M230813 MED23-07-062 (**Appendix 5**)). All participants involved in the study provided both written and verbal agreement after being thoroughly informed about the study.

2.9 Sample collection

Fingerstick capillary blood samples were drawn from all participants (n=501) and venous blood samples were drawn from 100/300 participants from the diabetic clinic and 200 participants from the infectious disease clinics (**Figure 2.1**).

To ensure the safety and accuracy of the samples, their collection, handling, testing, and storage were carried out in accordance with standard laboratory practices and the manufacturer's guidelines (105). Participants were kept updated and made comfortable throughout the procedure.

Before starting the blood collection, hands were thoroughly washed, and gloves were worn to maintain an aseptic technique. The required materials for capillary blood collection included alcohol swabs, cotton balls, and lancets. For venous blood collection, the materials included EDTA blood collection tubes, a tourniquet, alcohol swabs, a sterile needle, vacuum device, gauze or cotton balls, and an adhesive bandage or tape. All necessary materials were gathered and ensured to be within easy reach before beginning the procedure.

For capillary blood collection, the procedure was as follows. The fingertip was cleaned with an alcohol swab and allowed to air dry to reduce discomfort and contamination. The skin was then quickly punctured with a lancet, avoiding squeezing the finger to prevent fluid contamination. A blood droplet formed, and gentle massaging around the site encouraged blood flow without squeezing, if necessary. The appropriate collection tool (transfer loop for LumiraDx (~15µl) or glass capillary for DCA Vantage was used to collect the blood. Afterward, a cotton ball was applied to the site with gentle pressure, and the lancet was immediately disposed of in a biohazard container (106). Samples were tested immediately or with regards to the LumiraDx, immediately or within 5hours of collection being kept in lysis buffer.

Venous blood samples were collected by trained phlebotomist according to the acceptable protocol for blood collection (105). The samples were collected into EDTA collection tubes

to prevent blood samples from clotting. The collection tubes were immediately labelled accordingly by placing a barcode sticker from the corresponding blood form and placing both the tube and form in the specimen bag. These were then kept in a styrofoam box containing ice packs to keep samples cold ensuring sample stability until they could be transported to the laboratory for testing. These samples were tested within 24hours of being collected to ensure accurate results.

2.10 HbA1c analysers used in Phase 2

The HbA1c measurement instruments used in phase 2 of the study include the LumiraDx and the SOC instruments used for determination of HbA1c measurement at the CMJAH hospital NHLS laboratory. The SOC instruments included: the DCA Vantage® Analyzer (Siemens Healthineers, Erlangen, Germany), Bio-Rad Variant™ II Haemoglobin Testing System (Bio-Rad laboratories Inc., California, United States) and the Premier Hb9210™ (Trinity Biotech Plc., County Wicklow, Ireland). The testing procedure for the LumiraDx and Bio-Rad Variant II have already been outlined in phase 1 of the study.

2.10.1 The DCA Vantage Analyzer testing procedure

The DCA Vantage® Analyzer (Siemens Healthineers, Erlangen, Germany) is a POC instrument for measuring HbA1c in whole blood. It is a SOC analyser at CMJAH diabetes clinic and testing on this instrument was carried out by a designated nurse at the clinic.

A small blood sample of 1 µl was collected from a finger prick using a glass capillary (within plastic capillary holder) ensuring to avoid air bubbles. The sides of the glass capillary were carefully wiped with a tissue to remove any excess blood, taking care not to touch the end of the glass capillary to maintain blood retention through capillary action. The capillary holder was then placed into a reagent cartridge until the holder gently snapped into place. The reagent cartridge barcode was scanned on the instrument by smoothly sliding it down until a beep indicated a successful scan. The cartridge was then inserted into the cartridge compartment of the instrument until a gentle click was heard or felt **(Figure 2.3)**. The flexible plastic tab was slowly and firmly pulled and removed out of the reagent cartridge, after which the compartment door was closed to initiate the test, which lasted six minutes. During instrument testing, the nurse operating the instrument logged into the analyser using their identifier and password and entered the patient details (which included hospital number, patient name, surname and date of birth). Once the test was completed, the cartridge was

removed by pushing and holding down the button on the right side and discarded in biohazardous waste. The machine displayed the results as percentage HbA1c on the screen and a printed copy.



Figure 2.3: The DCA Vantage test procedure for HbA1c measurement from a capillary blood sample**.** 1) Sample is collected from patient using glass capillary, 2) The sides of the glass capillary were carefully wiped with tissue to remove excess blood, 3) The capillary holder is inserted into reagent cartridge, 4) The reagent cartridge barcode is scanned on the instrument and 5) The cartridge is inserted into the cartridge compartment of the instrument.

2.10.2 Changeover from using Bio-Rad Variant II to the Premier Hb9210

Towards the end of the study, the CMJAH NHLS Laboratory acquired a new instrument for the measurement of HbA1c. As a result, the last 26 samples of the study were measured on the new instrument, the Premier Hb9210™ (Trinity Biotech Plc., County Wicklow, Ireland). The laboratory performed a method validation to ensure the interchangeability of these two methods (**Appendix 8 & 9**).

The Premier Hb9210™ is an automated laboratory analyser for the quantifiable measurement of HbA1c in whole blood. It uses test principle of boronate affinity and high-performance liquid chromatography (HPLC) to measure HbA1c. It separates glycated haemoglobin from non-glycated haemoglobin based on their chemical properties, the analyser then employs HPLC to further separates and quantify the haemoglobin fractions after the initial boronate affinity separation. A spectrophotometer detector then measures the absorbance of the eluates at 413± 2nm to determine the concentration of HbA1c (107). Testing on this instrument was done by a designated laboratory technician at the CMJAH NHLS chemical pathology laboratory. The operations of the system were controlled by a computer, which processed the detector signals, calculated the concentrations of glycated proteins, and generated corresponding reports. The percentage of HbA1c was determined by analysing the peak areas and utilizing a two-point calibration method. This approach ensured accountability to the

"International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)" method and the NGSP certification, for accurate quantification of HbA1c levels.

2.11 Clinical Evaluation

Clinical samples (capillary and venous blood specimens) were tested on the LumiraDx, and the SOC assays used for measuring HbA1c at the CMJAH diabetic clinic and laboratory. Testing was performed after samples had been collected from participants (**Figure 2.4**). The HbA1c measurements of each sample on the LumiraDx was compared to the measurement on the SOC.

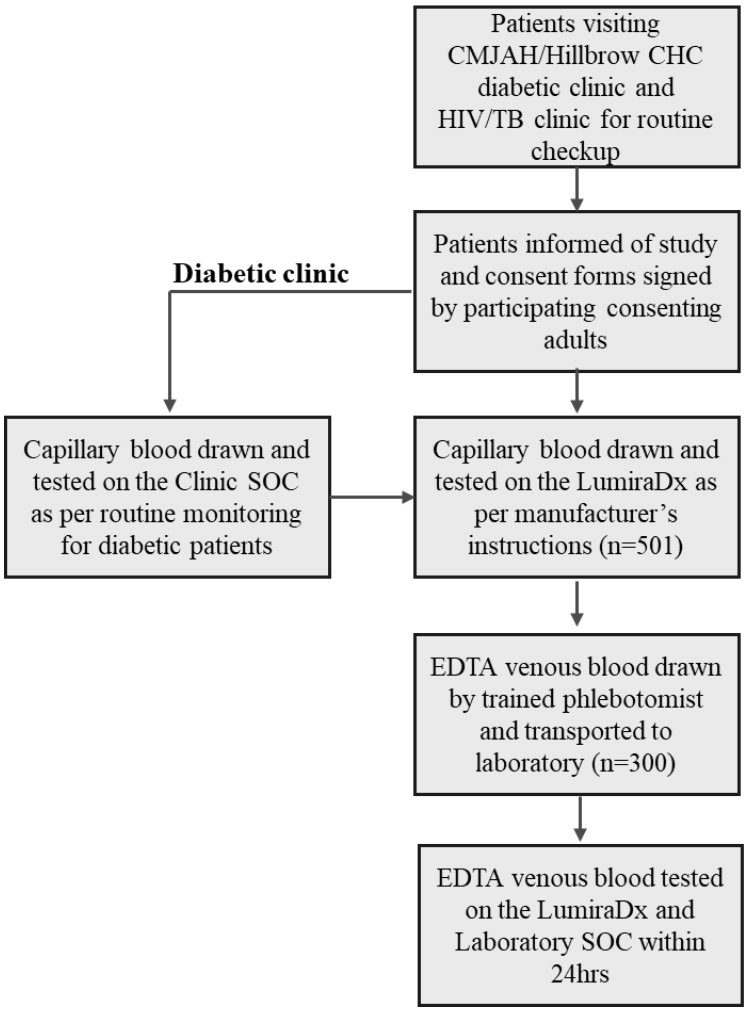


Figure 2.4: Study workflow of the clinical evaluation phase. CMJAH - Charlotte Maxeke Johannesburg Academic Hospital, CHC- Community Health Centre, HIV- Human immunodeficiency virus, TB- Tuberculosis, SOC- standard of care, EDTA- Ethylenediaminetetraacetic acid

2.11.1 Comparison between SOC testing and LumiraDx for participants from the Diabetes clinic

A total of 300 participants were enrolled from the CMJAH Diabetic Clinic. Among them, 100 participants were requested to provide an EDTA venous blood sample alongside a capillary blood sample. Participants were asked to complete a brief questionnaire by answering a few questions. (**Appendix 4**). Participants were tested on the LumiraDx and clinic SOC (DCA Vantage® Analyzer), using capillary samples collected by finger stick. The EDTA venous blood samples collected from the 100 participants, were tested on the LumiraDx and the laboratory SOC.

2.11.2 Comparison between SOC testing and LumiraDx for participants from Infectious Diseases clinics.

A total of 100 participants from the HIV clinic and 100 participants from the TB clinic were enrolled from the CMJAH HIV/TB clinic and the TB clinic at Hillbrow. Participants were requested to give a fingerstick capillary blood sample and a single EDTA venous blood sample. They were also requested to fill out a brief questionnaire. The capillary blood samples were tested on the LumiraDx at the clinic, while the EDTA venous blood samples were tested using both the LumiraDx and the laboratory SOC.

2.12 Data and statistical analysis

All data was recorded on data collection sheets, entered on the REDCap database (The REDCap Consortium, Vanderbilt) and transferred to Microsoft Excel (Microsoft® Excel® for Microsoft 365 MSO (Version 2412 Build 16.0.18324.20092) 64-bit). The statistical analyses were performed using MedCalc® Statistical Software version 23.1.7 (108–110).

For precision, the intra- and inter-variability was compared to the manufacturer's precision claims as well as Westgard (Ricos) criteria and the Clinical Laboratory Improvement Amendments (CLIA) as outlined in **Table 2.2**. The data was analysed by determining the mean, standard deviation (SD) and percentage coefficient of variation (%CV). The mean indicated the average of repeated measurements, while the SD showed value dispersion around the mean. The %CV normalizes SD relative to the mean, therefore it was used as the standard measure of precision and to determine whether precision of the LumiraDx HbA1c test was acceptable.

Table 2.2: Allowable error and precision limits employed in this evaluation (111,112)

Analyte	**Manufacturer Claim**		**Westgard*and CLIA#**				
Glycated Hemoglobin (HbA1c)	**Precision (Mean % CV)**		**Biological Variation**		**Allowable Bias (%)**	**Desirable total Allowable Error (TAE) (%)**	**Minimal total Allowable Error (TAE) (%)**
	Level 1 control	Level 2 control	%median CVi (Lower CI-higher CI)	%median CVg (Lower CI-higher CI)			
	5.8	2.9	1.9*	5.7*	1.5*+	3*	8#

* Westgard Criteria; # CLIA, CVi intra variability, CVg inter-variability, TAE- Total allowable error, CLIA - Clinical Laboratory Improvement Amendments

The degree of linearity was determined by analysing a linearity plot that compared expected HbA1c values with measured HbA1c values. The plot allowed for an assessment of the overall linearity of the test and the quantification of the coefficient of determination (R^2), slope and y-intercept of the regression line. A high value R^2, close to 1, indicated good linearity, whereas a lower R^2 value suggested deviations from linearity. Linearity was deemed acceptable if R^2 value was 0.95 or higher, as this is indicative of a strong linear correlation between the analyte concentration and the method's response (113). The manufacturers claim that linearity was established across the measuring range of 4.0% to 14.0%.

To assess for lot-to-lot variation, we compared the results for each lot with the results from the Laboratory SOC by Bland-Altman (mean difference), Concordance Correlation Coefficient (Pc) (**Table 2.3**), mean bias to determine whether each lot was within clinically acceptable bounds and thus if they can be used for testing. We then further compared the three lots with each other by determining the mean, SD and CV for each lot and analysing these for differences to assess the consistency between the different lots. A Kruskal-Wallis test was used to assess potential differences between the lots with significance level set at 0.05 (114). Passing-Bablok plots were included for visual inspection of data(115).

To determine the accuracy and interchangeability of the LumiraDx with the SOC tests used, the following analysis were performed: Bland-Altman analysis, Passing-Bablok regression, concordance correlation analysis, percentage similarity (116) and analytical error.

The mean difference (bias), SD of differences and limits of agreement (LoA) were determined from a Bland-Altman analysis (117). These metrics helped in understanding systematic errors, variability of differences, and the overall interchangeability of the methods (118,119). Bland-Altman and Passing-Bablok scatter plots were included for visual inspection of the agreement which allowed for identification of any patterns or trends in the differences between the method (115). The extent to which data sets line up along the 45° line across the origin (as determined by the Pc) was ascertained using concordance correlation, which also yielded an overall measure of accuracy (Cb, bias correction factor) and precision (P, Pearson) (120). The percentage similarity was computed to assess the level of agreement between the methods by measuring the proportion of data points where the results from both methods were closely aligned. The total analytical error was determined as a measure of the overall error associated with the measurement between the LumiraDx HbA1c test and SOC reference method.

Thereafter performing overall agreement analysis between LumiraDx HbA1c test and Laboratory SOC testing used as reference method, the analysis was then stratified by HIV status and TB status to identify any discrepancies in the agreement. The subgroups were: 1) HIV-positive, 2) TB-positive and 3) HIV and TB co-infected. The statistical results of the subgroups were compared to those of the overall data to determine whether there were significant differences in the agreement as observed by concordance correlation, percentage similarity, mean bias and analytical total error (**Table 2.3**).

