The gold standard under the microscope: navigating HbA1c standardisation and discrepancies

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Introduction

Diabetes is no longer just a medical concern; it is a major global health crisis [1]. Cases are projected to reach 853 million by 2050, with the vast majority living in low- and middle-income communities, representing a massive shift in global demographics and disease burden [2]. For these patients, the impact is deeply personal. Undiagnosed or misdiagnosed diabetes leads to devastating complications such as retinopathy, loss of vision and amputations that could have been prevented.

At the heart of this diagnostic journey sits a single, critical marker - Haemoglobin A1c (HbA1c). For decades, it has been the cornerstone of diabetes management and since 2011 also advocated by the WHO for the diagnosis of diabetes at a value of 48mmol/mol (6.5%), in line with American Diabetes Association (ADA) recommendations [3].

HbA1c is formed by the non-enzymatic binding of glucose to haemoglobin and reflects average blood glucose levels over approximately 3 months, particularly the preceding 30–60 days. Major studies, including the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS), demonstrated that lower HbA1c levels are associated with reduced risk of diabetes-related microvascular and macrovascular complications [4,5]. Despite its widespread use, analytical variation remains a silent disruptor in clinical care. Without rigorous standardisation, not all HbA1c results mean the same thing. This translates to real consequences: patients could be told they are living with diabetes when they are not, or worse, have a diagnosis missed entirely.

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Beside analytical variation there is also the non-glycaemic biological variation of HbA1c. It refers to differences in HbA1c levels that occur independently of blood glucose concentrations. While HbA1c is primarily used as an indicator of average glycemia over the previous 2–3 months, several biological factors can influence its value without reflecting true changes in glucose control. These factors include variations in red blood cell lifespan, age, ethnicity, genetic traits, anemia, hemoglobin variants, and certain medical conditions. Understanding non-glycemic biological variation is important because it can lead to HbA1c values that either overestimate or underestimate an individual's actual glycemic status, potentially affecting the diagnosis and management of diabetes.

Specifically, research into non-glycaemic biological variation of HbA1c reveals a sobering reality: even if a laboratory achieves perfect analytical performance, with zero bias and zero imprecision, the inherent biological variation among individuals means that the risk of misclassification remains as high as 11% [6]. This highlights the importance of considering the full clinical picture when making a diagnosis, rather than relying on a single parameter.

This article explores how the global medical community is moving from chaos in HbA1c results to global standardisation, how nations like Thailand are embracing quality assurance, and what clinicians must do when the laboratory result doesn't match the clinical picture.

Speaking the same language: the evolution of standardization

Historically, if a patient had their blood drawn in two different laboratories, the results could vary significantly. The inter-laboratory coefficients of variation (CV), a statistical measure of precision, frequently exceeded 15-20%. In practice, a patient whose results showed good control at one hospital could be flagged as "high-risk" at another.

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) represents true scientific standardisation. It defines the analyte with absolute precision: glucose attached to the N-terminal valine of the haemoglobin beta chain. The IFCC system is traceable to a primary reference method using pure A1c and A0 (non-glycated haemoglobin) as a standard [7]. It provides the "scientific truth" and the metrological foundation that ensures a test measures exactly what it claims to measure.

To understand the current state of diabetes diagnostics, we must look back at the initial absence of a global standard. Before the IFCC standard, there were no pure calibrators and no primary reference method, meaning laboratories operated in a landscape of testing where results were often incomparable.

The National Glycohaemoglobin Standardization Program (NGSP) chose a pragmatic path of harmonisation. They decided to align all methods to the specific testing method used in the landmark DCCT and UKPDS studies [3,4].

This was a vital move to ensure that patient results could be linked to the known risks of long-term complications, even before a true metrological system existed.

Today, these two systems do not compete; they co-exist through a necessary compromise. To ensure global consistency, a master equation was established to link the NGSP's clinical harmonisation with the IFCC's scientific standardisation [8].

The workflow that maintains this alignment is complex but vital. It moves from the patient's sample up to the clinical laboratory, then to manufacturers, and finally to reference laboratories. While regions like the United States prefer NGSP units (%) for their familiarity, and others prefer IFCC units (mmol/mol), the underlying standardisation ensures that these units are mathematically convertible and scientifically valid [9].

Today, thanks to a concerted international effort of the NGSP and the IFCC, the variability of HbA1c between different HbA1c methods has dropped to around 3.5% [10].

The Role of External Quality Assurance

While standardisation is the responsibility of manufacturers, ensuring their instruments are calibrated to the IFCC primary reference method before they ever leave the factory, the laboratory's role is to verify that these instruments perform correctly in the real world. This is the domain of External Quality Assurance (EQA).

The purpose of an EQA scheme is effectively a global reality check. It ensures that the standardisation promised by manufacturers holds true in clinical practice. Fundamentally, EQA asks: does a blood sample tested in Bangkok yield the same result as one tested in London? Without this independent verification, local variables can lead to inconsistencies that compromise patient care.

Thailand offers a compelling case study on how a nation can implement EQA to check the analytical performance of different HbA1c methods. Recognising that inconsistencies among Thai laboratories were threatening the accuracy of diabetes monitoring, the Department of Medical Sciences launched the National External Quality Assurance (EQA) Haemoglobin A1c Programme in 2016 [11].

This intervention was distinct in its design. Unlike some EQA schemes that rely on processed samples, the Thai model collaborated with major university hospitals such as Ramathibodi and Siriraj and a certified IFCC and NGSP reference laboratory to implement an accuracy-based programme using fresh blood panels. Fresh whole blood has a greater commutability than lyophilized material. Although lyophilized material has the advance of longer stability, the differences observed between analytical methods with fresh whole blood are more likely to reflect true methodological differences rather than artifact introduced by sample processing like lyophilization It was innovative because it was accuracy-based, with values assigned using four IFCC and NGSP secondary reference measurement procedures. In many EQA schemes, results are compared only with those of laboratories using the same analytical method, without establishing whether the reported values are actually correct or accurate. This approach, in contrast, enabled the assessment of true analytical accuracy rather than merely method-specific agreement.

