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Invited critical review

Current aspects in hemoglobin A1c detection: A review

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a pressing health issue that threatens global health and the productivity of populations worldwide. Despite its long-recognized role in diabetes management, glycated hemoglobin (HbA1c) only received WHO endorsement as a T2DM diagnostic tool in 2011. Although conventional plasma-specific tests have long been utilized to diagnose T2DM, the public should be informed that plasma-specific tests are not markedly better than HbA1c tests, particularly in terms of variability and convenience for diagnosing diabetes. In the midst of the debates associated with establishing HbA1c as the preeminent diabetes diagnostic tool, unceasing efforts to standardize HbA1c tests have played an integral part in achieving more efficient communication from laboratory to clinical practice and thus better diabetes care. This review discusses the current status of HbA1c tests in the diagnosis, prevention, treatment and management of T2DM across the globe, focusing on increasing the recognition of glycated hemoglobin variants with effective utilization of different HbA1c methods, updating the current status of HbA1c standardization programs, tapping into the potential of POC analyzers to establish a cost-effective HbA1c test for diabetes care, and inspiring the advancement of HbA1c biosensors for future clinical usage.

Contents

1. Introduction: significance of the study . . . 202
2. Biological challenges encountered in HbA1c tests: glycated hemoglobin and its variants . . . 203
   2.1. Plasma-specific tests versus HbA1c test for diagnosis . . . 203
   2.2. Current trends in glycated/glycosylated hemoglobin (HbA1c) tests for diagnosing diabetes . . . 204
3. Towards standardization of HbA1c tests . . . 205
4. Limitations of HbA1c tests in laboratory settings . . . 205
   4.1. Point-of-care (POC) technologies: reliability in doubt . . . 206
   4.2. New ideas for HbA1c tests: glycated hemoglobin biosensors . . . 208
5. Prediabetes in predicting incident diabetes: is HbA1c an effective screening tool? . . . 208
6. Glycated hemoglobin in the treatment and long-term management of diabetes . . . 209
7. Conclusions . . . 209
8. Future perspectives . . . 209
Conflict of interest . . . 210
Acknowledgments . . . 210
Appendix A. Supplementary data . . . 210
References . . . 210

1. Introduction: significance of the study

Type 2 diabetes mellitus (T2DM) is a global epidemic health issue. Approximately 439 million adults (7.7% of the world's population aged 20–79 years) are estimated to be afflicted with diabetes by 2030 [1]. As a consequence of population growth, longer life expectancy, and lifestyle changes, the estimated 54% increase in T2DM incidence worldwide by 2030 is concerning [1]. Although the spreading of the chronic disease itself is worrisome, the medical complications and socioeconomic impacts associated with diabetes are as fearful as the disease itself. Individuals with diabetes are at increased risk of developing

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microvascular (mainly retinopathy, nephropathy, and neuropathy) and macrovascular (especially stroke and coronary artery) diseases. Acute episodes such as diabetic ketoacidosis, hyperosmolar coma, and severe hypoglycemia can lead to morbidity and mortality [2], which could become a socioeconomic burden.

Diabetes is a metabolic disorder that consists of multiple etiologies characterized by chronic hyperglycemia with disturbances in the carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [3]. The diagnosis of diabetes can be very complicated, due to variations in diabetic symptoms. Some diabetic patients can be asymptomatic, whereas others have evident symptoms. In general, the diagnostic tests for type 2 diabetes mellitus can be grouped into two distinct categories, namely plasma-specific tests and the whole blood glycated hemoglobin (HbA1c) test. The HbA1c test has been the subject of multiple controversies, typically focusing on the precision and accuracy associated with different commercialized detection methods. Nonetheless, the role of glycated hemoglobin as a distinctive biomarker for type 2 diabetes mellitus is undeniable. Hence, a biological understanding of the role of HbA1c is necessary before a discussion of the detection methods.

2. Biological challenges encountered in HbA1c tests: glycated hemoglobin and its variants

Some biological problems can interfere with HbA1c assays, particularly when glycated hemoglobin variants are present, when the red blood cells' turnover rate is affected by morbidities, or when the glycation rate is influenced by genetic and dietary factors (for a detailed description please refer to Hare et al. [4]). In general, a healthy individuals' hemoglobin can be categorized into the following three groups: HbA (adult hemoglobin, 97%), HbA2 (2.5%) and HbF (fetal hemoglobin, 0.5%) [5]. The majority of HbA is not glycated (approximately 94%), and approximately 6% of HbA can be categorized as glycated hemoglobin [5]. In other words, as the main glycated component (HbA1a and HbA1b together contribute approximately 1%), HbA1c composes approximately 5% of the total hemoglobin in a healthy individual [6] (Fig. 1).

In general, glycation occurs spontaneously and non-enzymatically when glucose reacts with the amine groups in proteins to form a stable ketoamine, the Amadori compound (refer to Fig. 2). Further oxidation and rearrangement result in more reactive species, also known as advanced glycation end-products (AGEs), which are thought to be involved in diabetic complications [7]. Of the glycated protein species, the glycated hemoglobin, HbA1c, was chosen to gauge glycemic control in diabetic patients due to the stability of its Amadori form. HbA1c forms when glucose attaches specifically to the N-terminal valine of the β subunit of hemoglobin. However, only 60% of glucose is attached to N-terminal β-chain valines. Glycation can also occurs on the lysine side chains either on the α or β chains [8,9].

The glycated hemoglobin variants exist due to congenital disorders of globin chain synthesis, called "hemoglobinopathies". Although some variants can directly interfere with the HbA1c test, some variants interfere by causing premature turnover of red blood cells. In cases where an individual is heterogeneous, the individual will be asymptomatic and have normal red cell survival. For example, HbS homozygosity leads to sickle cell anemia, which involves premature turnover of red blood cells, whereas those who are heterozygous for the sickle cell allele are asymptomatic [10]. Because variants can lead to over or underestimation of glycated hemoglobin, it is very important to perform screening for hemoglobinopathies before HbA1c definitive tests, particularly for those of Mediterranean, African or Southeast Asian heritage, who exhibit a higher prevalence of hemoglobinopathies [11].

2.1. Plasma-specific tests versus HbA1c test for diagnosis

There are different tests for the measurement of glucose levels. Because tests employed in clinical settings depend on clinical decisions, variation in diabetes diagnostic tests is common across different health systems. Conventionally, the fasting plasma glucose (FPG), 2-hour post glucose loading with oral tolerance test (OGTT) and random plasma glucose (RPG) are categorized as plasma-specific tests, in which the plasma glucose is measured.