To assess the use of the LumiraDx HbA1c test in diagnosis of diabetes, A sub-analysis was done comparing results of the LumiraDx with the Laboratory SOC at the HbA1c concentration levels of 6.3%, 6.4% and 6.5%. Bland-Altman, percentage similarity and total analytical error were used for this assessment (**Table 2.3**)

Table 2.3: The acceptability criteria set for accuracy assessment and method comparison.

Bland-Altman	Mean difference and SD of differences within ±0.5% and Limits of Agreement (LoA) within ±1.0%.
Passing-Bablok	Slope should be close to 1, intercept should be close to zero. The 95% CIs for the slope and intercept on should include 1.00 and 0.00, respectively.
Concordance correlation	Pc < 0.9: Poor, Pc = 0.9–0.95: Moderate, Pc = 0.95–0.99: Substantial, Pc > 0.99: Almost perfect.
Percentage similarity	95% to 105%, within ±5%
Allowable Total Error	Optimal: 3% Minimal: 8%

Chapter 3 Results

Phase 1: Laboratory Evaluation

3.1 Precision (inter- and intra-variability)

The instrument variability assessed by testing five venous samples on three LumiraDx instruments was within the manufacture's claim as well as the Westgard acceptance criteria apart from Sample 5 which had inter-instrument variability (CVg) of 9.42% which is outside the acceptable range (**Table 3.1**). The variability of both high and low controls was within the manufacturer's claims, as well as the acceptance criteria set by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and Westgard. Minimal variability was eminent for both the low control (expected range 5.5-6.6) and for the high control (expected range 9.6-11.8) (**Table 3.2**). There were no reported results outside the expected ranges (**Table 3.2**). The analytical errors for control 1 and control 2 were acceptable with control 1 being above optimal allowable error but within the minimal allowable error (**Table 3.3).**

Table 3.1: Statistical analysis of data to determine the instrument variability of the LumiraDx HbA1c test using residual venous blood samples.

		HbA1c sample				
		Sample 1 (4.4 – 4.6%)	Sample 2 (5.8 – 6.2%)	Sample 3 (6.1 – 6.4%)	Sample 4 (6.9 – 7.3%)	Sample 5 (10.4 – 13.6%)
Intra-instrument variability	Mean	4.37	5.97	6.27	7	10.60
	SD	0.06	0.06	0.06	0.1	0.17
	%CV	1.32	0.97	0.92	1.43	1.63
Inter-instrument variability	Mean	4.38	5.98	6.23	7.07	11.73
	SD	0.08	0.11	0.07	0.07	1.13
	%CV	1.91	1.80	1.15	0.94	9.42
Manufacture's claim	CVi			2.9		
	CVg			2		
Westgard criteria	CVi			1.9		
	CVg			5.7		
Acceptable (Yes or No)		Yes	Yes	Yes	Yes	No

CVi intra variability, CVg inter-variability, SD- Standard deviation, %CV- percentage coefficient of variation

Table 3.2: Statistical analysis of data to determine the precision of the HbA1c test using LumiraDx controls.

Manufacturer claims						Westgard acceptable criteria		Within-run variability (CVi)						Between-run variability (CVg)					
Control 1			Control 2			CVi	CVg	Control 1			Control 2			Control 1			Control 2		
Mean	SD	CV%	Mean	SD	CV%			Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%
5.8	0.12	2.1	10.6	0.14	1.3	1.9	5.7	5.94	0.05	0.92	10.54	0.05	0.51	5.93	0.06	1.07	10.55	0.07	0.66

CVi - intra variability, CVg - inter-variability, SD - Standard deviation, %CV - percentage coefficient of variation

Table 3.3: The calculated bias and analytical error for the QC samples

%Bias		Total Analytical error (TAE)		Westgard*and CLIA# acceptable criteria	
Control 1	Control 2	Control 1	Control 2	Optimal Allowable Total Error (ATE)	Minimal Allowable Total Error (ATE)
2.24	0.57	4.01	1.41	3%*	8%#

* Westgard, # CLIA, TAE -Total analytical error, CLIA - Clinical Laboratory Improvement Amendments

3.2 Assessment of Linearity

The linearity of the LumiraDx HbA1c test was found to be excellent, as indicated by the high R^2 value of 0.99. No data points deviated significantly from the expected linear trend, suggesting that the measured HbA1c values closely aligned with the expected HbA1c values across the range of concentrations measured (**Figure 3.1**). The samples tested ranged from 4.6 to 11.5% HbA1c. The curve revealed slope=0.868 and y-intercept=0.483.

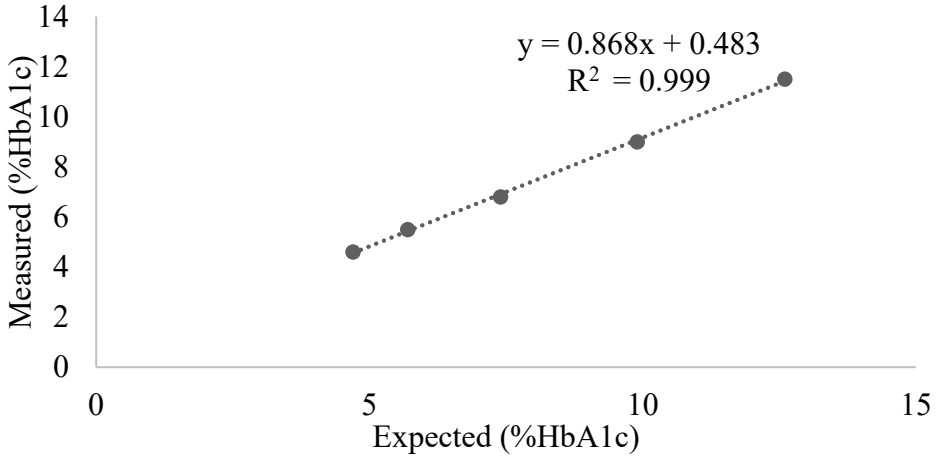


Figure 3.1: Linearity curve for assessment of HbA1c measurement performed on LumiraDx using patient venous samples (n=5). The y-axis represents the measured HbA1c concentration (%) while the x-axis represents the expected HbA1c concentration (%) for each of the samples. The dotted line shows the line of best fit. The formula for the line is shown along with the R^2 value.

3.3 Assessment of Accuracy

Method comparison was done on a total of 67 paired results of samples tested in the laboratory evaluation. HbA1c values measured, ranged from 4.2% to 12.5% with median of 6.5% and interquartile range (IQR) of 1.05. **Table 3.4** summarises the results from data collected which includes concordance correlation, Bland–Altman, percentage similarity analysis and Analytica Total Error (ATE). Analysis was done for the overall data and two sub-groups: non-diabetic (<6.5%) and diabetic (≥6.5%). All categories were within allowable error. Notably, the last category had a poor agreement score.

Table 3.4: Statistical analysis for method comparison presented for the overall dataset in the laboratory evaluation for accuracy and clinically relevant sub-groups.

		Category		
		Overall (Venous) n = 67	Non-diabetic (<6.5%) n = 27	Diabetic (≥6.5%) n = 40
Concordance Correlation	Correlation coefficient (95%CI)	0,98 (0,97 to 0,99)	0,95 (0,89 to 0,98)	0,97 (0,95 to 0,99)
	Pearson ρ (precision)	0.98	0.96	0.98
	Bias correction factor C_b (accuracy)	1.00	0.99	1.00
	Agreement score	Substantial	Substantial	Substantial
Bland-Altman	Bias (mean difference)	0.06	0.11	0.02
	SD of differences	0.36	0.22	0.43
	LLOA	-0.65	-0.32	-0.82
	ULOA	0.76	0.54	0.86
Percentage similarity	Mean % similarity	100.62	101.06	100.32
	SD	2.13	2.10	2.13
	%CV	2.13	2.07	2.12
Calculated mean bias		1.24	2.12	0.15
%CV (imprecision from controls)		1.07	1.07	1.07
Calculated TAE		3.00	3.89	1.92
Westgard*, CLIA#	Optimal Allowable Error		3%*	
	Minimal Allowable Error		8%#	
Acceptable		Yes	Yes	Yes

* Westgard, # CLIA, CI - confidence interval, SD - standard deviation, LLOA -lower limit of agreement, ULOA - upper limit of agreement, CV - coefficient of variation; TAE - total analytical error

3.3.1 Accuracy assessment of overall results

The LumiraDx HbA1c assay in the laboratory evaluation showed a substantial overall agreement compared to the SOC HbA1c results with a correlation coefficient of 0.98. The overall precision (ρ>0.95) and accuracy (Cb=0.9982) were good. The absolute bias was small (0.06) (**Figure 3.2A**) with low variability observed, as seen in the SD of differences and limits of agreement (**Table 3.4**) (all within acceptable limits). There only two outliers and >95% of the values were below the 2% SD of the mean difference (bias) (**Figure 3.2A**). The

95% confidence intervals for the slope and intercept on Passing-Bablok regression analysis (**Figure 3.2B**) included 1.00 and 0.00, respectively. A percentage similarity of 100.62 % was observed and analytical total error of 3% which was within acceptable criteria.

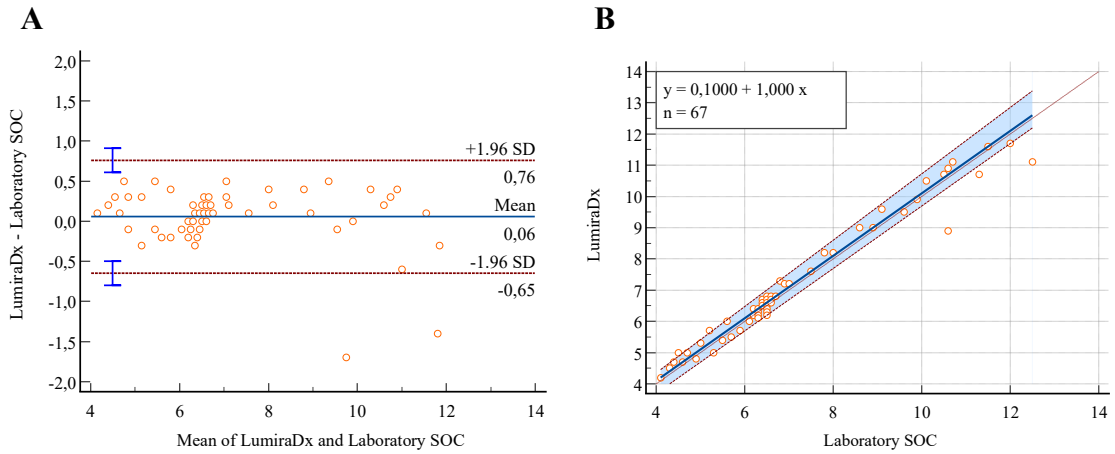


Figure 3.2: Bland-Altman and Passing-Bablok plots for accuracy assessment in the laboratory evaluation phase. **(A)** Bland-Altman plot of differences between LumiraDx and Laboratory SOC versus the mean of the two measurements. The mean difference (bias) is represented by the parallel solid line to the X axis. The limits of agreement (dotted lines), from -1.96*s* to +1.96 are shown as well as the 95%CI of the limits off agreement. **(B)** Passing-Bablok regression plot of the LumiraDx compared to the Laboratory SOC. The blue shaded space represents the 95%CI, the blue solid line is the line of best fit (regression line) whilst the dotted line is the 1:1 line. The formula for the regression line and sample size are shown.

3.4 Lot-to-lot variation.

Of the 60 samples measured using three different lots, 9 samples were excluded from the analysis as they gave an indefinite HbA1c measurement (<4% or >14%) for one or more lots. Thus, comparison between the different lots was carried out on 51 paired results of samples tested. HbA1c values measured ranged from 4.5% to 11.5% with median of 6.5% and IQR of 1.7. **Table 3.5** summarises the results. All three lots had substantial agreement score with the reference method with correlation coefficients of 0.98, 0.96 and 0.97, respectively. The absolute bias was higher for candidate lot 2 (0.32) than the other two lots (lot 1= 0.09, lot 3=0.03) (**Table 3.5**). For the Bland-Altman analysis, the mean difference (bias), SD of differences and limits of agreement were within acceptable bounds for all three lots (**Table 3.5**). The Passing-Bablok regression plot revealed 95% CI for the slope and intercept included 1.00 and 0.00, respectively, for lot 1 only (**Figure 3.3**).

Table 3.5: Statistical analysis for method comparison presented for the data results in the assessment of LTLV.

