Evaluation of the In2it® analyzer for HbA$_{1c}$ determination

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Abstract

Aim. – The prominent role of HbA$_{1c}$ in the follow-up of glycaemic balance in patients with diabetes mellitus necessitates the use of robust and reliable methods of assay. The purpose of this study was to evaluate the In2it® analyzer, a new device allowing HbA$_{1c}$ evaluation within 10 min, using 10 µL of blood, in the laboratory or clinical unit, using an affinity-based method.

Methods. – The analytical performance of the In2it® analyzer was tested for precision, interference and linearity, and correlated with two other analyzers – the high-performance liquid chromatography (HPLC)-based Variant II™ analyzer in a laboratory, and the immunology-based DCA 2000® analyzer in a clinical unit – for practicability and its compliance with good laboratory practices.

Results. – HbA$_{1c}$ assay is linear from 4 to 14%, with coefficients of variations ranging from 2.4 to 3.9%. In2it® correlation was satisfactory with both the HPLC Variant II® ($r^2 = 0.974$, $P<0.001$) and DCA 2000® ($r^2 = 0.794$, $P<0.001$) analyzers although, with the latter, unpredictable differences were randomly observed. However, the method is free of interference from common haemoglobin variants, labile glycated haemoglobin and carbamylated haemoglobin, hyperbilirubinaemia ($<520$ µmol/L) and hypertriglyceridaemia ($<6$ mmol/L). The practicability of the analyzer is good. However, software specifications need to be upgraded, especially for quality-control management, traceability of results and data safety.

Conclusion. – The In2it® analyzer is suitable for HbA$_{1c}$ assay in small laboratory series and for point-of-care testing, and its analytical performance is satisfactory overall. However, several issues related to software need to be improved for optimal application. Also, special attention should be paid concerning the possibility of underestimation of results in cases of high hypertriglyceridaemia.

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Keywords: Diabetes mellitus; Evaluation; HbA$_{1c}$; Affinity chromatography; Point-of-care testing; In2it® analyzer

Résumé

Évaluation de l’automate In2it® pour le dosage de l’HbA$_{1c}$.

Objectifs. – L’importance de l’HbA$_{1c}$ dans le suivi de l’équilibre glycémique des patients diabétiques nécessite l’utilisation de méthodes de dosage robustes et performantes. Nous avons évalué l’analyseur In2it®, nouveau appareil de dosage de l’HbA$_{1c}$ utilisable au laboratoire ou en biologie délocalisée par chromatographie d’affinité en dix minutes, sur 10 µL de sang.


Résultats. – Le dosage d’HbA$_{1c}$ est linéaire de 4 à 14%, avec des coefficients de variation compris entre 2,4 et 3,9%. La corrélation avec la méthode de CLHP Variant II® est satisfaisante ($r^2 = 0.974$, $P<0.001$) de même qu’avec l’appareil d’immunodosage DCA 2000® ($r^2 = 0.794$, $P<0.001$). Cependant, dans le second cas, des différences aléatoires ont été constatées. La méthode n’est pas affectée par la présence d’hémoglobines anormales courantes et ne présente pas d’interférences avec l’hémoglobine glyquée labile, l’hémoglobine carbamylée, l’hyperbilirubinémie (jusqu’à 520 µmol/L) et l’hypertriglycéridémie (jusqu’à 6 mmol/L). La praticabilité de l’appareil est bonne. Les aspects logiciels concernant la gestion des contrôles de qualité, la traçabilité des résultats et la sécurisation des données doivent faire l’objet de développements.

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Conclusion. – L’appareil In2it® est adapté au dosage de l’HbA1c au laboratoire pour de petites séries et en biologie délocalisée. Ses performances analytiques sont globalement satisfaisantes. Plusieurs points restent néanmoins à améliorer pour son utilisation optimale. Une attention particulière doit être portée à la sous-estimation possible des résultats en cas d’hypertriglycéridémie importante.