The most widely accepted type 2 diabetes mellitus diagnostic tests are the fasting plasma glucose (FPG) and the 2-hour plasma glucose by the oral glucose tolerance test (OGTT). Because not all patients show symptoms of diabetes, the use of a random plasma glucose test, which involves apparent symptoms, such as unexplained weight loss, increased thirst, and a high level of glycosuria, is not practical for the diagnosis of diabetes in all cases [13]. While both FPG (diagnostic of diabetes at plasma glucose level ≥126 mg/dL or 7.0 mmol/L [14]) and OGTT (diagnostic of diabetes at plasma glucose level ≥200 mg/dL or 11.1 mmol/L [14]) are commonly used diagnostic tests, the associated advantages, such as low cost and the popularity of automated laboratory machines, make FPG the preferred test. Even so, FPG is commonly affected by biological variations, preanalytical variations such as extended incubation time of the blood samples (glycolysis can occur), and analytical variations (in which the serum instead of plasma glucose concentration is measured) [15]. While analytical variations can occur when serum instead of plasma is used for the glucose measurement, findings have concluded that the glucose measurement in serum and plasma do

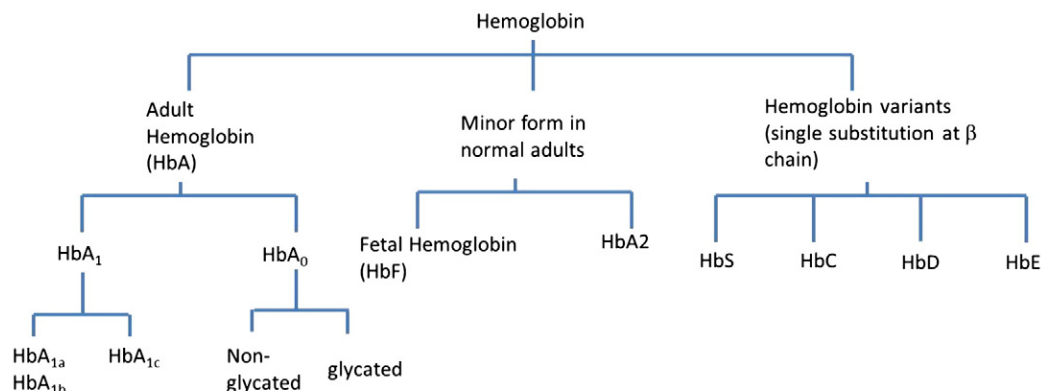

Fig. 1. Hemoglobin and its variants. Hemoglobin can exist in mainly three forms in healthy adults, HbA contributes to around 97% of the whole pool, while the rest of hemoglobin can exist in HbF or HbA2 forms. HbA, HbF, and HbA2 are unique with the different subunits. HbA has two α and β subunits (ααββ), while HbF is made up of two α and γ subunits (ααγγ), and HbA2 is consist of two α and δ subunits (ααδδ). On the other hand, hemoglobin variants exist at the expense of single amino acid substitution at the beta chain of HbA. The variants shown in the figure are the general ones; there is a span of variants that could be silent while interfering with HbA1c tests (not shown in figure). Glycation occurs mostly on HbA1 species and some on HbA_0 species; majority of the glycated HbA1 species is the HbA1c fraction [5,12]. Glycation can also occur on the variants as long as the glucose-binding moiety is not affected by the substitution.

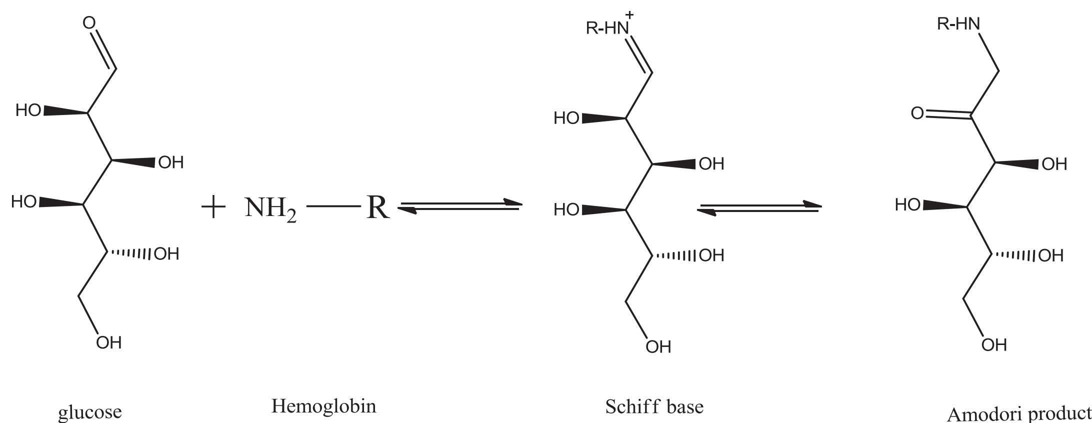

Fig. 2. Formation of glycated hemoglobin. Along the lifespan of a red blood cell (120 days), the hemoglobin is constantly in contact with glucose in the blood stream. Glucose reacts nonenzymatically with the hemoglobin amine group, and when it specifically binds to valine of beta chain of hemoglobin, it undergoes the intermediate stage (Schiff base) and becomes stable Amodori product, which is known as HbA1c (the glycated hemoglobin). There are whole series of membrane proteins that undergo the same glycation reaction in the blood stream, most of them undergo further oxidation and rearrangement to become a more reactive species, advance glycation end-products (AGEs) (not shown in the figure) that are perceived to be responsible for the long term diabetes complications.

not differ substantially [16]. However, clinical organizations do not recommend the measurement of serum glucose for the diagnosis of diabetes [16].