		Category		
		Lot 1 n = 51	Lot 2 n = 51	Lot 3 n = 51
Mean		7.24	7.48	7.18
SD		1.80	2.24	1.91
%CV		0.25	0.30	0.27
Concordance Correlation	Correlation coefficient (95%CI)	0.98 (0.97 to 0.99)	0.96 (0.94 to 0.97)	0.97 (0.94 to 0.98)
	Pearson ρ (precision)	0.98	0.99	0.97
	Bias correction factor C_b (accuracy)	1.00	0.97	1.00
	Agreement score	Substantial	Substantial	Substantial
Bland-Altman	Bias (mean difference)	0.09	0.32	0.03
	SD of differences	0.35	0.49	0.49
	LLOA	-0.59	-0.64	-0.94
	ULOA	0.76	0.54	0.86
Percentage similarity	Mean % similarity	100.73	101.84	100.09
	SD	2.18	2.47	3.35
	%CV	2.17	2.43	3.34
Calculated mean bias		0.01	0.04	0.00
%CV (imprecision from controls)		1.07	1.07	1.07
Calculated TAE		1.77	1.80	1.76
Westgard*, CLIA#	Optimal Allowable Error		3%*	
	Minimal Allowable Error		8%#	
Acceptable		Yes	Yes	Yes
Kruskal- Wallis test			p-value = 0.97	

* Westgard, # CLIA, CI- Confidence interval, SD- standard deviation, LLOA- lower limit of agreement, ULOA- upper limit of agreement, %CV- percentage coefficient of variation, ATE- Analytical total error, TAE – Total analytical error, CLIA - Clinical Laboratory Improvement Amendments

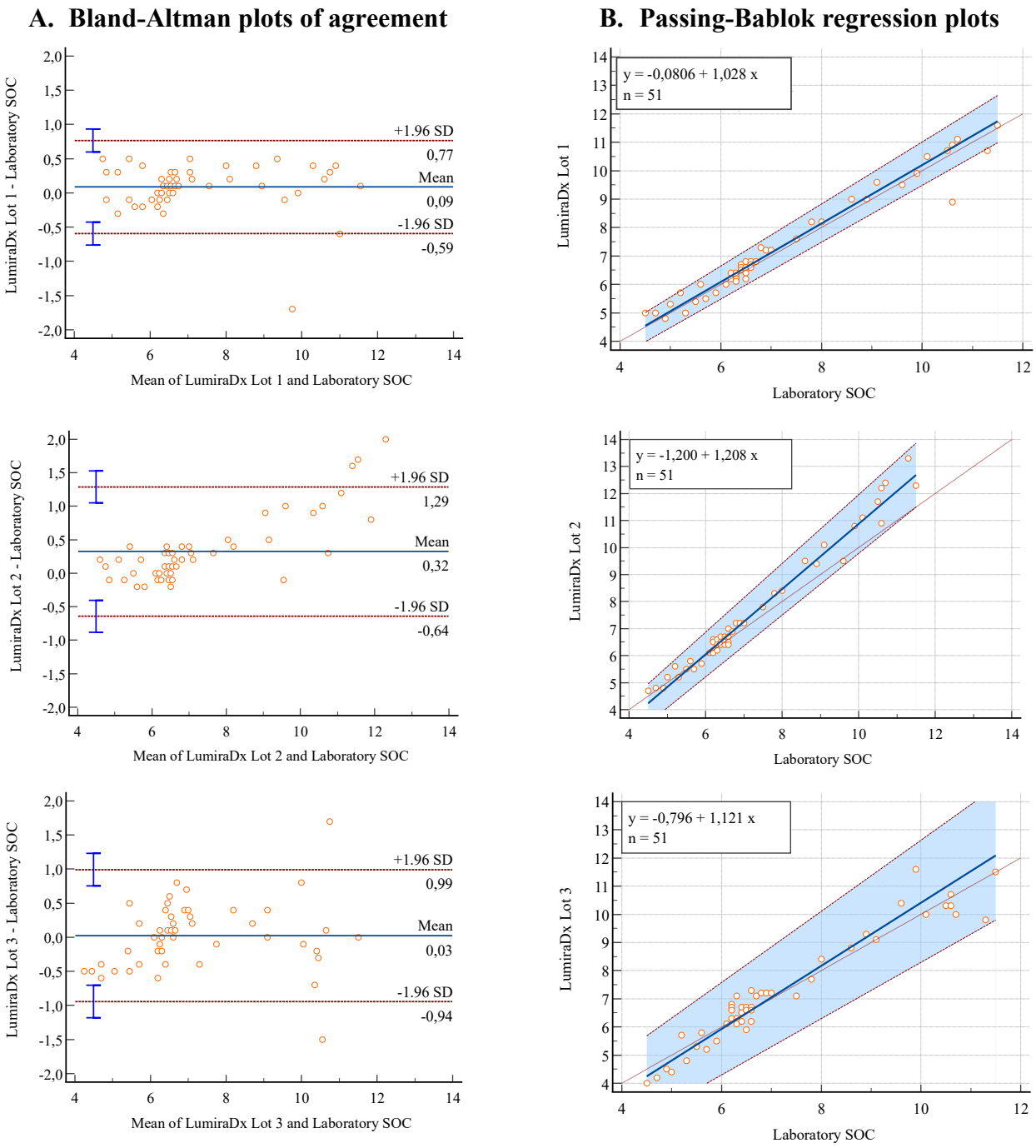


Figure 3.3: Bland-Altman and Passing-Bablok plots for LumiraDx kit lot 1, 2 and 3. **(A)** Bland-Altman plots of differences between LumiraDx lot 1, 2 and 3 and Laboratory SOC versus the mean of the two measurements. The mean difference (bias) is represented by the parallel solid line to the X axis. The limits of agreement (dotted lines), from -1.96*s* to +1.96 are shown as well as the 95%CI of the limits off agreement. **(B)** Passing-Bablok regression plots of the LumiraDx lot 1, 2 and 3 compared to the Laboratory SOC. The blue shaded space represents the 95%CI, the blue solid line is the line of best fit (regression line) whilst the dotted line is the 1:1 line. The formula for the regression lines and sample sizes are shown.

3.5 Results of the bridging study for the changeover of test strips

In the bridging study conducted to facilitate the transition from the old test kit to the new kit, we evaluated several approaches. The concordance correlation coefficient (CCC) was calculated, yielding a value of 0.99 (95%CI: 0.98 to 0.99), indicating a high level of agreement between the two assay kits. The Bland-Altman analysis revealed that the mean difference between the two kits was minimal, at -0.23, with limits of agreement falling within the acceptable limits of ±1.0, suggesting that the new kit performed comparably to the old one.

Percentage similarity analysis demonstrated an overall similarity of 101% in results, further supporting the interchangeability of the kits. Additionally, the analytical error was assessed against external criteria, revealing that the new assay kit met the predefined acceptance criteria, with an analytical error rate of 1.79%. Collectively, these results indicate that the new assay test kit is a reliable replacement for the old kit, ensuring continuity in the quality of testing outcomes.

Phase 2: Clinical evaluation

3.6 Description of study population

The study included a total of 501 participants of which 58 were excluded from the analysis (**Figure 3.4**). The participants' age varied from 18 to 88 years, with a median age of 48 years (IQR: 22). The weight of the participants varied, with a median weight of 76kg (IQR:25). There were slightly more female (52.6%) than male (47.4%) participants. In terms of HIV status, 174 participants (39%) were HIV-positive, 199 (45%) were HIV-negative, and 70 (16%) had an unknown HIV status. There were 99 (22%) TB-positive participants, and 61 (14%) participants were co-infected with HIV and TB (**Table 3.6**).

The median HbA1c level for the cohort tested using the Clinic SOC (DCA Vantage) was 8.6% (IQR: 3.2), with 88% of participants in this cohort classified as having diabetes (HbA1c ≥ 6.5%). For the cohort tested using the Laboratory SOC, the median HbA1c level was 6.0% (IQR: 2.2) with 36% of this cohort classified as having diabetes (HbA1c ≥ 6.5%) (**Table 3.6**).

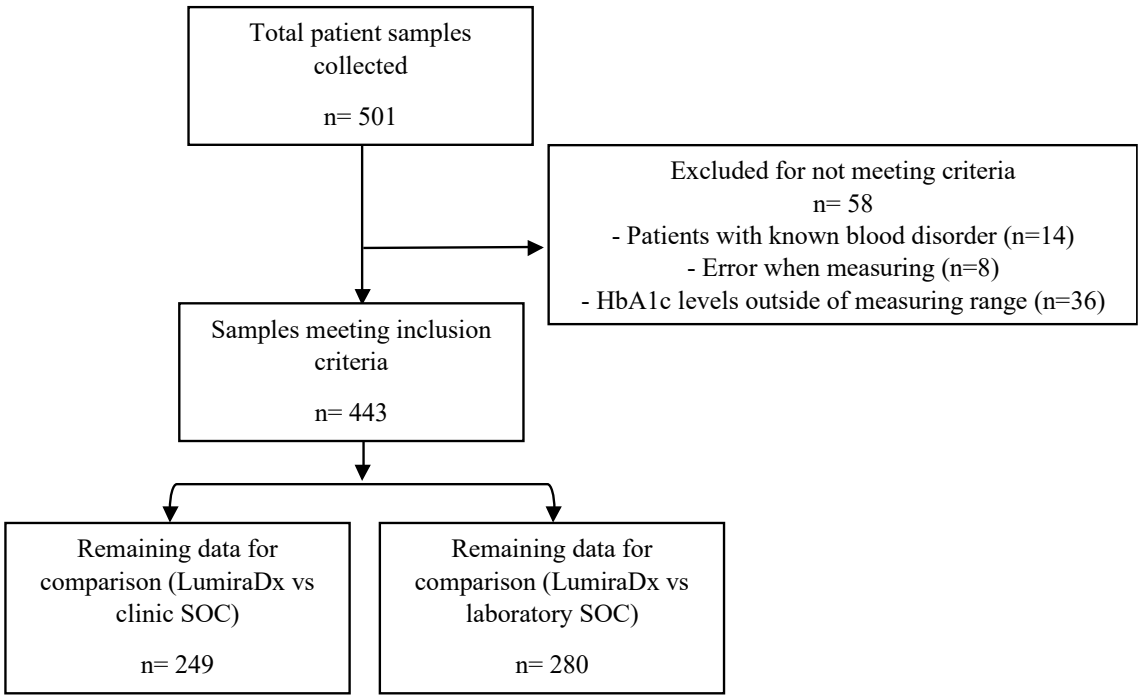


Figure 3.4: Flow chart showing the results included in the analysis of the data

Table 3.6: Descriptive analysis of the overall clinical study population

Variable	Total Population (n= 443)
Age, median [IQR] (Year)	48 [22] (18 to 88years)
Sex	
Male, % (n)	47.4 (210)
Female, % (n)	52.6 (233)
Weight, median [IQR]	76 [25] (14% (n=62) unknown)
HIV-positive	(n=174)
Male, % (n)	44.5 (78)
Female, % (n)	55.5 (96)
HIV-negative	(n=199)
Male, % (n)	49.7 (99)
Female, % (n)	50.3 (100)
HIV status unknown, % (n)	16 (70)
TB-positive	(n=97)
Male, % (n)	61.9 (60)
Female, % (n)	38.1 (37)
HIV and TB co-infection	n=61
TB infected, no HIV	n=38
HbA1c (%)	
FS (Clinic SOC), median [IQR]	8.6 [3.2] (n=249) (range: 5.2 - 14%)
Venous (Laboratory SOC), median [IQR]	6.0 [2.2] (n=280) (range: 4.6 - 13.5%)

IQR- interquartile range, HbA1c – glycated haemoglobin, SOC- Standard of care, FS- fingerstick

3.7 Method comparison

Method comparison was done on a total of 443 paired results of samples. This total included 249 results used in the comparison of the LumiraDx with the Clinic SOC and 280 results used for comparison of the LumiraDx with the Laboratory SOC. **Table 3.7** summarises the method comparison results from data collected and includes concordance correlation, Bland–Altman, percentage similarity analysis and analytical error. There were six categories with a substantial agreement score, two with moderate agreement score and three with poor

agreement score. The mean difference (Bias) as determined by Bland-Altman analysis were all negative and ranged from 0.01 to 0.25. The calculated mean biases ranged from 0.01 to 0.04 while the percentage similarities ranged from 98.14% to 98.78%. The total analytical error was within acceptable criteria for all the categories analysed (ranging from 1.77 to 1.80).

3.7.1 Method comparison between LumiraDx and the Clinic SOC

The LumiraDx HbA1c assay showed a moderate overall agreement compared to the Clinic SOC with a correlation coefficient of 0.93. The Bland-Altman analysis demonstrated that the mean difference between the methods was negative with magnitude of 0.3 units, with limits of agreement above the acceptable limit of ±1.0 (**Figure 3.5**). A Passing-Bablok regression analysis revealed a linear relationship between the two methods (slope = 0.94 (95%CI: 0.9 to 0.1), intercept = 0.31 (95%CI: -1.0 to 0.6)) (**Figure 3.5**). Percentage similarity analysis showed an overall similarity of 98.85%. The analytical error met the predefined acceptance criteria, with an analytical error rate of less than 1.79%. All categories exhibited analytical errors which were within the predefined optimal and minimal acceptable total analytical error (**Table 3.7**).

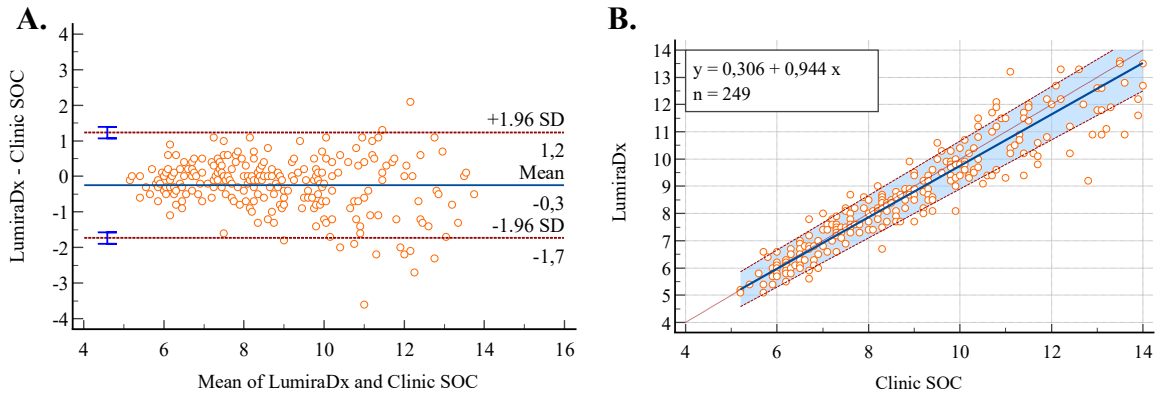


Figure 3.5: Bland-Altman and Passing-Bablok plots for accuracy assessment in the clinical evaluation phase by comparing with the Clinic SOC (DCA Vantage). **(A)** Bland-Altman plot of differences between LumiraDx and Clinic SOC versus the mean of the two measurements. The mean difference (bias) is represented by the parallel solid line to the X axis. The limits of agreement (dotted lines), from -1.96*s* to +1.96 are shown as well as the 95%CI of the limits off agreement. **(B)** Passing-Bablok regression plot of the LumiraDx compared to the Clinic SOC. The blue shaded space represents the 95%CI, the blue solid line is the line of best fit (regression line) whilst the dotted line is the 1:1 line. The formula for the regression line and sample size are shown.