The results of this program provided a clear view of the landscape. The data highlighted that while the bias (the difference between the average test result and the reference value) was often small, the overall CV (variability) remained high. This underscored that while labs were generally aiming at the right target, their precision needed tightening.

The future of this model is ambitious. It includes expanding the EQA network beyond major hubs to community hospitals and linking this data to a nationwide e-health system. This initiative aims to bridge the gap between rural and urban healthcare, ensuring that a patient receives a consistent high level of diabetes care regardless of where the participating labs are located.

Continuous Glucose Monitoring (CGM) and HbA1c: a more consistent and complete glycemic picture

A revolution in wearable technology, like CGM, has introduced a new variable for the lab and the clinician to reconcile.

These CGM devices offer a wealth of data that a single lab test cannot, specifically capturing real-time fluctuations and hypoglycemic trends. While an HbA1c result provides a three-month average, it can mask dangerous "lows" if they are balanced out by "highs." CGM fills this gap by recording the "time in range" and identifying patterns of hypoglycemia that require immediate clinical intervention [12].

A key metric generated by these devices is the Glucose Management Indicator (GMI). It is important to distinguish this from a real-time glucose reading. GMI is a derived number; it is an estimate of what a patient's HbA1c would likely be, calculated based on the average glucose values captured by the CGM over the previous 14 days [13].

However, clinicians are increasingly facing a confusing scenario where the patient's GMI says one thing, but the laboratory HbA1c result says another. Because the GMI is a "predicted A1c" based on a short two-week window of glucose data, while the lab-measured HbA1c is a physical measurement of glycated haemoglobin over three months, the two markers are frequently discordant.

Understanding this difference is critical: the lab-measured HbA1c remains the gold standard for long-term risk, but the CGM and its GMI offer the granular detail necessary to adjust daily therapy and catch the "hidden" lows that a laboratory test simply cannot see.

To resolve this discordance, one must understand that these two tools measure different aspects of biology.

- HbA1c is a weighted average of glucose levels over the past 3 months [3]. It is fundamentally tied to red blood cell (RBC) biology. It captures the overall glucose exposure but misses the peaks and valleys.
- GMI captures real-time trends, hypoglycaemic events, and variability [13]. However, it is calculated from glucose measured in interstitial fluid, not blood, and is subject to sensor lag and user-related factors (e.g., wear time, sensor adequately immobilised).

Authors of a recently published paper suggested that a difference of ≥ 0.8% (9 mmol/mol) between the GMI and the HbA1c is the threshold for clinical significance [14]. Smaller variations typically reflect normal biological and analytical differences. When values diverge significantly, systematic evaluation becomes essential, and clinicians must investigate.

Investigating discordance between GMI and HbA1c readings

The investigation requires a dual approach, checking both the technology and the biology:

- Sensor check: Was CGM sensor wear time sufficient to ensure reliable GMI estimation? Were there "compression lows" caused by sleeping on the sensor?
- Patient check: Are there conditions altering the lifespan of red blood cells? Is the patient anaemic? Is the patient pregnant? Is there a haemoglobin variant present?

Ultimately, GMI cannot replace HbA1c, but rather a complementary tool. HbA1c provides the validated average that guides diagnosis and major treatment decisions. CGM reveals patterns informing day-to-day management. When they align, confidence increases. When they diverge, investigation reveals important clinical information that neither measure alone would provide.

When the Laboratory is "Right" but the Result is "Wrong"

Even with analytical accurate HbA1c methods fully traceable to the IFCC primary reference method and accurate sensors, biological interference can lead to results that are analytically correct but clinically misleading. Two specific scenarios often arise that highlight the need for laboratory-informed care.

Scenario A: The Haemoglobin Variant

Genetic variants in haemoglobin, such as HbJ Baltimore, can dramatically affect HbA1c measurements. Depending on the assay method used (for example, High-Performance Liquid Chromatography (HPLC) versus Immunoassay), variants can produce falsely low or high HbA1c results [15].

- The Risk: A patient might be treated for diabetes they do not have, risking dangerous hypoglycaemia. Conversely, a diabetic patient might be left untreated because their HbA1c looks falsely normal.
- What to look out for: In these instances, the HbA1c is analytically correct but physiologically irrelevant. Alternative markers, such as Fructosamine, Glycated Albumin and or CGM data, become essential.

A commitment to laboratory-informed care

The journey to optimised diabetes care is not solely about better drugs or newer technology; it is about confidence in the numbers that guide our decisions. The transition from the era of poorly harmonized HbA1c testing to global standardization has been a triumph of scientific collaboration, but the work is not finished.

Laboratories must continue to commit to strategic method selection, choosing assays based not just on cost, but on interference patterns and manufacturer support. Mandatory participation for laboratories and users of point-of-care devices in accuracy-based EQA programmes is non-negotiable for maintaining accuracy.

For clinicians, the path forward involves a shift in perspective. A test result is not a verdict; it is a piece of data that must be interpreted within the context of the patient's unique physiology. By understanding the limitations of HbA1c, the nuances of GMI, and the potential for interference, healthcare professionals can ensure that every diagnosis is accurate and every treatment plan is safe. The synergy between robust standardisation and clinical awareness is the only way to ensure patient safety. The ultimate goal is simple yet profound: to ensure every HbA1c result is traceable, comparable, and clinically meaningful, driving reliable and equitable diabetes care worldwide.

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