Although the OGTT has long been established as one of the diagnostic modalities for diabetes, compared with FPG, it is less favored as a plasma glucose test in clinical settings [15]. In fact, the WHO discouraged the use of the OGTT for the diagnosis of diabetes due to its inconvenience, high cost, and poor reproducibility [17]. Due to its principle of testing the efficiency of carbohydrate metabolism, patients who undergo the OGTT need intensive preparation before the test. In addition to requiring a 10- to 16-hour fast, the test has to be performed in the early morning between 7 and 9 am, which can be very tedious for both patients and clinicians [15]. As previously mentioned, the casual plasma glucose (random plasma glucose, RPG) indicates diagnosis of type 2 diabetes when the plasma glucose is ≥200 mg/dL (11.1 mmol/L) in combination with apparent classical diabetes symptoms. Although the random plasma glucose test can be carried out anytime, it is very insensitive as a screening tool for diabetes [18]. To maximize the test's sensitivity, a 3-year epidemiological study suggested that the cutoff should be adjusted to RPG ≥130 mg/dL (7.2 mmol/L) to provide good yield and minimize false positives [19]. While some argue that casual plasma glucose is very convenient to avoid a long fast, standardized HbA1c tests offer a better alternative that remains convenient while offsetting the sensitivity issues associated with RPG.

Despite being considered the preferred plasma-specific T2DM diagnostic test, the biological variability of FPG is 14%, indicating that the test is neither perfectly stable nor free of laboratory variability [20]. Additionally, the comparison of both the FPG and HbA1c tests with the OGTT revealed no evidence that FPG is superior to the HbA1c test [21]. Because there is no specific evidence to endorse the superiority of the FPG test, the wide implementation of the HbA1c test as a diagnostic tool for type 2 diabetes should be considered.

2.2. Current trends in glycated/glycosylated hemoglobin (HbA1c) tests for diagnosing diabetes

Given the ease of testing without prolonged patient preparation, low intra-individual biological variability (stable in the presence of sudden glycemic variations, thus providing a better reflection of the plasma glycemic status over the past 2–3 months), and greater reproducibility [22], the HbA1c test is rapidly replacing the conventional approach for diagnosing diabetes. This shift has a huge impact on the public health sector, which is accustomed to the conventional plasma-specific tests.

In 2010, the ADA (American Diabetes Association) endorsed HbA1c as a diagnostic criterion for diabetes [23]. The ADA selected a result of 6.5% as the diagnostic value for type 2 diabetes mellitus (T2DM), gauged by the development of diabetic retinopathy, which increases steeply at ≥6.5% [23]. In 2011, the HbA1c test was endorsed by the WHO as a diabetes diagnostic test, provided that the measurements are performed by standardized HbA1c tests that passed the stringent quality assurance tests [24]. As such, HbA1c has officially become a diabetes diagnostic tool.

Although its role as a diabetes biomarker is undisputable, HbA1c has yet to receive the designation of "the" diabetes diagnosis tools across the globe due to initial difficulties in standardizing HbA1c assays. For example, the China Guideline regarding type 2 diabetes in 2010 did not recommend the HbA1c test for the diagnosis of diabetes due to inconclusive results in the Chinese population and the lack of a standardized HbA1c measurement nationwide [22]. More longitudinal epidemiological studies have to be conducted to identify the demographic and ethnic factors that may potentially contribute to complications in using HbA1c in type 2 diabetes diagnosis. Even after the role of HbA1c was recognized, arguments regarding the fluctuations in HbA1c levels due to genetic and biological variations, coexisting medical complications, assay interference and high costs associated with its wide application in health systems have continued [25]. Instead of debating the plausibility of implementing HbA1c as a diagnostic tool, it is more realistic to resolve the problems and adjust the diagnostic cutoffs according to demographic, anthropometric, or laboratory measurements to maintain consistency without losing the sensitivity of the HbA1c tests.

Studies have been conducted to mitigate the disputes by combining the results of the HbA1c test with the preferred plasma-specific test, namely the fasting plasma glucose test (FPG). An eight-year longitudinal prospective study of the Chinese population concluded that the HbA1c test was better at predicting the incidence of diabetes than the fasting plasma glucose test in clinical practice, the study suggested that the combination of FPG, HbA1c and WC (waist circumference) be used to accurately diagnose diabetes [26]. Additionally, a study conducted in Tokyo found that the combination of FPG at 6.1–6.9 mmol/L and 6.0–6.4% HbA1c levels yielded a prediction of an absolute progression to type 2 diabetes over the course of five years [27], indicating that the combination of plasma and HbA1c tests can be used to improve the diagnosis efficiency.

In short, HbA1c tests have huge potential to aid the diagnosis and screening of type 2 diabetes. Although there were some obstacles initially, intensive efforts to standardize HbA1c methods are slowly gaining traction in clinical laboratories across the globe. As such, better adaptation

of the HbA1c test as a diagnostic tool is to be expected when all HbA1c methods are standardized against the IFCC (International Federation of Clinical Chemistry) reference methods.

3. Towards standardization of HbA1c tests

Ever since the Diabetes Control and Complications Trial (DCCT) [28] and the UK Prospective Diabetes Study (UKPDS) [29] showed a good relationship between glycemic control and reduced outcome risks, the precise measurement of HbA1c (long used as a glycemic management test) was highly sought after. Although HbA1c methods flourished, the lack of a primary reference method led to inconsistent interpretations of the HbA1c level. In the absence of an international standard method to measure HbA1c, few countries have initiated national standardization programs to set a reference method. For example, Mono-S ion-exchange chromatography was employed as the standard method in Sweden, whereas the Japan Diabetes Society (JDS) used two calibrators with JDS-assigned values [30]. In an effort to standardize HbA1c tests, the NGSP (National Glycohemoglobin Standardization Program) started out as the subcommittee of the AACC (American Association for Clinical Chemistry) in 1996, with a network of reference laboratories calibrated against the DCCT reference values [30]. Secondary Reference Laboratories (SRLs) under the NGSP work specifically with manufacturers to standardize HbA1c kits or methods and compare data directly with the DCCT results for method certification [31]. The IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) Working Group, whose purpose is to perfect reference methods for higher-level calibration systems, established a higher-order reference method in 2001 (refer to Section 4 for a discussion of the IFCC reference method). Although both the IFCC and NGSP have established distinctive laboratory networks, continuous monitoring within and between IFCC and NGSP laboratory networks allows harmonization of HbA1c results worldwide [32]. To monitor the effectiveness of the NGSP in harmonizing the HbA1c results, it is evaluated by the College of American Pathologists (CAP) twice per annum [31]. Through the constant assessment and scaling up of the certification criteria, the CVs (coefficient variants) within and between laboratories have decreased, justifying the routine use of HbA1c tests to aid in the clinical decisions on diabetes management and diagnosis [32].