Table 3.7: Method comparison results from data collected in the clinical evaluation

Category	(n)	Concordance correlation			Bland-Altman				Percentage similarity			Calculated mean bias	%CV (imprecision from controls)	Calculated Total Analytical error (TAE)	Optimal ATE/Minimal ATE
		Correlation coefficient (95%Cl)	Pearson ρ (precision)	Bias correction factor C_b (accuracy)	Bias	SD of differences	LLOA	ULOA	mean % similarity	%SD	%CV				
LumiraDx vs Clinic SOC	249	0,93 (0,91 to 0,95)	0,94	0,99	-0,25	0,76	-1,73	1,23	98,85	3,71	3,75	0,02	1,07	1,79	
LumiraDx Venous vs Laboratory SOC (Overall)	280	0,98 (0,98 to 0,99)	0,99	1,00	-0,05	0,36	-0,75	0,64	99,49	2,60	2,61	0,01	1,07	1,78	
LumiraDx FS vs Laboratory SOC (Overall)	280	0,97 (0,97 to 0,98)	0,98	0,99	-0,21	0,41	-1,01	0,58	98,35	2,86	2,91	0,03	1,07	1,80	
LumiraDx Venous vs Laboratory SOC (Diabetic)	100	0,97 (0,96 to 0,98)	0,98	0,99	-0,01	0,45	-0,89	0,88	99,78	2,57	2,58	0,00	1,07	1,77	
LumiraDx FS vs Laboratory SOC (Diabetic)	100	0,95 (0,93 to 0,97)	0,96	0,99	-0,20	0,54	-1,26	0,85	98,73	3,02	3,06	0,03	1,07	1,79	3%* / 8%#
LumiraDx Venous vs Laboratory SOC (Non-diabetic)	180	0,81 (0,75 to 0,85)	0,82	0,98	-0,08	0,29	-0,64	0,48	99,33	2,60	2,62	0,01	1,07	1,78	
LumiraDx FS vs Laboratory SOC (Non-Diabetic)	180	0,70 (0,62 to 0,76)	0,78	0,90	-0,22	0,31	-0,83	0,39	98,14	2,76	2,81	0,04	1,07	1,80	
LumiraDx Venous vs Laboratory SOC (HIV+)	164	0,97 (0,96 to 0,98)	0,97	1,00	-0,08	0,33	-0,73	0,57	99,36	2,72	2,74	0,01	1,07	1,78	
LumiraDx FS vs Laboratory SOC (HIV+)	164	0,95 (0,94 to 0,96)	0,96	0,99	-0,22	0,37	-0,94	0,50	98,23	2,81	2,86	0,04	1,07	1,80	
LumiraDx Venous vs Laboratory SOC (TB +)	97	0,91 (0,86 to 0,94)	0,91	0,99	-0,07	0,30	-0,66	0,52	99,44	2,75	2,77	0,01	1,07	1,78	
LumiraDx FS vs Laboratory SOC (TB+)	97	0,86 (0,79 to 0,90)	0,89	0,96	-0,19	0,33	-0,84	0,46	98,37	2,98	3,03	0,03	1,07	1,80	

* Westgard, # CLIA, SOC- standard of care, FS- fingerstick, HIV+ - HIV positive, TB+ - TB positive, CI- Confidence interval, SD- standard deviation, LLOA- lower limit of agreement, ULOA- upper limit of agreement, %CV- percentage coefficient of variation, ATE- Allowable total error, TAE – Total analytical error

3.7.2 Method comparison between LumiraDx and the laboratory SOC for overall results

The method comparison study evaluated the interchangeability of the LumiraDx HbA1c using both capillary and venous samples against the Laboratory SOC. For capillary samples, the concordance correlation coefficient (CCC) was calculated at 0.97, yielding a substantial agreement score. The Bland-Altman analysis for capillary samples revealed a negative mean difference of 0.21, with limits of agreement falling within the limits of ±1.0. In contrast, the venous sample analysis yielded a slightly higher CCC of 0.98, also reflecting substantial agreement with the laboratory standard. The Bland-Altman results for venous samples showed a small negative mean difference of 0.05 units, with limits of agreement within ±1.0.

Further analysis using Passing-Bablok regression demonstrated a linear relationship for both capillary (slope = 1.00 (95%CI: 1.00 to 1.04), intercept = -0.20 (95%CI: -0.43 to -0.20)) and venous (slope = 1.03 (95%CI: 1.00 to 1.06), intercept = -0.28 (95%CI: -0.48 to -0.10)) samples (**Figure 3.6**). Percentage similarity analysis indicated that the capillary samples exhibited a similarity of 98.35%, while venous samples showed 99.49% similarity to the Laboratory SOC. The analytical error for both blood sample types met the predefined acceptance criteria, with analytical error rates of 1.80% and 1.78%, respectively (**Table 3.7**).

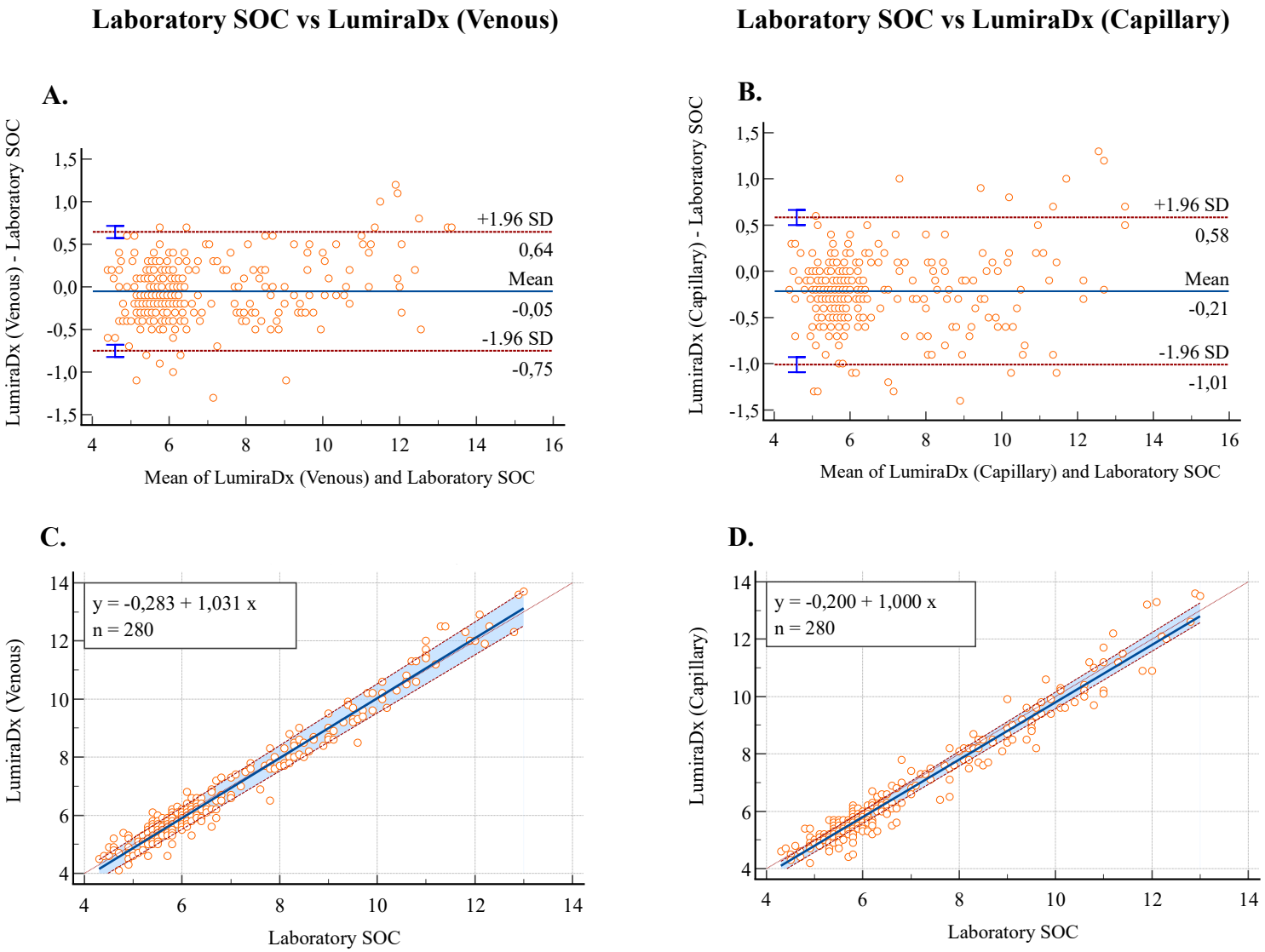


Figure 3.6: Bland-Altman and Passing-Bablok plots for accuracy assessment in the clinical evaluation phase by comparing with Laboratory SOC for the overall clinical data results. **(A and B)** Bland-Altman plots of differences between LumiraDx and Laboratory SOC versus the mean of the two measurements. The mean difference (bias) is represented by the parallel solid line to the X axis. The limits of agreement (dotted lines), from -1.96*s* to +1.96 are shown as well as the 95%CI of the limits off agreement. **(C and D)** Passing-Bablok regression plots of the LumiraDx compared to the Clinic SOC. The blue shaded space represents the 95%CI, the blue solid line is the line of best fit (regression line) whilst the dotted line is the 1:1 line. The formula for the regression line and sample size are shown. **Left:** Comparison using venous sample tested on Lumira and same venous sample tested on Laboratory SOC. **Right:** Comparison using capillary sample tested on Lumira and venous sample tested on Laboratory SOC taken both from same patient.

3.7.3 Method comparison between LumiraDx and the laboratory SOC for diabetic participants

Sub-analysis of the diabetic category (HbA1c≥6.5%) showed a substantial agreement between the LumiraDx and Laboratory SOC for both capillary and venous samples with concordance correlation coefficients of 0.95 and 0.97, respectively. The Bland-Altman analysis for capillary samples revealed a negative mean difference of 0.20 while venous samples revealed a small negative bias of 0.01 with limits of agreement (for both) falling within the limits of ±1.0. Passing-Bablok regression demonstrated a linear relationship for both capillary (slope = 1.07 (95%CI: 1.00 to 1.14), intercept = -0.82 (95%CI: -1.43 to -0.20)) and venous (slope = 1.11 (95%CI: 1.06 to 1.17), intercept = -1.02 (95%CI: -1.48 to -0.64)) samples (**Figure 3.7).** Percentage similarity analysis indicated that the capillary samples exhibited a similarity of 98.73%, while venous samples showed 99.78% similarity to the Laboratory SOC. The analytical error for both blood sample types met the predefined acceptance criteria, with analytical error rates of 1.79% and 1.77%, respectively (**Table 3.7**).

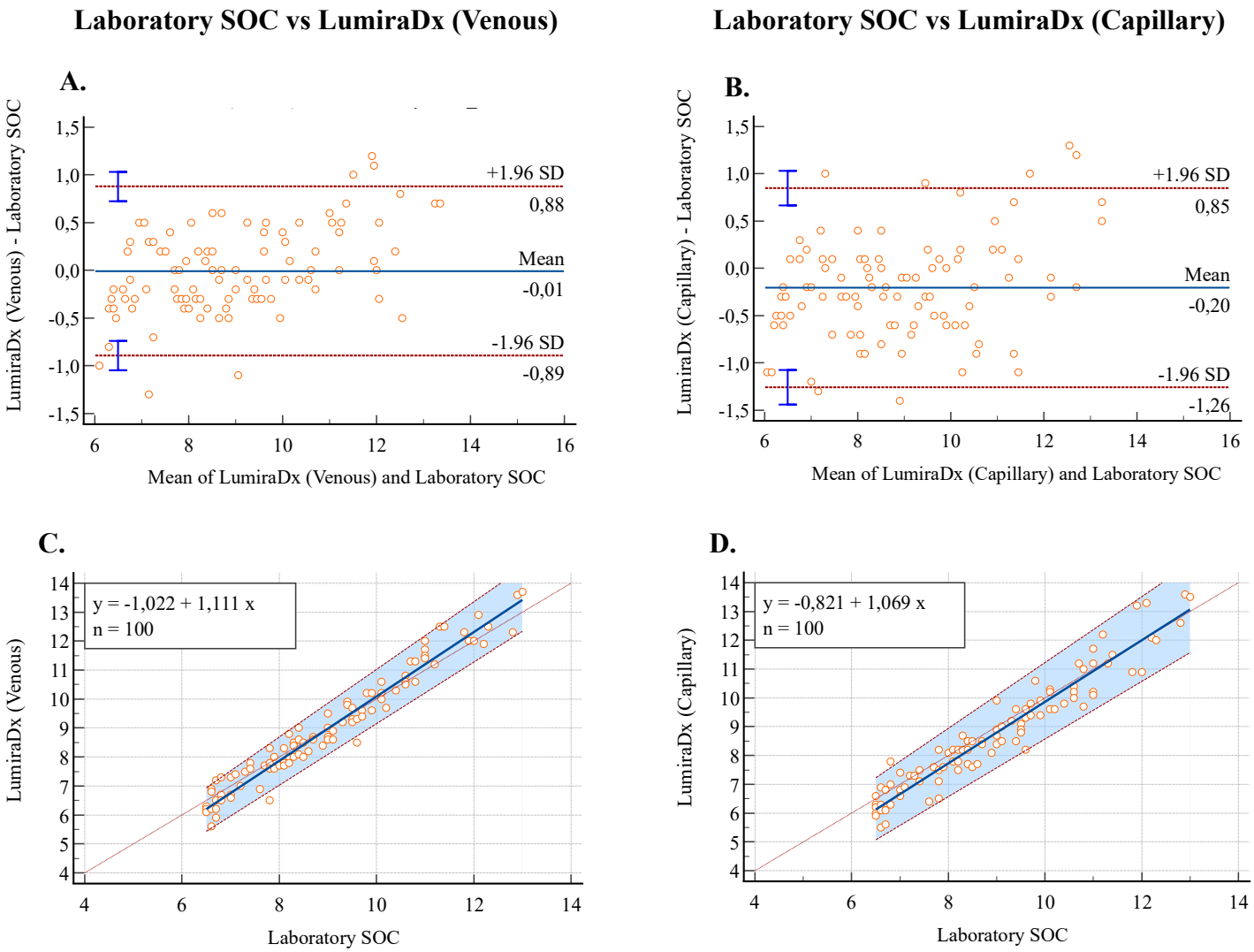


Figure 3.7: Bland-Altman and Passing-Bablok plots for sub-analysis of accuracy in the clinical evaluation phase for the diabetic category (HbA1c≥6.5%). **(A and B)** Bland-Altman plots of differences between LumiraDx and Laboratory SOC versus the mean of the two measurements. The mean difference (bias) is represented by the parallel solid line to the X axis. The limits of agreement (dotted lines), from -1.96*s* to +1.96 are shown as well as the 95%CI of the limits off agreement. **(C and D)** Passing-Bablok regression plots of the LumiraDx compared to the Clinic SOC. The blue shaded space represents the 95%CI, the blue solid line is the line of best fit (regression line) whilst the dotted line is the 1:1 line. The formula for the regression line and sample size are shown. **Left:** Comparison using venous sample tested on Lumira and same venous sample tested on Laboratory SOC. **Right:** Comparison using capillary sample tested on Lumira and venous sample tested on Laboratory SOC taken both from same patient.