Mots clés : Diabète sucré ; Évaluation ; HbA1c ; Chromatographie d’affinité ; Biologie délocalisée ; Analyseur In2it®

1. Introduction

Measurement of HbA1c is key in the follow-up of patients with diabetes mellitus. In addition to being a retrospective marker of recent glycaemic control, HbA1c is correlated with the risk of long-term complications in both type 1 and type 2 diabetes [1,2]. The development of compact devices for point-of-care testing (POCT) has significantly improved the efficiency of diabetes management, allowing the appropriate guidance of patients for their therapeutic management at the time of medical consultation [3,4]. To provide the optimal information, these devices need to cope with the standard criteria of good laboratory practices such as analytical performance, traceability of results, data safety, identification procedures and quality control.

In the present study, we evaluated the In2it® analyzer, a new compact device allowing HbA1c measurement in both the clinical unit and the laboratory.

2. Materials and methods

2.1. Materials

The In2it® device (Bio-Rad Laboratories, Marne-La-Coquette, France) uses a boronate affinity method to separate glycated haemoglobin from non-glycated haemoglobin. This compact analyzer uses ready-to-use test cartridges containing sample reagent, wash solution, boronate affinity resin and elution buffer.

Red blood cells are automatically lysed in the cartridge, and a period of incubation allows the binding of glycated haemoglobin to the boronate affinity resin. The non-glycated fraction is collected first, and evaluated photometrically at 440 nm. The glycated fraction is then eluted and evaluated under the same conditions.

The HbA1c percentage is calculated using the following algorithm: HbA1c (%) = M × [A glycated fraction/100]/[A glycated fraction + A non-glycated fraction] + C. Here, M and C represent the slope and intercept factors used to correct the crude value. The method is based on the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method. Results are expressed in DCCT (Diabetes Control and Complications Trial)-derived units, but will be available as IFCC units (mmol/mol) by 1st January 2011 [5].

2.2. Samples

Blood samples were collected for routine HbA1c assay in EDTA-containing tubes (reference number 367657, Becton Dickinson, Le Pont De Claix, France). Evaluation in the clinical unit used 10 μL of fingertip capillary blood taken with a key supplied with the test cartridges. No further samples were necessary for this study, and no samples were stored after the assays.

2.3. Precision study

Intraday precision was determined by 20 assays of HbA1c from the same blood samples in the same series by the same technician. Between-day precision was determined by daily measurements of HbA1c, using two control materials supplied by the manufacturer (normal-level control [CQL1] and high-level control [CQL2], Bio-Rad Laboratories), in 52 different series by different technicians.

2.4. Correlation study

HbA1c results obtained with the In2it® analyzer were compared with the results obtained with ion-exchange high-performance liquid chromatography (HPLC), used routinely in the laboratory for HbA1c assay (Variant II®, kit 270.2101 NU, Bio-Rad Laboratories), in 100 blood samples collected in EDTA-containing tubes. HbA1c values were distributed across the entire range of measurements. All comparative assays were performed within a 6-h time period.

Correlation between the results obtained with the In2it® analyzer and the DCA 2000® (Siemens Medical Solutions Diagnostics, Puteaux, France) analyzer, a compact device for immunological assay of HbA1c [2,3], was also performed, using 24 samples of fingertip capillary blood.

2.5. Linearity study

Linearity was studied by measuring HbA1c levels in a series of blood samples, prepared by serial sample dilutions, with a high of 15.9% and a low of 4.4%. Samples were previously adjusted to obtain the same total haemoglobin concentration (126 g/L).

2.6. Interference evaluation

Interference by labile HbA1c (LA1c) was determined by measuring HbA1c in blood samples incubated with 2.5 M of glucose for 30, 60 and 90 min at 37 °C. The amount of LA1c formed was estimated, using the HPLC Variant II® analyzer. Interference by LA1c was also studied by measuring the HbA1c of a blood sample before and after elimination of LA1c, as described elsewhere [6].

Interference by carbamylated haemoglobin (CHb) was evaluated by incubating red blood cells with 0.25 mM and 0.5 mM of KCN (control) or of KCNO for 3 h at 37 °C. CHb levels were also estimated using the HPLC Variant II® device [7].
The effect of bilirubin was studied by mixing red blood cells with various dilutions of hyperbilirubinaemic plasma to obtain bilirubin concentrations ranging from 0–520 μmol/L. The effect of triglycerides was studied by spiking red blood cells with various dilutions of triglyceride-rich plasma to obtain triglyceride concentrations ranging from 0–40 mmol/L.