Because the IFCC and NGSP utilize different reference methods for standardizing the HbA1c methods (the NGSP network reports in %HbA1c, and the IFCC network reports in mmol HbA1c/mol Hb), the absolute numbers reported are different between the two groups. The IFCC reference system's measurements are typically 1.5–2.0% units lower than the NGSP values [33], possibly due to the higher specificity of IFCC methods. Although they are more accurate, IFCC methods are more time consuming and comparatively higher in cost and are not meant for routine clinical usage. Therefore, the NGSP values prevail as the better assessment tool for diabetes diagnosis and management (due to the traceability to UKPDS and DCCT), and most clinical settings utilize NGSP units to report HbA1c values. To avoid confusion, a master equation was established (Eq. 1) to relate the IFCC results to the NGSP results. However, Japan's and Sweden's national standardization programs established master equations with different conversion numbers (please refer to Eqs. 2 and 3) [30].

$$\mathrm{NGSP}\,(\%) = 0.09148 \times \mathrm{IFCC}\left(\frac{\mathrm{mmolHbA1c}}{\mathrm{mmol\,Hb}}\right) + 2.512 \tag{1}$$

$$\mathrm{JDS}(\mathrm{Japan\,Diabetes\,Society,\,in\,\%}) = 0.0927 \times \mathrm{IFCC} + 1.73 \tag{2}$$

$$\mathrm{Mono\ S}(\mathrm{Sweden,\,in\,\%}) = 0.0989 \times \mathrm{IFCC} + 0.88 \tag{3}$$

To facilitate the communication of HbA1c analysis, the A1c-Derived Average Glucose (ADAG) Study Group found a more user-friendly way to deliver the result from A1C assays in an attempt to obtain better patient compliance in routine self-monitoring for better diabetes management [34]. By establishing a linear relationship between HbA1c and average glucose (AG) levels, the ADAG proposed to report HbA1c as estimated average glucose (eAG, in mg/dL or mmol/L) [34], and the conversion equation is shown below [33].

$$\mathrm{eAG}\left(\frac{\mathrm{mg}}{\mathrm{dL}}\right) = (28.7 \times \mathrm{HbA1c\%}) - 46.7 \tag{4}$$

With a true reference method developed by the IFCC, global consensus was reached regarding the use of the IFCC reference method for calibration in clinical laboratories [35]. Three standards were established based on the presentation of HbA1c results to clinicians, patients, and scientific journals. First, in clinical laboratories worldwide, the HbA1c SI unit will be expressed in IFCC unit (mmol/mol, with no decimals), and both the IFCC-and NGSP-derived units (%, with one decimal) shall be reported. Second, the HbA1c conversion table with the IFCC and NGSP units should be easily accessible to the diabetes community. Third, journals are recommended to require manuscripts to report HbA1c in both SI (IFCC) and NGSP units [35]. As a result of global standardization, countries such as Sweden and Japan, which are famous for their own HbA1c reference methods, have switched and adapted these standards. For example, Sweden, which used to utilize its national reference method, namely Mono S, agreed to report HbA1c results using the IFCC units (mmol/mol) starting in January 2011 [36]. The Japan Diabetes Society switched to reporting HbA1c results in NGSP(%) instead of JDS values starting in 2013 [37].

Overall, the effort to standardize HbA1c tests has led to a global consensus on conveying HbA1c results for better diabetes care. Although the choice of reporting in IFCC, NGSP, or eAG units still varies by country, with the recognition of a common language for HbA1c results, the implementation of HbA1c tests across the globe should not be too far away.

4. Limitations of HbA1c tests in laboratory settings

In 2001, the IFCC working group first described a higher-order reference method that was later accepted worldwide as the true reference method. The IFCC reference method includes the following three phases: (1) cleavage of hemoglobin by endoproteinase, (2) separation of the β-chain glycated and non-glycated N-terminal hexapeptides, and (3) quantification by HPLC and electrospray ionization mass spectrometry or HPLC and capillary electrophoresis with UV (for a detailed description, please refer to the IFCC publication) [38]. HbA1c tests can be categorized into two main groups: those based on charge differences (cation exchange matrices and capillary electrophoresis) and structural differences (affinity chromatography and immunoassays). The HbA1c tests commonly utilized in laboratory settings typically involve the pretreatment of blood samples before analysis.

Although glycation is an irreversible reaction, the formation of the glucose adduct at the N-terminus of the β chain contributes to additional negative charges in order to allow HbA1c to flow through negatively-charged cation-exchange matrices [8]. Then, spectrophotometric analysis of the eluents will provide results as the percentage of each hemoglobin species in the samples [39]. In general, cation-exchange chromatography provides high precision with rapid hemoglobin separation [32]. With buffer systems at pH 6.2 and below, cation-exchange assays were found to be sufficiently sensitive to quantify αβ dimers, which may be glycated on both β chains [40]. Although some cation-exchange HPLC methods are able to indicate the presence of hemoglobin variants, they lack sufficient resolution to recognize specific glycated hemoglobin variants. In cases in which the method cannot resolve the peaks, different HPLC methods have distinct responses. For example, the Bio-Rad Variant II thalassemia method shows additional peaks as specific HbA1c variants [41], whereas other HPLC methods, such as

HA-8140 HPLC, describe hemoglobin variants in a chromatogram as "variant Hb" [42].

Although most cation-exchange HPLC methods can now resolve the most common HbA1c variants and have been widely employed in clinical settings, they are still affected by the high prevalence of hemoglobinopathies, particularly in certain ethnicities. Different detection methods such as affinity separation and immunoassays can then be used. Affinity chromatography, which reacts boronic acid with the cis-diol groups of glucose bound to hemoglobin, measures the total glycated hemoglobin and is therefore considered to be less affected by the presence of variants. Affinity chromatography assays are more stable (less affected by pH and temperature) compared with cation-exchange HPLC. However, affinity chromatography does not distinguish amongst the glycated hemoglobin species; thus, they are highly associated with the overestimation of HbA1c when the total glycated hemoglobin value is taken as the value of HbA1c [43]. As such, affinity separation methods commonly give 40–50% higher HbA1c values compared with cation-exchange assays [8]. However, using the newly established guidelines by NACB (National Academy of Clinical Biochemistry), all affinity assays that measure the total glycated hemoglobin have been calibrated to report the HbA1c equivalents in order to harmonize HbA1c results [16]. In addition, the seemingly non-specific boronate affinity method can be very useful in cases in which hemoglobin variants are present. In fact, it can be employed as a comparative method in cases in which cation-exchange HPLC (the DCCT reference method is Bio-Rex 70 resin cation-exchange HPLC, and NGSP uses the same reference method [31]) yields bizarre chromatograms ("abnormal separation", additional peaks, too low HbA1c concentrations or those above the nondiabetic range) [44].