3.7.4 Method comparison between LumiraDx and the laboratory SOC for non-diabetic participants

The sub-analysis of the non-diabetic category (HbA1c<6.5%) showed a poor agreement between the LumiraDx and Laboratory SOC for both capillary and venous samples with concordance correlation coefficients of 0.70 and 0.81, respectively. The Bland-Altman analysis for capillary samples revealed a negative mean difference of 0.22 while venous samples revealed a small negative bias of 0.08 with limits of agreement (for both) falling within the limits of ±1.0. Passing-Bablok regression demonstrated a linear relationship for both capillary (slope = 1.00 (95%CI: 1.00 to 1.17), intercept = -0.20 (95%CI: -1.15 to -0.20)) and venous (slope = 1.09 (95%CI: 1.00 to 1.25), intercept = -1.62 (95%CI: -1,5250 to -0.10)) samples **(Figure 3.8**). Percentage similarity analysis indicated that the capillary samples exhibited a similarity of 98.14%, while venous samples showed 99.33% similarity to the Laboratory SOC. The analytical error for both blood sample types met the predefined acceptance criteria, with analytical error rates of 1.80% and 1.78%, respectively (**Table 3.7**).

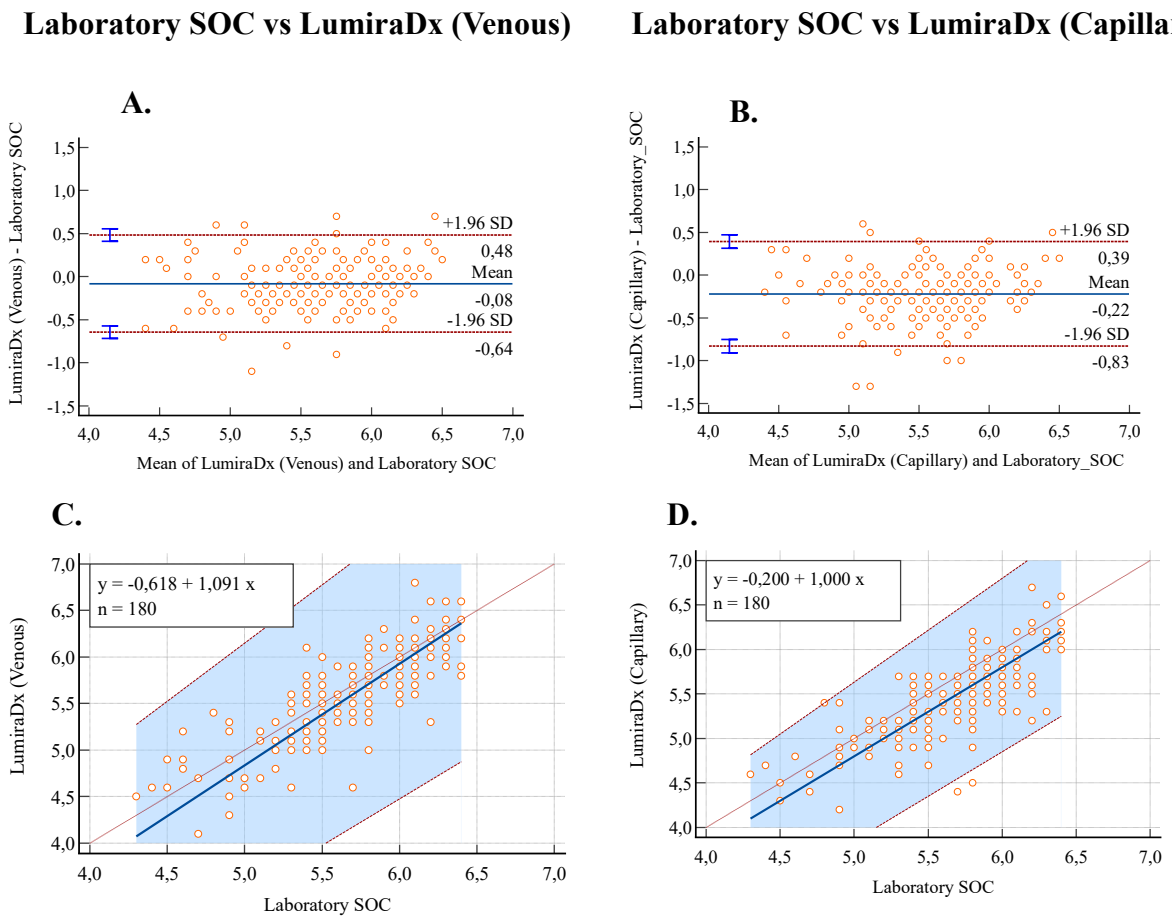


Figure 3.8: Bland-Altman and Passing-Bablok plots for sub-analysis of accuracy in the clinical evaluation phase for the non-diabetic category (HbA1c<6.5%) **(A and B)** Bland-Altman plots of differences between LumiraDx and Laboratory SOC versus the mean of the two measurements. The mean difference (bias) is represented by the parallel solid line to the X axis. The limits of agreement (dotted lines), from -1.96*s* to +1.96 are shown as well as the 95%CI of the limits off agreement. **(C and D)** Passing-Bablok regression plots of the LumiraDx compared to the Clinic SOC. The blue shaded space represents the 95%CI, the blue solid line is the line of best fit (regression line) whilst the dotted line is the 1:1 line. The formula for the regression line and sample size are shown. **Left:** Comparison using venous sample tested on Lumira and same venous sample tested on Laboratory SOC. **Right:** Comparison using capillary sample tested on Lumira and venous sample tested on Laboratory SOC taken both from same patient.

3.8 Impact of HIV and TB on concordance of the methods

The sub-analysis of HIV-positive, TB-positive and co-infected participants, when compared to the overall data from the study (results when using capillary blood for testing), revealed the following: The HIV-positive, TB-positive and co-infected subgroups showed substantial (Pc

= 95), poor (Pc= 86) and poor (Pc= 88) agreements score, respectively, while overall results showed substantial agreement with a higher correlation coefficient of 0.97 (**Table 3.7**). The Bland-Altman analysis revealed slightly lower and one high mean difference (bias) of 0.22, 0.19 and 0.15 for HIV-positive, TB-positive and co-infected, respectively, compared to 0.21 (**Table 3.7**). The mean difference remained negative in the sub-group with limits of agreement still falling within the limits of ±1.0. The Passing-Bablok regression analyses all included the values 0 and 1 for the 95%CI of the slopes and intercepts apart for the 95%CI of intercept for the TB-positive cohort (-1,15 to -0,05) (**Figure 3.9**). The percentage similarity analysis revealed similarities of 98% for all the groups which was the same for the overall dataset. All groups met the predefined acceptance criteria for analytical error, with rates of 1.8% for HIV-positive and TB-positive participants and 1.79% for the co-infected participants (**Table 3.8**).

Table 3.8: Results for comparison between Laboratory SOC and LumiraDx in HIV-positive, TB- positive and co-infected participants.

		Category		
		HIV positive n = 164	TB positive n = 97	HIV and TB co-infected n = 61
	Correlation coefficient (95%CI)	0,95 (0,94 to 0,96)	0,86 (0,79 to 0,90)	0,88 (0,81 to 0,93)
Concordance Correlation	Pearson ρ (precision)	0.96	0.89	0.90
	Bias correction factor C_b (accuracy)	0.99	0.96	0.98
	Agreement score	Substantial	Poor	Poor
Bland-Altman	Bias (mean difference)	-0.22	-0.19	-0.15
	SD of differences	0.37	0.33	0.32
	LLOA	-0.94	-0.84	-0.77
	ULOA	0.50	0.46	0.47
Percentage similarity	Mean % similarity	98.23	98.37	98.76
	SD	2.81	2.98	2.85
	%CV	2.86	3.03	2.88
Calculated mean bias		0.04	0.03	0.02
%CV (imprecision from controls)		1.07		
Calculated TAE		1.80	1.80	1.79
Westgard*, CLIA#	Optimal Allowable Error		3%*	
	Minimal Allowable Error		8%#	
Acceptable		Yes	Yes	Yes

* Westgard, # CLIA, CI- Confidence interval, SD- standard deviation, %CV- percentage coefficient of variation, TAE – Total analytical error, CLIA - Clinical Laboratory Improvement Amendments

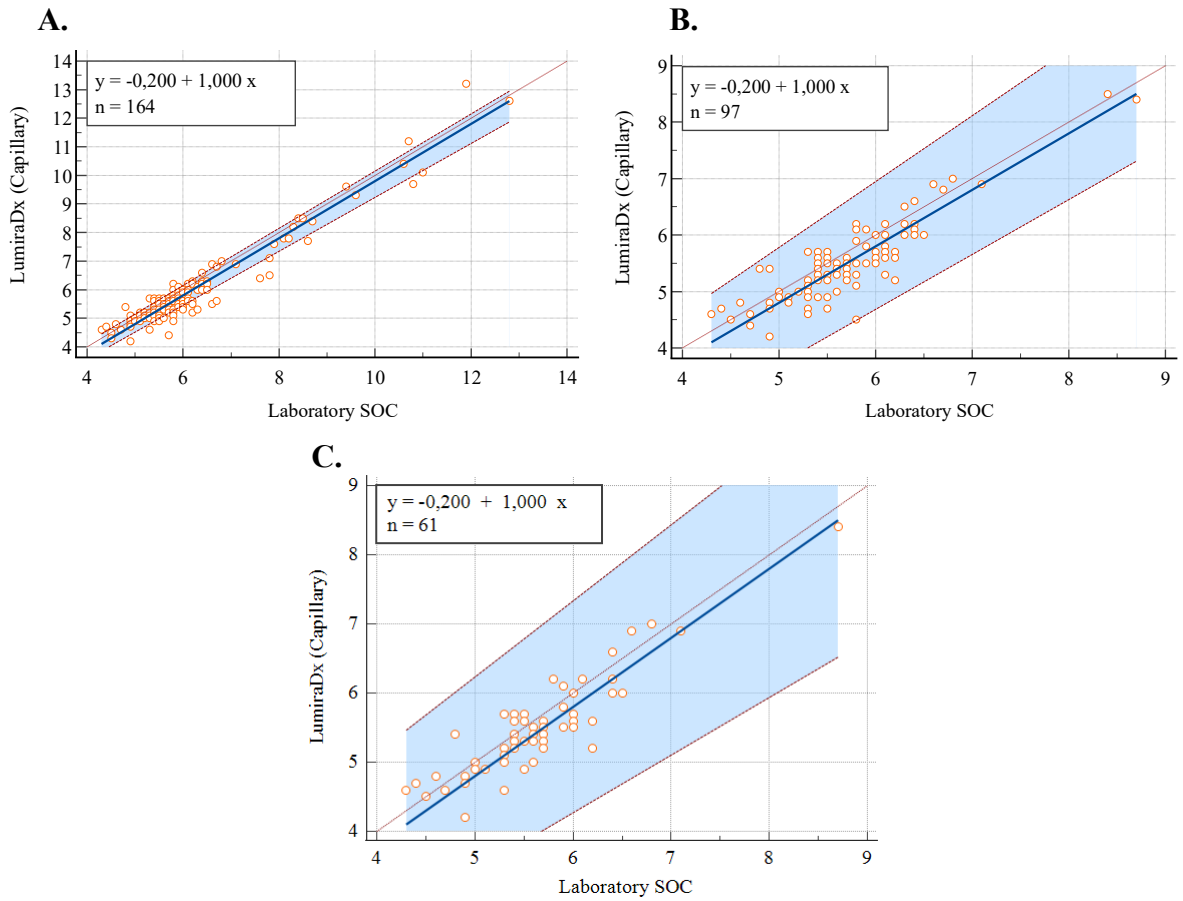


Figure 3.9: Passing-Bablok regression plots of the LumiraDx compared to the Laboratory SOC for **(A)** HIV-positive, **(B)** TB-positive and **(C)** co-infected participants. The blue shaded space represents the 95%CI, the blue solid line is the line of best fit (regression line) whilst the dotted line is the 1:1 line. The formula for the regression line and sample size are shown.

3.9 Analysis of diagnostic capability

To assess whether the LumiraDx HbA1c test is suitable for use in diagnosis of diabetes, a sub-analysis was done to evaluate performance at concentrations of 6.3%, 6.4% and 6.5% using data from phase 1 and phase 2 of the study. The Bland-Altman revealed a negative mean difference of -0.06 with SD of differences of 0.22 and limits of agreement falling within the limits of ±1.0 (mean ±1.96SD: -0.50 to 0.40) (**Figure 3.10**). Percentage similarity analysis indicated a similarity of 99.53% (SD: 1.74, %CV: 1.75) of the LumiraDx HbA1c test to the Laboratory SOC. The analytical error met the predefined acceptance criteria, with analytical error rate of 1.77%.

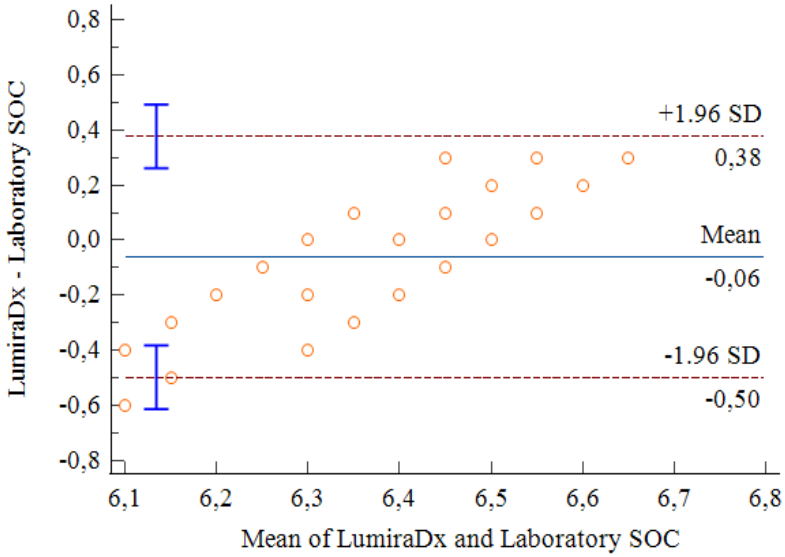


Figure 3.10: Bland-Altman plot of differences between LumiraDx and Laboratory SOC versus the mean of the two measurements for HbA1c concentrations from 6.3% to 6.5% as obtained on the Laboratory SOC. The mean difference (bias) is represented by the parallel solid line to the X axis. The limits of agreement (dotted lines), from -1.96SD to +1.96SD are shown as well as the 95%CI of the limits off agreement.

Chapter 4 Discussion

There is a growing need for more widespread diabetes diagnosis, as 45.4% of South Africans with the condition remain undiagnosed (7,96). This may be owing to the limited availability and convenience of standard laboratory testing (69). POC testing presents an attractive alternative due to its greater convenience, typically using small benchtop analysers and requiring a small capillary blood sample obtained from a fingerstick which is considered less invasive than venipuncture (97). Furthermore, it can offer results within a few minutes leading to timely diagnosis. Most importantly, clinical studies have confirmed that POC testing can improve patient glycaemic control compared to standard laboratory testing (61,121,122). It has thus been endorsed for use in monitoring glycaemic levels and guiding outpatient decisions for patients with diabetes (123). However, the use of POC testing to diagnose diabetes is limited and is not yet recommended due to concerns about the analytical performance of these devices relative to standard laboratory methods, and their issue with dependency on reagent lot variation (81,84). This study aimed at evaluating the analytical performance of the LumiraDx POC analyser for the measurement of HbA1c for use in diagnosis and monitoring of diabetes and to further determine if results are affected by lot variation. Furthermore, analysis was conducted to assess whether HIV or TB affects method concordance.

4.1 Performance of LumiraDx in the Laboratory

The run precision (within-run and between-run variability) was acceptable. There were no measurements falling outside the expected ranges for both controls, which speaks positively to the consistency of the measurements. The analytical error of the high control was optimal, while the analytical error of the low control was not optimal but remained within the minimal acceptable threshold as set by external criteria. This indicates a reliable testing precision. The evaluation of the LumiraDx HbA1c test found that both within-run (repeatability) and between-run variabilities were within the bounds of the manufacturer's claims and fulfilled the acceptance criteria based on the CLIA and the Westgard guideline. For instrument variability most samples measured fell within the predefined acceptance criteria, except for one sample, which had a very high inter-instrument variability of 9.42%. This was the sample with the highest HbA1c level (11.7%). It is worth noting that the high inter-instrument variability was influenced by the observed SD for this sample which was the highest of all samples meaning there was a wide variability in the repeated measurements taken, which is

not ideal. This suggests reduced instrument precision at higher concentrations; however, this variability is unlikely to have significant clinical implications. This is because patients exhibiting HbA1c levels in the same range as this sample (10,4 - 13,6%) are typically classified as poorly controlled diabetics, regardless of minor variations in testing results as their HbA1c is well above 7% (31). Previous research has exhibited that the precision performance of HbA1c POC analysers varies significantly across different instruments. While some analysers show high precision and meet quality standards, others exhibit variability that may affect clinical decision-making (81,124–127).

The LumiraDx HbA1c test showed excellent linearity over the measuring range of 4.6 to 11.5% ($R^2 = 0.99$) meaning a substantial proportion of the variability in the results can be explained by the linear relationship between the measured HbA1c and the expected HbA1c in samples. Furthermore, there was no significant lot variation observed as the Kruskal-Wallis test revealed a high p-value indicative of absence of a statistically significant difference between the different lots. The three lots further demonstrated substantial agreement with the Laboratory SOC results and thus performed comparably with the reference method. The results between the different lots were consistent with one another. Similarly, in the study by Jeong et al. assessing the accuracy of three different lots of another POC HbA1c test, high correlation (correlation coefficients all >0.98) was observed between the results of the lots with the reference value (128).

The accuracy assessment performed in the laboratory evaluation phase, showed substantial overall correlation of the LumiraDx HbA1c test with the Laboratory SOC method. Furthermore, the agreement between the LumiraDx HbA1c test and the Laboratory SOC was excellent with a slight overestimation of the results indicated by an observed minor bias of +0.06. The consistent relationship observed between the two methods shows that the LumiraDx HbA1c test reliably predicts Laboratory SOC test results, indicating its accuracy.

A sub-category analysis across different ranges (diabetic and non-diabetic) showed comparable results to the overall analysis with minor differences. The agreement between the POC analyser and the Laboratory SOC was excellent in the both the non-diabetic and diabetic range, indicating that the POC analyser is reliable across the different ranges and can be used to differentiate between non-diabetic and diabetic.

The laboratory evaluation demonstrated satisfactory performance of the LumiraDx HbA1c test, with acceptable precision, accuracy, linearity across the relevant measuring range (4.6 – 11.5%), and no significant lot-to-lot variation. These findings propose that the results obtained by the LumiraDx HbA1c test can be accurately interpretated and reliable conclusions can be obtained for clinical decision making.

4.2 Performance of LumiraDx in the clinical context

4.2.1 Performance of the LumiraDx relative to the Clinic SOC and Laboratory SOC

In the clinical evaluation phase of the study, the LumiraDx HbA1c test was found to be comparable to both the Clinic SOC and Laboratory SOC. It was more comparable to the Laboratory SOC than the Clinic SOC, showing substantial agreement with the Laboratory SOC and moderate agreement with the Clinic SOC. Furthermore, a higher bias was observed between the LumiraDx and Clinic SOC. This lower comparability with the Clinic SOC may be due to variations in capillary blood collection techniques performed by different operators. The observed biases indicated an underestimation of HbA1c measurement by the LumiraDx HbA1c test. However, this was not clinically relevant as the bias was within the predefined acceptance criteria. This finding aligns with literature, as a meta-analysis done in 2016 found that most POC analysers tend to have a negative bias (underestimation) as compared to results of laboratory reference methods (79). It is worth noting that in the laboratory evaluation phase, for accuracy, the results indicated an overestimation of HbA1c measurement by LumiraDx however in the clinical evaluation, an underestimation was observed. This may be due to the strength of the analysis attributed to the difference in sample size with the clinical evaluation having a much greater sample size and thus statistical power (129). Nevertheless, clinically insignificant biases were observed. The assessment of the LumiraDx against SOC methods indicated no relative or differential bias and systematic bias and that the LumiraDx has similar performance to the SOC methods. The analytical errors calculated were well within the predefined criteria set using the CLIA and Westgard guidelines.

No major difference in performance was observed when capillary blood samples was used for comparison with the Laboratory SOC (which only uses venous samples) as opposed to venous blood samples. However, slightly subpar results were observed when results of

capillary blood samples were used for the comparison versus when venous blood samples were used. This discrepancy could be attributed to variations in blood collection techniques or inherent differences in sample types. Notably, the analytical error of the LumiraDx HbA1c test was consistently <2% across all data, regardless of the sample type.

4.2.2 Performance in the diabetic and non-diabetic subcategory analyses

In the sub-analysis of the diabetic and non-diabetic cohort, the diabetic group exhibited substantial correlation between the LumiraDx and the Laboratory SOC method. The concordance correlation coefficient was at least 0.95 using both capillary and venous sample types. In contrast, for the non-diabetic group, there was poor correlation between the two methods: the concordance corelation coefficient was 0.81 and 0.70 for capillary and venous sample types, respectively. This suggests that the LumiraDx HbA1c test may be less reliable at lower HbA1c levels. However, Concordance Correlation Coefficient alone may not provide complete information about the relative contributions of bias and correlation. It is recommended to use it alongside other statistics for a more comprehensive assessment (130). The Bland-Altman analysis exhibited a small negative bias for the diabetic (-0.01) and non-diabetic (-0.08) group for venous sample type whereas a bigger negative bias was observed for both groups for capillary sample type (-0.20 and -0.22). The negative bias observed indicates that, on average, the LumiraDx HbA1c underestimates the results of the Laboratory SOC. The larger negative bias for capillary samples in both categories indicate a more pronounced underestimation, raising concerns about reliability of capillary sample testing, more especially because clinical decision is likely to rely on results obtained capillary blood when POC analyser is used. However, these biases are minimal and fall within the predefined acceptable range, indicating that the LumiraDx HbA1c test remains fairly accurate for both sample types and for distinguishing between diabetic and non-diabetic patients. The 95% confidence intervals for the estimated biases contained zero, indicating the absence of differential or relative bias. This suggests that while there are differences in agreement between the two sub-categories and sample types, these differences do not appear to be clinically significant in terms of bias.

4.2.3 Performance of the LumiraDx in HIV and TB infected individuals

A supplementary sub-analysis was conducted for study participants infected with HIV and TB to ascertain whether any differences in the performance of the LumiraDx HbA1c test would emerge within these specific population cohorts. This analysis employed the same

methodological approach as the overall analysis, allowing for a comparative evaluation of the findings.

In the HIV-infected cohort, a substantial correlation was still evident between the LumiraDx HbA1c test and the Laboratory SOC method. A slight increase in bias was noted, with values of -0.05 in the overall analysis compared to -0.08 in the HIV cohort for venous sample type, and -0.21 for the overall analysis versus -0.22 for the HIV cohort for capillary sample type. The observed bias was marginally higher in the HIV cohort. Notably, the analytical errors remained consistent with those identified in the overall analysis.

In the TB infected cohort, the degree of agreement observed was lower than that identified in the overall analysis, with a poor correlation of 0.86 for capillary blood sample type. This indicates a decrease in the agreement between the LumiraDx HbA1c test and the Laboratory SOC for this cohort. A study conducted by Lawson et al. among adults presumed to have TB also revealed poor agreement between POC and Laboratory HbA1c results (131). Notably, a sizeable proportion of this cohort consisted of participants with low HbA1c values, ranging from 4.3% to 8.7% (mean = 5.7%). As previously observed in the sub-analysis of the non-diabetic group, which exhibited HbA1c values ranging from 4.3% to 6.4% (mean = 5.7%), the correlation was also poor. These findings suggest that at lower HbA1c values, the agreement between the LumiraDx HbA1c test and the Laboratory SOC may be suboptimal. Like the HIV cohort, minor differences in bias were observed within the TB cohort to the overall study population. A higher negative bias was identified for venous sample types, increasing from -0.05 (overall) to -0.07 (TB-cohort), while a lower negative bias was noted for capillary sample types, decreasing from -0.21 (overall) to -0.19 (TB-cohort). The analytical errors remained consistent with those recorded in the overall analysis. This finding corresponds with a study done by Huangfu et al. among TB patients showing that differences observed between the POC and laboratory HbA1c results was within an acceptable error margin (132).

Similar findings were observed for the HIV and TB co-infected cohort. These results do not raise any concerns for the usage of the LumiraDx HbA1c test for individuals with HIV and/or TB as the presence of these infections did not influence the comparative performance of the LumiraDx HbA1c test with the Laboratory SOC. This corresponds with a study which showed that the POC HbA1c were higher compared to Laboratory SOC, irrespective of the HIV status (132).

4.3 The performance of the LumiraDx HbA1c test for diagnosis and monitoring of diabetes

The potential for managing diabetes mellitus using the LumiraDx HbA1c test has been demonstrated through its acceptable precision and accuracy. This study indicates that the LumiraDx HbA1c test is useful for diagnosing and managing diabetes in clinical settings, providing a reliable foundation for healthcare providers to trust its results. Notably, the analysis of HbA1c concentrations between 6.3% and 6.5% (close to the diagnostic cut-off) shows minimal bias of -0.06, supporting its use for diagnostic purposes. However, a proportional increase in the difference between the two methods was observed, indicating that the bias is not constant and may vary with the different HbA1c levels. This variability suggests that while the test is reliable within a specific range, careful interpretation is necessary to ensure accurate clinical decision-making. Furthermore, the analytical error is <2%, aligning with recommended standards for HbA1c methodologies used in diagnosis. While POC tests are not yet recommended for diagnosis, this study highlights their potential to meet stringent diagnostic criteria, making them valuable for both monitoring and diagnosing diabetes (133).

4.4 Summary of results

The study assessed the LumiraDx HbA1c test's performance by assessing precision, accuracy, linearity and lot-to-lot variation in a laboratory environment and thereafter determining its interchangeability with SOC methods in a clinical setting. Excellent precision and good linearity were observed across a clinically relevant range of HbA1c concentrations. Secondly, no statistically significant lot variation was observed in the measurement of HbA1c using different batches of the LumiraDx HbA1c kit. Thirdly, accuracy as assessed in the laboratory was satisfactory with good correlation observed between the LumiraDx HbA1c test and the Laboratory SOC. Finally, the LumiraDx HbA1c test was deemed interchangeable with the Clinic SOC and Laboratory SOC, with the LumiraDx HbA1c test slightly underestimating the measurement. The results also showed that the test would be appropriate for use in patients infected with HIV and/or TB as no significance difference in method concordance was observed. Additionally, the results met the standard analytical guidelines for using HbA1c methods in diagnosis.

4.5 Strengths and Limitations of the study

The study's strength rests in its relevance to the day-to-day treatment of individuals with diabetes, particularly through its clinical performance evaluation. By utilizing both venous blood and the recommended capillary blood for POC testing, the study provides a comprehensive overview of the LumiraDx HbA1c test's effectiveness in a real-world context. This dual approach not only reflects the practical scenarios healthcare providers face but also reinforces the versatility of the LumiraDx HbA1c test in different clinical settings.

However, there are notable limitations that warrant consideration. While the results indicate acceptable LTLV, it is essential to acknowledge that this assessment was conducted in a laboratory setting using venous blood samples rather than a clinical environment. Laboratory conditions often differ significantly from real-world clinical scenarios, where factors such as patient variability, operator experience, and sample handling can influence results. Therefore, conducting LTLV assessments in a clinical setting would provide a more accurate depiction of the test's reliability and lot consistency in everyday practice. Furthermore, including a greater number of lots in the assessment could have strengthened the findings, as this would permit for a more thorough evaluation of the LumiraDx HbA1c test's performance across different manufacturing batches.