Immunoassays, particularly immunoturbidity assays, involve an agglutinator and antibody-coated latex particles. When HbA1c is present, it reacts with the antibody to inhibit agglutination, hence reducing the turbidity of the sample. However, the pitfall of immunoassays is that they are sensitive to HbF interference. For example, a high concentration of HbF (due to hereditary persistence [11]) has been shown to cause more than 20% underestimation of the HbA1c values measured by a DCA 2000 analyzer (a clinically significant value) [45]. Although HbA tends to be glycated at the β chain terminal valine, HbF consists of a γ chain (with glycine at its terminus) and is preferentially acetylated rather than glycated, which essentially leads to the underestimation of glycated hemoglobin in individuals [45]. For a comprehensive discussion of interference in particular methods, please refer to the table on the NGSP website [46].

In addition to the constant effort to standardize well-established lab methods to detect HbA1c, attempts to perform more effective measurements of HbA1c in lab settings continue. For example, drop-coating deposition Raman (DCDR)-spectroscopy has been found to be very specific and sensitive for the detection of HbA1c; with a detection limit approximately 15 fold lower than the lowest physiological concentrations associated with clinical settings [47]. Additionally, with the incorporation of nanotechnologies, novel systems such as a microfluidic magnetic bead-based immunoassay have been proposed to increase the accuracy of HbA1c detection [48].

In short, end-users should be aware of possible interference by other HbA1c variants, particularly if the assays employed can under- or overestimate HbA1c values in a clinically significant way. In addition to providing a given population with the right choice of assays, constant monitoring of the analytical performance of the assay and quality control is important. To achieve the goals of intra-laboratory CV <2% and inter-laboratory CV <3.5%, the bottom line of performance monitoring and quality control is to perform tests on at least two control materials with different mean values at the beginning and at the end of the day's run [16]. In addition to maintaining the standardization efforts for the NGSP-certified methods, the development of detection methods that function based on different principles should be encouraged.

4.1. Point-of-care (POC) technologies: reliability in doubt

Laboratory techniques, such as cation-exchange HPLC and capillary electrophoresis, are not very useful with regard to the detection of HbA1c in situ (please refer to Table 1 for a list of available POC analyzers). The bulky machines and laborious preparations are not a good match for point-of-care technologies, which are more mobile. Undeniably, with good mobility, POC devices are attractive for nursing practitioners to obtain HbA1c values in situ and thus allow better patient care. However, the adoption of POC analyzers in clinical settings is quite controversial due to doubt regarding their accuracy.

There are a variety of commercially available POC tools. However, only few devices are calibrated precisely enough to gain NGSP certification. According to Lenters-Westra, the DCA Vantage (Siemens Medical Solutions Diagnostics, New York) and Afinion (Axis-Shield, Norway) are the only devices that are able to meet the NGSP criteria with imprecision of <3% CV with two different reagent lots, indicating that their equivalent analytical performance is equivalent to that obtained with laboratory-based methods [49]. In general, central laboratory methods are often subject to positive bias, whereas POC methods tend to exhibit

Table 1
Point-of-care A1c devices.[a]

Point-of-care instruments for A1c tests	Manufacturer	Types of methods/assay time (min)	Detection range and coefficient variants (CV)
In2it*	Bio-rad, California	Affinity separation/10 min	HbA1c (10 μL whole blood): 4% to 14% (20 mmol/mol to 130 mmol/mol), CV 2.4–3.9% [56]
DCA vantage*	Siemens Medical Solutions Diagnostics, New York	Immunoassays/6 min	HbA1c (1 μL whole blood): 2.5% to 14% (4 mmol/mol to 130 mmol/mol) [57], CV <3% [49]
Afinion*	Axis-Shield, Norway	Affinity separation/3 min	HbA1c (1.5 μL whole blood): 4% to 15% (20 mmol/mol to 140 mmol/mol), CV <3% [58]
Nyocard	Axis-Shield, Norway	Affinity separation/3 min	HbA1c (5 μL whole blood): 4% to 15% (20 mmol/mol to 140 mmol/mol)[58], CV <5% [60]
GDX/Micromat II*	Bio-Rad, California	Affinity separation/5 min	HbA1c (10 μL whole blood): 4% to 15% (20 mmol/mol to 140 mmol/mol), CV 2.93–4.65% [61]
Clover	Infopia, Korea	Affinity separation/5 min	HbA1c (4 μL whole blood): 4% to 14% (20 mmol/mol to 130 mmol/mol), CV <3% [62]
InnovaStar	DiaSys, Germany	Agglutination/7 min	HbA1c: 4% to 15% (20 mmol/mol to 140 mmol/mol), CV <3% [63]
A1CNow+*	Metrika, Bayer, California	Immunoassay/5 min	Accurate between 7% and 8.5% (53 mmol/mol to 69 mmol/mol) [55]
Quo-test	Quotient Diagnostics, UK	Affinity separation/4 min	HbA1c: 4% to 15% (20 mmol/mol to 140 mmol/mol), CV <3% [64]

[a] Point-of-care (POC) devices play very significant role in aiding the patience compliance of self-monitoring and also allow the practitioners to perform routine checking in situ on patients who are not convenient in moving around for blood testing. POC analyzers which are in par with laboratory machineries in HbA1c testing provide a more practical way for better clinical monitoring in order to perform better personalized treatments (*CLIA-waived POC technologies [53]).

Table 2
HbA1c biosensors.[a]

Compound detected	Characteristics	Types of biosensors	Detection range/limits	Sensitivity	Limitations
Fructrosyl valine (FV)	-Detect: End product from decomposition of glycated hexapeptides -Biological recognition element: Enzyme fructosyl amine oxidase (FAO)	Electrochemical (amperometric) with magnetic nanoparticles	-Detection range: 0 to 2 mM FV -Detection limit: 0.1 mM for FV (Chawla et al., 2011) [74]	Not available	-Lack of reproducibility and stability
		Electrochemical (amperometric) with zinc oxide nanoparticles–polypyrrole film	-Detection range: 0.1 to 3 mM FV -Detection limit: 50 uM FV (Chawla et al., 2012) [73]	38.42 μA/mM	
		Disposable iridium-modified electrochemical biosensor (amperometric)	-Detection range: 0 to 2 mM of FV -Detection limit: Not available (Fang et al., 2008) [72]	21.5 μA/mM cm^2	
Glycated hemoglobin (HbA1c)	-Detect: HbA1c -Biological recognition element: Anti-HbA1c antibody	Electrochemical (potentiometric) immunosensors with mixed SAMs wrapped nano-spheres array	-Detection range: 50 to 170.5 ng/mL HbA1c -Detection limit: Not available (Xue et al., 2010) [67]	94.73 μV/(ng/mL)	-Reproducibility is of concern
	-Detect: Fc-IgG-HbA1c immunocomplexes -Biological recognition element: anti-HbA1c antibody	On-chip electrochemical flow immunoassay	-Detection range: up to 500 μg/mL -Detection limit: Not available (Tanaka et al., 2007) [75]	Not available	-Laborious pretreatment
	-Detect: HbA1c -Biological recognition element: Anti-HbA1c antibody	Sandwich immunoassays on polydimethylsiloxane-based antibody microarrays	-Detection range: 10–100 ng/mL -Detection limit: 3.58 ng/mL (Chen et al., 2012) [68]	4–5 orders of magnitude higher	-Long incubation time (2 h)
	Boronic acid-based HbA1c Biosensors				
	Detect: HbA1c -Biological recognition element: Ferroceneboronic acid	Electrochemical (piezosensor) immunoassay	-Detection range: 0 to 20% -Detection limit: >5% (standard deviation 20%) (Halamek et al., 2007) [76]	Not available	-Low sensitivity -Low specificity
	-Detect: HbA1c -Biological recognition element: Ferroceneboronic-acid labeled anti-HbA1c antibody	Electrochemical (piezosensor) immunoassay	-Detection range: 4 to 13% -Detection limit: Not available (Halamek et al., 2007) [66]	Sensitivity was increased at three fold compared to without antibodies (Halamek et al., 2007)	-Low sensitivity
	-Detect: Wide range of glycoproteins, for example HbA1c -Biological recognition element: Boronic-acid	Disposable biochip	Not available (Son et al., 2006) [77]	Not available	-Laborious pretreatment on sample matrix. -High sample volume was required (50 μL)
	-Detect: Wide range of glycoproteins, for example HbA1c -Biological recognition element: Boronic-acid -Coupled with GOx (glucose oxidase) backfilling assay	Electrochemical	-Detection range: 2.5 to 15% HbA1c -Detection limit: Not available (Song et al., 2009) [70]	Not available	-Lack of specificity to HbA1c
	-Detect: Glycoproteins -Biological recognition element: Self-assembled monolayer (SAM) thiophene-3-boronic acid	Affinity biosensors with impedance measurement	-Detection range: 1 to 100 ng/μL -Detection limit: 1 ng/μL of HbA1c (Hsieh et al., 2013) [71]	Not available	-Lack of stability
	-Detect: HbA1c -Biological recognition element: phenylboronic acid	SPR (surface plasmon resonance) biosensor	-Detection range:0.43 to 3.49 μg/mL -Detection limit: 0.01 μg/mL (Liu et al., 2008) [69]	Not available	-Expensive instrumentation

[a] The table shows examples of fabricated HbA1c biosensors. HbA1c biosensors can be grouped under two main groups: the Fructrosyl valine (FV) and HbA1c biosensors. The FV biosensors measure the end product from the decomposition of glycated hexapeptides (after proteolysis of HbA1c), while the HbA1c biosensors measure concentrations of HbA1c directly. The HbA1c biosensors typically involve immunoglobulins as the biological recognition elements, and some of them utilize the biomimetic ability of boronic acid to selectively measure HbA1c.

negative bias. Hence, the relative biases of POC and CL (central lab) methods are potentially unacceptable in cases in which different principle-based methods are employed interchangeably [50]. Using different methods can lead to complicated data analysis issues, but the 18% false negatives obtained by HbA1c POC devices, which can lead to high incidence of missed diagnosis of diabetes, is a concern [51].

In addition to inconsistent performance, clinical doubt regarding the HbA1c values obtained using POC devices has obstructed the wide application of POC devices in clinical settings. POC devices are susceptible to reagent variations across different batches [52]. POC devices are often perceived to not be on par with standard laboratory tests in performing HbA1c measurements. Due to the unstable analytical performance of some POC analyzers, clinical practices are advised to always compare the POC methods to the laboratory methods before adopting POC methods in routine practice [51]. Although the 18% false negative results obtained using POC devices remain a pressing issue, the potential of the POC devices for incorporation in clinical practice should not be overlooked. Therefore, more attention should be given to the ongoing effort to increase the accuracy and decrease the variability between batches of commercialized POC methods. Hence, the waived status of the assessment of POC methods by the Clinical Laboratory Improvement Amendments (CLIA) causes concern that the quality of POC methods may be compromised.

Clinical Laboratory Improvement Amendments was established in 1988 to ensure the accuracy and reliability of a method regardless of the places where the test is performed [53]. Under the law, waived tests are defined as laboratory examinations cleared by the Food and Drug Administration (FDA) and safe for household use [53]. With CLIA-waived status, POC devices are not required to undergo assessment under other schemes, such as CAP surveys (refer to Section 3 for discussion). Thus, the concern is that the analytical performance consistency of POC devices is not known, rendering diabetes care in the general population less effective. As of today, the ADA (American Diabetes Association) has excluded the use of POC analyzers for the diagnosis of diabetes due to the variability in analytical performance.

With advances of technology, the analytical performance of POC devices can be improved, and with the revised CLIA-waived status creating the obligation to participate in external quality control schemes, POC devices have huge potential to become reliable diagnosis tools in the near future. However, because the therapeutic modality is often decided by HbA1c values, the negative bias of POC analyzers could affect clinical decisions. Therefore, pharmacists and medical practitioners should be cautious in relying solely on POCs. Even if most CLIA-waived POC devices exhibit a satisfactory correlation to laboratory methods, it is often advisable to perform a POC test and a laboratory test to avoid misjudgments in practice. Again, it may take some time before POC devices are ready for diabetic diagnosis purposes; however, for diabetic care and management purposes, POC devices are reliable [54] and more cost-effective [55] and yield a higher patient-satisfaction level, due to the decentralized system to provide rapid and same-visit results.