Another limitation of the study is its focus on the impact of HIV and TB on the relationship between the two measurement methods. While this focus provides valuable insight into the applicability of the LumiraDx HbA1c test as a substitute for SOC testing within these specific population groups, it restricts the generalizability of the findings. The study does not address whether HbA1c is an appropriate measure of glycaemic levels for these populations, which is a crucial consideration for effective diabetes management. There are studies indicating that HbA1c measurements may be underestimated or overestimated in these population groups (134,135). Incorporating an additional measure of glucose levels for comparison would have offered further insights into the accuracy of HbA1c in showing glycaemic control, especially in patients with coexisting conditions such as HIV or TB.

Lastly, extending the range for linearity with more samples could enhance the robustness of the findings. A broader range of samples would provide a more detailed understanding of the test's performance across various HbA1c levels, thereby improving its applicability in both diagnostic and monitoring contexts. Overall, while this study lays a solid foundation for the use of the LumiraDx HbA1c test in medical practice, addressing these limitations in future

research will be vital for maximizing its effectiveness and reliability in managing diabetes mellitus across diverse patient populations.

4.6 Conclusion

In conclusion, both the laboratory and clinical evaluations of the LumiraDx HbA1c test demonstrated its excellent performance, establishing its interchangeability with SOC methods. This robust analytical performance highlights the value of the LumiraDx HbA1c test for accurately and reliably measuring HbA1c levels in diverse patient populations, including those with diabetes, without diabetes, and individuals with HIV or TB. The test is effective for both diagnosing diabetes and monitoring glycaemic levels in diabetic patients.

For future studies, it would be valuable to examine the performance of the LumiraDx HbA1c test in the presence of haemoglobin variants, especially if the analyser is intended for use in populations with an elevated prevalence of these variants. Additionally, exploring a larger and more varied sample size would strengthen the assessment of the LumiraDx HbA1c test's performance across different demographic groups and clinical settings. Longitudinal studies could evaluate the long-term effectiveness and reliability of the LumiraDx HbA1c test in routine clinical practice. Integrating POC instruments into routine practice will enable timely diagnosis and interventions, thus enhancing diabetes management and improving overall patient health (71). As healthcare increasingly emphasizes precise diagnostics, maintaining rigorous standards will be essential to ensure patient safety and effective treatment outcomes.

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Appendices

Appendix 1: Benchtop HbA1c POC testing technology landscape

Technology	**Principle**	**Sample type and size**	**Time of result**	**Measuring range**	**Precision**	**Additional tests available**
Abbott (USA) 1. Afinion	Boronate affinity	Capillary whole blood, anticoagulated venous whole blood 1.5ul	3 min	4.0–15.0% / 20–140 mmol/mol	<3%	Lipid panel, ACR, CRP
2. Nycocard Reader II	Boronate affinity	Capillary whole blood, anticoagulated venous whole blood 5ul	3 min	4.0–15.0% / 20–140 mmol/mol	<5%	CRP
Aidian (Finland) QuikRead go	Immunoassay	Capillary whole blood, anticoagulated venous whole blood 1 ul	5min 50sec	4.0–15% / 20–151 mmol/mol	<3%	CRP, CRP+Hb, wrCRP, wrCRP+Hb, Strep A, iFOBT
Arkray Inc. (Japan) The Lab 001	Capillary electrophoresis	Capillary whole blood, anticoagulated venous whole blood 1.5 µL	1.5min	4.0–16.0% / 20–151 mmol/mol	Total Error ≤ 6.0%.	No
Boditech Med (Republic of Korea) ichroma II	Immunoassay	Capillary whole blood, anticoagulated venous whole blood 5 µL	12min	4.0–15% / 20–140 mmol/mol	<5%	More than 50 different assays available
Green Cross MEDIS Corp. (Republic of Korea) CERA STAT 2000	Boronate affinity	Capillary whole blood, anticoagulated venous whole blood 5 µL	<3min	3.0–15.0% / 9–140 mmol/mol	<3.1 %	No
DiaSys (Germany) InnovaStar	Enzymatic	Capillary whole blood, anticoagulated venous whole blood 10ul	<5min	3.0–14.0% / 9–130 mmol/mol	<2% at 6.24% DCCT	CRP, glucose/haemoglobin
EKF Diagnostics (United Kingdom) 1. Quo-Lab	Boronate affinity	Capillary whole blood, anticoagulated venous whole blood 4ul	4min	4.0–15% / 20–140 mmol/mol	<3% at 7% A1c	No
2. Quo-Test	Boronate affinity	Capillary whole blood, anticoagulated venous whole blood 4ul	4min	4.0–15% / 20–140 mmol/mol	<3% at 7% A1c	No
HemoCue (Sweden) HbA1c 501	Boronate affinity	Capillary whole blood, anticoagulated venous whole blood 4ul	4min	20–130 mmol/mol	<3%	No
HUMAN Gesellschaft für Biochemica und Diagnostica GmbH (Germany) HumaMeter A1c	Boronate affinity	Capillary whole blood, anticoagulated venous whole blood 4ul	4min	4.0–15.0% / 20–140 mmol/mol	<3% at 7%A1c	No

i-SENS Inc. (Republic of Korea) A1Care	Enzymatic	Capillary whole blood, anticoagulated venous whole blood 2.5ul	4min 20 sec	4.0–15.0% / 20–140 mmol/mol IFCC	< 3%	ACR
Nova Biomedical (USA) Allegro	Immunoassay	Capillary whole blood 1.5ul	<7min	4.0–14.0% / 20–130 mmol/mol	<4.5%	Lipid panel, blood glucose, blood creatiine, PT/INR, CRP, urine creatinine, urine albumin
OSANG Healthcare (Republic of Korea) Clover A1c Plus	Boronate affinity	Capillary whole blood, anticoagulated venous whole blood 4ul	5min	4.0–14.0% / 20–130 mmol/mol	<3%	No
Roche Diagnostics (Switzerland) Cobas b 101	Immunoassay	Capillary whole blood, anticoagulated venous whole blood 2ul	5min 20sec	4.0–14.0% / 20–130 mmol/mol	<4%	Lipid panel, CRP
Siemens Healthineers (Germany) DCA Vantage	Immunoassay	Capillary whole blood, anticoagulated venous whole blood 1ul	6min	2.5–14.0% / 4–130 mmol/mol	<3%	ACR

Appendix 2: Participant Information sheet

UNIVERSITY OF THE WITWATERSRAND - JOHANNESBURG

School of Pathology
Department of Chemical Pathology

7 York Road, Parktown 2193 Telephone 011 7172561/2000

NATIONAL HEALTH LABORATORY SERVICES

PO Box 1038, Johannesburg 2000 Telephone 011 489 8505 /9000

Participant Information Sheet

Project title: Validation of the LumiraDx for the measurement of HbA1c for Diabetes monitoring and diagnosis.

Good day

My name is _____and I am working with some clinicians who are assisting in the detection of chemical biomarkers to diagnose and monitor health at the University of the Witwatersrand. We would like to invite you to participate in a research study to look at new and faster ways to detect such markers so that improved care for patients can be provided.

How can you help?

We would appreciate it if you could donate the following samples to us at the time you are seen:

1. At least 2 tubes (approximately 2 teaspoons) of venous blood by venipuncture: This is a standard of care blood collection procedure. The health care worker will place a needle into your arm and collect 10mL of blood. This will take less than five minutes.

2. 1 x finger stick blood (1 drop)

This is blood collected routinely as part of your normal visit to the diabetic clinic. Additionally, you will be invited to answer a few questions by filing in a questionnaire which should not take longer than 5 minutes. With your consent, your patient results may be obtained from the NHLS system including your HIV results if there are any.

What are we going to do with the samples you give us?

The sample taken will be used to evaluate a point of care test that detects blood sugar levels. The testing that will be undertaken will be for glycated hemoglobin (HbA1c)

which is a biomarker that gives indication of average blood sugar levels for the past 3 months. It is associated with diabetes mellitus.

Voluntary Participation

Participation in this study is entirely optional. You may opt not to participate in or exit this study at any time, without stating a reason. Your decision to leave the study will not impact the medical care you receive from your routine health services. If you do not take part in the study, we would like to record your age and gender, so we can check that the study is not unfairly excluding people.

If you agree to take part, but we find that there is a reason that we cannot include you in the study, we would like to use the information we collect today (such as information about your health, and test results). This is so we can understand whether any particular groups of people have been left out of the study.

Confidentiality

All information obtained during the course of this study, including hospital records, personal data and research data will be kept strictly confidential. Your personal information will not be shared with anyone unless they have received permission from the investigators and an authorized human research ethics committee. Under data protection law (POPI) “Protection of Personal Information Act 2013” your study site will be responsible as ‘data controller’ of your personal information to ensure that your information is safeguarded. Data that may be reported in scientific journals will not include any information that identifies you as a participant in this study. The information might also be inspected by the University of the Witwatersrand, Human Research Ethics Committee (HREC). These records will be utilized by them only in connection with carrying out their obligations relating to this study.

What are the potential risks of participating in the study?

The study will be conducted under rigorous safety settings to safeguard both you and the health practitioners who will be taking the samples. All health practitioners will be trained in phlebotomy and in the use of the POC device. You may experience minor discomfort when blood is being collected routinely:

Venipuncture:

Taking blood may result in pain, bleeding, or bruises where the needle enters your body. Rarely, taking blood may cause fainting or infection.

Finger stick:

Finger sticks may cause some slight irritation.

Only the medical professionals in charge of your clinical treatment and the laboratory that receives your samples and makes the diagnosis will have access to your name

and the clinical information you submit. Additional information will be made anonymous.

Potential Benefits

While taking part in this study won't have any immediate benefits for you, it will help us develop a new test that will help us diagnose and monitor diabetes at the site of patient care.

Cost of Participation

There is no charge for this test, and it is an entirely voluntary procedure.

Insurance

The University of the Witwatersrand has insurance cover for this study.

Contact details for any queries or concerns:

The Investigators in charge of this study are Ofentse Lesito, Dr Ngalula Kone and Prof Jaya George. They may be reached for answers to questions concerning this study at the following contact numbers:

Ofentse Lesito (MSc (Med) candidate): 079 474 7422

Dr Ngalula Kone (Supervisor): 011 489 8499/8427

Prof Jaya George (Supervisor): 082 325 7493

South African Health Products Regulatory Authority (SAHPRA)

In-country regulatory authority

Email: Boitumelo.Semete@sahpra.org.za

CEO Tel: 012 842 7629/26

The Human Research Ethics Committee (HREC) at the University of the Witwatersrand has approved the quality improvement procedure after it was presented to them. The protection of the rights and dignity of all individuals who consent to serve as research subjects as well as the objectivity of the study is one of the committee's main responsibilities.

If you have any questions about your rights as a study participant or if you would like to place a complaint regarding this study, please contact the Chairperson of this Committee, Professor Paul Ruff, on telephone number 011 717 2301, or by e-mail at Clement.Penny@wits.ac.za. The telephone numbers for the Committee secretariat are 011 717 2700/1234/2656/1252 and the e-mail addresses are Zanele.Ndlovu@wits.ac.za,Rhulani.Mkansi@wits.ac.za, Mapula.Ramaila@wits.ac.za and Iain.Burns@wits.ac.za.

The Declaration of Helsinki, which deals with the principles directing physicians in biomedical research involving human subjects, was followed in the study's format (last updated: October 2013). If you want to look at a copy, you can get one from me.

Thank you for reading this study information sheet.

Appendix 3: Participant Consent Form

UNIVERSITY OF THE WITWATERSRAND - JOHANNESBURG

School of Pathology
Department of Chemical Pathology

7 York Road, Parktown 2193 Telephone 011 7172561/2000

NATIONAL HEALTH LABORATORY SERVICES

PO Box 1038, Johannesburg 2000 Telephone 011 489 8505 /9000

INFORMED CONSENT

Project title: Validation of the LumiraDx for the measurement of HbA1c for Diabetes diagnosis and monitoring.

1. I have been given a Participant Information Sheet which explains the nature and processes involved in this study, which is attached hereto.
2. I was given time to read it, or had it read to me, in the language I best understand.
3. I was given time to ask any questions I wanted to and found any answers given to me to be reasonable and satisfactory.
4. I believe I fully understand why the study is being conducted and what the intended outcomes will be.
5. I understand that there will be no immediate benefit to me, should I agree to participate, nor will I receive any payment; conversely, participation will not cost me anything but my time.
6. I understand that, even if I initially consent to take part in the study, I may subsequently withdraw at any time and would not be required to give any reasons; if that happened, any data collected about me for the purposes of the study would immediately be destroyed, unless I give consent for it to be retained.
7. I have been given a range of contact details, listed below. If I require further information or become concerned about any aspect of this study, I am free to speak to any of these contacts.
8. I give permission for the Principal Investigator to access my TB and HIV status from the NHLS system.

Contact details:

Ofentse Lesito, Principal Investigator, telephone no. 079 474 7422, or by e-mail at 2088694@students.wits.ac.za,

Dr Ngalula Kone, Supervisor, on telephone no. 011 489 8499/8427, or by e-mail at ngalula.kone@nhls.ac.za

Prof Jaya George, Supervisor, on telephone no. 082 325 7493, or by e-mail at jaya.george@wits.ac.za

Dr Riffat Munir, Supervisor, on e-mail at rmunir@witshealth.ac.za

Professor P Ruff, Chairperson of the Human Research Ethics Committee (Medical) at the University of Witwatersrand, who may be contacted via any one member of the secretariat. The telephone numbers for the Committee secretariat are 011 717 2700/1234/2656/1252 and the e-mail addresses are Zanele.Ndlovu@wits.ac.za, Rhulani.Mukansi@wits.ac.za, Mapula.Ramaila@wits.ac.za and Iain.Burns@wits.ac.za.

Name of Participant: _____

Date: _____

Place: _____

Signature or mark: _____

Witnessed by:

Name of Witness: _____

Signature: _____

Date: _____

Appendix 4: Participant Questionnaire

UNIVERSITY OF THE WITWATERSRAND - JOHANNESBURG

School of Pathology
Department of Chemical Pathology

7 York Road, Parktown 2193 Telephone 011 7172561/2000

NATIONAL HEALTH LABORATORY SERVICES

PO Box 1038, Johannesburg 2000 Telephone 011 489 8505 /9000

Validation of the LumiraDx for the measurement of HbA1c for Diabetes diagnosis and monitoring.