4.2. New ideas for HbA1c tests: glycated hemoglobin biosensors

Unlike other methods, HbA1c biosensors always involve a layer of biological recognition elements that create specificity and selectivity towards HbA1c. Because the designs of HbA1c biosensors often involve miniaturized set-ups, it is fair to anticipate a revolution of accurate and cost-effective HbA1c biosensors in POC instruments in clinical settings.

The main group of HbA1c biosensors are those designed to detect HbA1c directly, and they can be categorized as amperometric, potentiometric, piezoelectric biosensors, and biochips [56]. Electrochemical biosensors that detect HbA1c directly typically involve an anti-HbA1c antibody as the biological recognition element and are commonly known as immunosensors. For example, by combining the principle of piezoelectricity and electrochemistry, Halámek et al. developed an HbA1c immunosensor using an antibody labeled in situ with ferroceneboronic acid (redox label) to amplify the electrochemical signal [57]. Utilizing nanotechnology, Xue et al. reported a miniaturized potentiometric HbA1c immunosensor based on mixed SAM (Self Assembled Monolayer)-wrapped nanospheres. The prototype was then tested for its consistency in a clinical setting to determine the potential of HbA1c biosensors in POC instruments [58]. Due to a constant urge to increase the sensitivity and specificity of HbA1c biosensors, a sandwich immunoassay was proposed to detect HbA1c at the nanomolar level at high specificity [59]. Expensive instrumentation, such as SPR (surface plasmon resonance) spectroscopy, has also been utilized to fabricate HbA1c biosensors for the robust detection of HbA1c with high sensitivity [60]. When conducting detection with high sensitivity, it would be wise to be cautious if the techniques utilized can compromise the rapidity of the results generated.

Boronic acid, typically used in affinity chromatography, is an excellent biomimetic molecule that is highly utilized as the biological recognition element in HbA1c biosensors to selectively bind to the sugar moiety of HbA1c. For example, Song and Yoon manipulated a boronic acid-modified electrode with a GO*x* (glucose oxidase) backfilling assay to quantitatively measure HbA1c [61]. Utilizing the biomimetic nature of boronic acid, Hsieh et al. also fabricated an affinity biosensor to measure HbA1c concentrations based on impedance [62]. Using a low-cost and low-volume sample, it was claimed to be feasible to develop the proposed HbA1c biosensor prototype into a sensitive point-of-care device [62]. However, because boronic acid binds to the cis-diol of any glycan moiety (HbA1c is only one of the glycan moieties), studies attempting to fabricate HbA1c biosensors should be cautious to not compromise specificity by using the cost-effective boronic acid in place of antibodies.

Fructosyl valine (FV), which is generated by the decomposition of glycated hexapeptides (the product of HbA1c proteolysis) has also been highly exploited in the development of HbA1c biosensors. By manipulating the enzyme fructosyl amine oxidase (FAO) as the biological recognition element, FV can be further catalyzed to produce hydrogen peroxide for electrochemical detection. Because FAO can be extracted from different organisms (marine yeast or bacteria such as *Escherichia coli* and *Arthrobacter* sp.), the fabrication of FV biosensors is constantly challenged by altered enzymatic activity, the sensitivity and reproducibility of the biosensors, and the operational potential and temperature [63]. To counter the weaknesses of FV biosensors, Fang et al. designed a disposable iridium-modified FV biosensor to detect HbA1c [63]. By incorporating nanotechnology in the fabrication of the FV biosensor, Chawla and Pundir managed to develop an amperometric biosensor for HbA1c with improved operational efficiency, higher stability and increased sensitivity [64]. For examples of different types of HbA1c biosensors and their analytical performance, please refer to Table 2.

In summary, the attempt to develop HbA1 biosensors is a positive effort to stimulate innovations for the creation of better point-of-care analyzers in the future. Although the stability of biomolecules as the recognition element has always been a concern in the reliability of detection, with the advancement of nanotechnology, more effective and consistent biosensors that can be as accurate as current lab HbA1c tests can now be developed.

5. Prediabetes in predicting incident diabetes: is HbA1c an effective screening tool?

Before one becomes hyperglycemic, one can be asymptomatic and remain dysglycemic for years. Dysglycemia occurs when the blood glucose level is higher than the normal level but does not reach the diabetic cutoff. In fact, the prediabetic stage is considered part of the continuum of dysglycemia. Prediabetes refers to IFG (impaired fasting glucose with FPG at 100–125 mg dL^{-1} or 5.6–6.9 mmol/L), IGT (impaired glucose tolerance with 2-hour oral glucose test at 140–199 mg dL^{-1} or 7.8–11.0 mmol/L) or an A1c of 5.7 to 6.4% (ADA, American Diabetes

Association prediabetes diagnostic range) [23]. Because type 2 diabetes is a progressive disease, in cases in which patients are asymptomatic, delayed intervention and therapy can lead to morbidities and premature death. In fact, studies have shown that prediabetic patients are associated with a 20% increase in cardiovascular diseases, with higher prevalence of neuropathy, chronic kidney disease and microvascular complications [65]. The Diabetes Prevention Project revealed that only 4.8% of individuals are properly diagnosed as prediabetic, and the majority of patients remain undiagnosed [66], reflecting the urgent need for an effective screening tool to recognize high-risk populations.

Because the HbA1c cutoff sensitivity depends on demography, ethnicity, age, and the detection methods chosen in a particular health system, it is not surprising that different countries may have variable values for prediabetes. For example, instead of utilizing the ADA cutoff, the CDA (Canadian Diabetes Association) has chosen 6.0 to 6.4% (42–46 mmol/mol) to define prediabetes [67]. To define prediabetic ranges with the HbA1c levels, Suzuki et al. performed a ROC (Receiver Operating Characteristic) curve analysis to reveal that A1C ≥5.7% is better than A1C ≥6.0% as the cut-off point to screen for prediabetes in Japanese patients [68].