Questionnaire

1. **How old are you?** _____
2. **What is your sex?**

   Male ☐ Female ☐

3. **Do you know your Body Mass Index (BMI)?** Yes ☐ No ☐

   If Yes, please state your BMI _____

   If No, please state your height (in meters) _____
4. **What is your weight?** (in kg) _____
5. **When last did you have your HbA1c level checked?** _____
6. **Do you have any known blood disorders?** (e.g. anemia, bleeding disorders such as hemophilia, blood clots, blood cancers) _____
7. **Have you had an HIV test before?**

   Yes ☐ No ☐ Prefer not to say ☐
8. **If you are comfortable, please share your HIV status by marking below.**

   Positive ☐ Negative ☐ Prefer not to say ☐
9. **If you are comfortable, please share your TB status by marking below.**

   Positive ☐ Negative ☐ Previous TB ☐ Unknown ☐

Appendix 5: HREC ethics certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Miss Ofentse Thoriso Lesito

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M230813 MED23-07-062

NAME: (Principal Investigator)	Miss Ofentse Thoriso Lesito
DEPARTMENT:	Chemical Pathology Charlotte Maxeke Johannesburg Academic Hospital
DEGREE:	Master of Science in Medicine
PROJECT TITLE:	*Validation of the LumiraDx for the measurement of HbA1c for Diabetes diagnosis and monitoring*
DATE CONSIDERED:	25/08/2023
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - Nicoleen.Potgieter@wits.ac.za
SUPERVISOR:	Dr N. Kone, Prof J. George and Dr R. Munir
APPROVED BY:	Mrs Matty Van Niekerk, Co-Chairperson, HREC (Medical)

DATE OF APPROVAL: 15/02/2024 **EXPIRY DATE:** 15/02/2029

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions.Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **August** and will therefore be due in the month of **August** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

16/02/2024
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 6: Approval from clinic to perform study

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Joburg
a world class African city

Research Committee of Johannesburg Health District

Enquiries: Prof S. Moosa |researchjoburg@gmail.com

DATE: 9 September 2024

ATT: Ms Ofentse Thoriso Lesito

EMAIL: ofentselesito765@gmail.com

Dear Sir/Madam

STUDY TITLE: Validation of the LumiraDx for the measurement of HbA1c for Diabetes diagnosis and monitoring

NHRD REF. NO.: GP_202408_063

OFFICIAL APPROVAL

The District Research Committee has reviewed your application. This letter serves as a final approval letter for this study.

The following conditions must be observed:

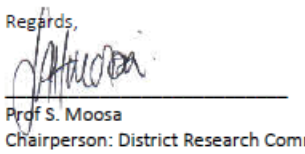
- The facilities in which the research will be conducted are listed below
- These facilities will be visited from: 2024/09/09 to 2029/02/15
- Participants' rights and confidentiality will be maintained all the time.
- Neither the District nor the facility will incur any additional cost for this study.
- No resources (Financial, material and human resources) from the above facilities will be used for the study.
- The study will comply with Publicly Financed Research and Development Act, 2008 (Act 51 of 2008) and its related Regulations.
- You will submit a copy (electronic and hard copy) of your final report. In addition, you will submit an annual progress report to the District Research Committee.
- If this is academic research then your supervisor and the University will ensure that these reports are being submitted timeously to the District Research Committee.
- The District must be acknowledged in all the reports/publications generated from the research and a copy of these reports/publications must be submitted to the District Research Committee.
- You will liaise with the manager/s listed below as relevant before initiating the study.

1

We reserve our right to withdraw our approval, if you breach any of the conditions mentioned above. Please feel free to contact us, if you have any further queries.

On behalf of the District Research Committee, we would like to thank you for choosing our District to conduct such an important study.

Regards,



Prof S. Moosa
Chairperson: District Research Committee
Johannesburg Health District
As delegated by Mrs M.L. Morewane, Chief Director, Johannesburg Health District, and Mr. Frans Moseane, Acting ED Health, City of Johannesburg

List of Facilities Approved

- Hillbrow CHC

List of Managers

Sub District/ Hospiital	Sub District Manager/ Area Manager	Contact No.	Cell phone	Email
ABCEF	Ms Prudence Mapeka		(084) 406 1917	prudence.mapeka@gauteng.gov.za

Appendix 7: Approval from hospital to perform study

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG
ACADEMIC HOSPITAL

Enquiries: Mr E Mosiamo
Email: edward.mosiamo@gauteng.gov.za
Tel: 011 488 4206
Ref: 1/7/2

To: Ofentse Thoriso Lesito

NHRD Ref: GP_202401_057

FINAL APPROVAL OF STUDY

STUDY TITLE: Validation of the LumiraDx for the measurement of HbA1c for Diabetes monitoring and diagnosis.

Permission to conduct the above-mentioned study at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) is granted subject to the following:

- Charlotte Maxeke Johannesburg Academic Hospital will not in any way incur or inherit costs as a result of the said study.
- Your study shall not disrupt services at the study sites.
- Strict confidentiality shall always be observed.
- Informed consent shall be solicited from participants in your study.

Before commencing data collection, please liaise with the relevant Head/s of Department and / or Unit to confirm arrangements, and agree on dates and times that suit all parties.

Once the research is completed, kindly forward the results to the CMJAH research committee Secretariat on cmjahresearch@gauteng.gov.za

Approved / Not Approved

Prof. Mary Kawonga
Chairperson: CMJAH Research Committee
Signed on behalf of the CEO, CMJAH
Date: Aug 6, 2024

Version2

Appendix 8: Evaluation of the Trinity Premier Hb9210

Quality Assurance
1 Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 386 6147 Fax: +27 (0)11 386 6296
Reference: QA Doc / Doc & policies
E-mail: patience.dabula@nhls.ac.za

Date: 21 July 2023

The Scientific Group (Pty) Ltd.
Cnr Epson Ave and Malibongwe Drive
Boundry Park
Northriding, 2169
South Africa

Attention: Alet Pieterse

Evaluation of the Scientific Group Trinity Premier Hb9210

Thank you for providing the NHLS with an opportunity to evaluate the Scientific Group Trinity Premier Hb9210.

The details of the evaluation were as follows:
HTA project Identification number: HTP22-33
Evaluation Site: NHLS, Inkosi Albert Luthuli Central Hospital, Department of Chemical Pathology
Evaluation Site contact person: Dr Verena Gounden
Evaluation Period: November 2022 to December 2022
]

Recommendation:
The Scientific Group Trinity Hb9210 is recommended for use in the NHLS and is deemed fit for clinical purpose. All labs introducing this analyser should perform own in house verification as per NHLS policy/Local SOPs
This report was independently reviewed by two members of CTAC (Dr Aye Aye Khine and Dr Siphokazi Gwiliza)

NB: Please see the full report for details.
Please be advised that the NHLS is not obligated to place the above product; the supplier would have to follow the NHLS Procurement procedures for all purchase and/or placement agreements.

Yours sincerely

Dr Verena Gounden

NHLS Chemistry Expert Committee Chair

Patience Dabula

National Manager: Quality Assurance

Appendix 9: Method Comparison report between the Bio-Rad Variant II and Premier Hb9210

Method Comparison: EP09- Trinity Biotech Premier Hb9210 for HbA1c

Analysis in R by Dr Sean Currin

31/07/2024

Method X (Old): BioRad Variant II

Method Y (New): Trinity Biotech Premier Hb9210 HbA1c Analyzer

Laboratory

Charlotte Maxeke JHB Academic Hospital Development Laboratory, Department of Chemical Pathology

Analyte

Glycated haemoglobin (HbA1c)

Method

Boronate Affinity HPLC

Protocol

- Method comparison according to CLSI EP09 protocol
- 50 patient samples were run over multiple days consisting of whole blood EDTA samples

Reagent kit used

Trinity Biotech Hba1c

Date of validation

10 - 31 July 2024

Explanation of any deviation from protocol

- A minority of samples were run that were processed more than 24 hours after collection due to the rarity of samples at the extremes of range, however most samples fell within the 24 hour period, the stability has been found to be acceptable for up to 7 days at room temperature regardless.
- The EP09 experiment took place over 21 days due to difficulty in finding samples at the extreme of ranges, the EP09 experiment also took place during a time of decreased sample load at the NHLS.

Conclusion

Acceptance criteria

- Allowable Bias: 1.8% (based on EFLM Biological Variation Desirable Specifications)
- Manufacturer claims: 0.3% HbA1C % units (i.e. absolute difference)

Note:

Total allowable error: 5% (based on National Glycohaemoglobin Standardisation program (NGSP))

Ideal Correlation coefficient (r) > 0.95

Performance

Absolute Bias: -0.14 %HbA1c units (95% CI: -0.22 to -0.06)

Percentage Bias: -1.8 % (95% CI: -2.68 to -0.92)

Correlation coefficient: 0.997 (95% CI: 0.995 - 0.998)

Comment

There is a negative bias of -1.8%. This negative bias is within EFLM Biological Variation Desirable performance specifications of 1.8%. However, 95% confidence intervals cross the 1.8% target, it is noted that 95% confidence intervals are within the 2.7% allowable bias for minimal performance specifications. The absolute bias of -0.14%HbA1c units, including 95% confidence limits, is within manufacturer claims of 0.3%HbA1c units bias.

The two methods show excellent linearity with a Pearson correlation coefficient of 0.997.

The two methods are comparable and the level of bias present is not clinically significant.

Recommendations

Fit for purpose

Accepted by

Dr Sean Currin

31/07/2024

Appendix 10: Quality Control for the analysers used in the study

1. LumiraDx (LumiraDx UK Ltd, London, UK)

The LumiraDx scanned the 2D barcode on each test strip to verify its expiration status. It also checked if the calibration file for the specific test strip lot was loaded; if not, the instrument prompted the user to install it. Once the correct calibration file was installed, the instrument gained access to all necessary information to process future tests with the same lot. Additionally, the LumiraDx instrument and HbA1c test strips have multiple built-in quality control features to ensure the accuracy of each test (Table 0**.1**). These functions verified that the sample volume was adequate and that the test strip's assay sequence was as expected. They also checked for any damage or prior use of the test strip. If any quality control checks failed, the instrument rejected the test and displayed an error message on the touchscreen.

Table 0.1: Features incorporated in the LumiraDx instrument to maintain the integrity of test results

Automated Functionality Checks	The instrument performs automated checks upon power-on and during operation, assessing the functionality of electrical components, heater performance, battery status, mechanical actuators and sensors, and optical system performance.
Continuous Monitoring	The performance of the test strip and control measures is continuously monitored throughout the test runtime.
Quality Control Testing	The instrument can execute quality control tests using LumiraDx Quality Control solutions to meet regulatory compliance requirements.

2. Bio-Rad Variant™ II Haemoglobin Testing System (Bio-Rad laboratories Inc. , California, United States)

The IQC procedure was performed at the start of each testing session and as needed throughout the run to verify system performance. A blank sample and two QC material consisting of a normal and an abnormal level, were used. The QC material (Bio-Rad, Lyphochek Diabetes Control, Bilevel) which is prepared from human whole blood, that came in a lyophilized form. To reconstitute the QC materials, a calibrated pipette was used to add 0.5 ml of deionized water to each vial. Subsequently, for 10 to 15 minutes the vials were placed on a blood mixing roller which gently swirled the vials to enable proper mixing and ensure homogeneity. To prepare the blank and the QC samples to be ran, 1.5 ml of diluent was mixed with 5 µl of the reconstituted QC material (control 1 (normal), was used for the blank). The QC results were evaluated to ensure that they fell within the acceptable range specified by the manufacturer.

3. DCA Vantage® Analyzer (Siemens Healthineers, Erlangen, Germany)

Calibration was essential for each new lot of DCA cartridges, with each box containing a unique calibration card specific to its lot number. Before using a new box of cartridges, the calibration card had to be scanned and saved in the analyser. Values for reagent calibration parameters were encoded on the calibration card provided with each reagent cartridge. If multiple boxes shared the same lot number, the calibration card only needed to be scanned once. Scanning of the card enabled the instrument to access the appropriate calibration parameter values and calibration curve for the reagent lot in use. Additionally, two levels of QC were analysed after the calibration process to ensure accurate performance.

The Internal Quality Control (IQC) procedure was performed daily using two levels of aqueous material: normal and abnormal. To reconstitute the QC materials, the vials were removed from the fridge, gently tapped to settle the powder at the bottom, and six drops of reconstitution fluid were added to the powder. The first drop of the reconstitution fluid was discarded. The vials were then closed and gently swirled for mixing, allowing the solution to sit for 15 minutes before use.

If the QC material had already been reconstituted, it was taken from the fridge and allowed to warm up at room temperature for 10 minutes, along with the cartridge. The QC card was scanned—one side being Normal (GREEN) and the other Abnormal (BLUE)—followed by

scanning the cartridge. A single drop of QC was placed onto a clean glove or cartridge foil using a plastic pipette and collected using a glass capillary. Excess sample was wiped off before placing the glass capillary into the cartridge. The cartridge was then inserted into the analyser with the label facing right, the foil tab was completely removed, and the cartridge compartment door was shut. This process was repeated for the next QC, ensuring the correct side of the card was scanned. If a QC test failed, the failed level was repeated, and checks were made to ensure the cartridges and QC had not expired. In the case of persistent failure, the manufacturer was contacted.

4. Premier Hb9210™ (Trinity Biotech Plc., County Wicklow, Ireland)

The QC samples were tested at the beginning of each testing session and as necessary to verify analytical performance during the testing process. The QC materials (Trinity Biotech Level I and Level II Haemoglobin A1c Controls) consist of hemolysate containing oxyhaemoglobin with low and high HbA1c values. These controls are derived from non-diabetic and glycated non-diabetic whole blood. To reconstitute the QC samples, a calibrated pipette is employed to add 500 µl of Trinity Biotech Diluent to each vial. Following this, the vials are placed on a blood mixing roller for approximately 10 minutes, allowing for gentle swirling that ensures proper mixing and homogeneity of the solution. To prepare the QC samples for analysis, 1490 µl of diluent is combined with 10 µl of the reconstituted QC material. The QC results are then evaluated to confirm that they fall within the acceptable range specified by the manufacturer, thereby ensuring the reliability and accuracy of the testing procedures.