However, compared to its counterparts, HbA1c appears to have lower screening power for prediabetes. In non-Hispanic white society, utilizing the ADA HbA1c prediabetic criterion, Lorenzo et al. found that the HbA1c test is less sensitive than the FPG and OGTT tests [69]. Coinciding with Lorenzo et al.'s findings, Zhou et al. also concluded that FPG performs better as a screening tool than the HbA1c test based on studies in Qingdao, China [70]. In contrast, a Bulgarian population-based study found a significant positive correlation between HbA1c and both the fasting plasma glucose test and OGTT by setting the HbA1c cutoff at ≥5.5% [71], indicating that the HbA1c test can be an equally effective screening tool. In fact, in an attempt to analyze the screening power of HbA1c, a review of 63 studies concluded that the HbA1c test is as effective as FPG as a screening tool [21]. More recently, in clinical practice, the HbA1c test was recognized to be equally good, and often better, as a screening tool compared with FPG [72]. Although the evidence is inadequate, HbA1c was recently found to have similar screening power as OGTT [72]. To enhance the screening power, Heianza et al. suggested that the use of both the HbA1c and FPG tests will allow more efficient recognition of individuals who are most likely to develop diabetes [27].

In short, the HbA1c test can be an effective screening tool for prediabetic individuals when the cutoffs are adjusted based on ethnicity, demography, gender, and age. To accurately screen for prediabetes, additional plasma glucose tests (namely the FPG and OGTT tests) are still necessary [73]. With the early detection of prediabetes, individuals can receive early intervention, efficiently preventing the progression to type 2 diabetes. Although the implementation of HbA1c as the sole screening tool remains premature, through the constant surveying and standardization of HbA1c methods using adjusted cutoffs, the HbA1c test has high potential to become a reliable independent screening tool for the detection of prediabetes and undiagnosed diabetes.

6. Glycated hemoglobin in the treatment and long-term management of diabetes

Given the impacts of T2DM on society, it is important to seek out the most suitable treatments to delay the progression of diabetes. There are a plethora of treatments available that cater to different groups of prediabetic or diabetic patients, including monotherapy with hypoglycemic drugs, combinations of drugs, insulin therapy and lifestyle changes, each of which can be initiated based on the glycemic status and patient age, pre-existing medical complications, and/or diabetic symptoms. To initiate treatment, clinicians could refer to the glycemic status reflected by HbA1c tests.

To strike a balance between side effects and effectiveness of delaying the progress of the disease, it is very important to personalize therapy for each T2DM patient. For example, metformin is as effective as lifestyle modification in individuals aged 24 to 44 years old or in those with BMI ≥35 kg/m^2, but these categories include only a small group of diabetic patients [74]. In reality, clinicians may be hesitant to treat patients aggressively, due to fear of hypoglycemic complications or uneasiness associated with adapting to a new therapy. The rule of "ABC" (A1C, Blood Pressure, Cholesterol) outlines the treatment goals for A1c (<7%, submit to modification based on individuals' adverse effects during glycemic management), blood pressure (≤129/79 mm Hg), and cholesterol (LDL <100 mg/dL in patients with ≥40 years of vascular disease) [75].

Although the role of the HbA1c test has long been recognized in the management of the treatment of T2DM, different organizations around the world have suggested different treatment goals for mitigating the cases of over- and under-treatment. For example, the ADA (USA) suggested a treatment goal of HbA1c <7%, whereas the JDS (Japan) aims to achieve 6.5% for a successful diabetes treatment [76].

Because the rules for glycemic control and treatment goals have always included HbA1c values for practical clinical decision on appropriate modalities, it is important for different countries and regions to have specific treatment goals for the HbA1c level in order to achieve more personalized and effective treatment.

7. Conclusions

Although there used to be many debates regarding the utilization of HbA1c tests to diagnose type 2 diabetes mellitus, it has now been recognized as reliable diagnostic biomarkers for type 2 diabetes. An IUPAC unit (IFCC unit, mmol/mol) for HbA1c has been established, and to standardize all of the methods, commercial methods are required to maintain traceability to IFCC reference methods. Although laboratory HbA1c methods have been utilized to perform HbA1c measurements, the need for bulky machines can decrease patients' compliance, thus affecting the effectiveness of diabetes management. However, due to biases associated with POC analyzers, clinical practitioners are hesitant to rely on POC analyzers as a stand-alone HbA1c method. To populate the use of point-of-care (POC) HbA1c analyzers in clinical settings, more efforts to create sensitive miniaturized HbA1c biosensors should be encouraged. Although the development of accurate POC devices is of utmost importance, future HbA1c tests should also consider cost and ease of interpretation in common households. Because type 2 diabetes is a progressive disease, effective screening of prediabetes is very important to reducing the cases of undiagnosed diabetes and to thus perform early treatment and management of the glycemic status.

8. Future perspectives

Although the traditional role of HbA1c in diabetes management has long been accepted, its contribution to the diagnosis of type 2 diabetes is only slowly gaining acceptance. With the current efforts directed at standardization, the authors predict that HbA1c tests will be used for diabetes diagnosis across the globe. The development of more consistent and accurate HbA1c methods remains an ongoing effort to maintain traceability to important diabetes studies. However, to improve the performance of POC analyzers, constant surveillance of batch-to-batch variation is necessary. By controlling the quality and maintaining the performance of different lots of POC devices, end users can gain confidence in utilizing POC devices for better diabetes management. With an assurance of quality, the employment of POC analyzers in clinical settings is anticipated. The quality control for CLIA-waived POC analyzers is very critical, because they can be very beneficial to diabetes management in developing countries that do not have sufficient health budget allocations to perform HbA1c laboratory tests nationwide. According to one survey, the set-up costs for the laboratory HbA1c test are 13.6 fold greater than those for the plasma glucose measurement [77], and POC analyzers that are on par with laboratory methods can be cost-effective and convenient, particularly in underserved regions.

With the effort to standardize the test and the advancement of technology, it is only a matter of time for the mobile analyzers to be recognized as effective and accurate tools for the diagnosis and management of diabetes in situ. HbA1c biosensor development should be encouraged to produce more efficient miniaturized devices that may be potentially useful POC analyzers. To tackle the implementation cost for HbA1c tests, attention should be paid to the use of cost-effective materials while fabricating HbA1c biosensors. HbA1c tests have the potential to be more convenient, user-friendly, accurate, and cost-effective in order to aid world-wide diabetes care.

Conflict of interest

The authors have no conflict of interest on the work.

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Appendix A. Supplementary data

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