Interference by abnormal haemoglobins was checked in samples containing HbS, HbD, HbC or HbE. Two samples with abnormally increased HbF (2.4% and 34.6%, respectively) were also studied. Samples were obtained by either routine laboratory procedures or provided by Bio-Rad Laboratories via Henri Mondor University Hospital, as described elsewhere [7]. The effect of total haemoglobin concentration was assessed by diluting red blood cells in 0.15 M of NaCl to obtain total haemoglobin concentrations ranging from 60–181 g/L.

3. Results

Coefficients of variation (CVs) of intraday precision ranged from 2.43 to 3.86%, whereas between-day CVs were 3.46% with the CQL1 sample (HbA1c = 5.20%) and 3.22% with the CQL2 sample (HbA1c = 9.95%). HbA1c values obtained with the In2it® and Variant II® analyzers were well correlated (P<0.001). Linear-regression analysis provided the equation y (HbA1c In2it®) = 0.945 × (HbA1c Variant II®) + 0.002, with a coefficient of correlation r2 = 0.974. The Bland–Altman plot of these data [8] is shown in Fig. 1A. Comparison of the In2it® and DCA 2000® analyzers (Fig. 1B), using a smaller number of samples, also showed an acceptable correlation (P<0.001): the equation of the linear-regression analysis curve was y (HbA1c In2it®) = 0.973 × (HbA1c DCA 2000®) + 0.237, with a coefficient of correlation r2 = 0.794.

The linearity of the In2it® method proved excellent between 4.7% and 13.2%, with an upper limit of 14% (Fig. 2). Also, as shown in Tables 1 and 2, LAC<14.2% and CHb<3.4% did not interfere with HbA1c determinations using the In2it® analyzer, nor did the haemoglobin variants tested induce major differences in the values obtained by the Variant II® analyzer (data not shown). Furthermore, no interference effect of bilirubin was found for concentrations less than 520 μmol/L (data not shown).
Fig. 2. Linearity of HbA1c determination using the In2it® analyzer.

not shown), whereas triglyceride concentrations greater than 6 mmol/L decreased HbA1c values (Fig. 3). HbA1c was not affected by total haemoglobin concentrations in the range of 72–181 g/L (data not shown).

4. Discussion

At present, HbA1c is considered the best and most suitable tool for retrospectively assessing the quality of glycaemic control in patients with diabetes [9,10]. The importance of HbA1c in patient education and treatment is related to its pivotal role in delaying the development of the long-term degenerative complications of the disease, and requires the availability of robust and reliable methods of determination.

Sophisticated techniques – especially those using HPLC – are routinely used in the laboratory, and are based on the internationally accepted IFCC reference method [5,11–13]. How well these methods are performing is periodically evaluated by quality control and proficiency-testing programmes [14]. In addition, some analyzers may be used in POCT. Such devices can be operated by trained nurses or physicians, thereby allowing HbA1c levels to be determined in outpatient units or doctors’ offices. They should also combine good practicability and analytical performance compatible with the current standards to ensure optimal patient care.

In the present study, a new compact analyzer for HbA1c determination was evaluated in both a laboratory and a clinical unit. The analyzer uses an affinity-based methodology that allows quantification of total glycated haemoglobin (GHB), and not HbA1c directly. The latter is calculated from the GHB value, using an equation that takes into account the traceability to the reference method [11]. The present device provides HbA1c values expressed according to the DCCT system. However, traceability to the IFCC method is ensured, as the analyzer can be reset to the IFCC calibration via the system’s software. At this time, IFCC values are expressed as percentage units, which is not recommended, but will be available as IFCC units (mmol/mol) by 1st January 2011 to comply with the terms of the 2007 American Diabetes Association (ADA)/European Association for the Study of Diabetes (EASD)/International Diabetes Federation (IDF)/IFCC consensus document [5].

On evaluating the precision of the method, the CVs were found to be compatible with the usual accepted limits (from

Table 1

Effects of labile HbA1c (LA1c) formed in vitro and in vivo on HbA1c assay.

<table>
<thead>
<tr>
<th></th>
<th>HbA1c (%)</th>
<th>LA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In2it®</td>
<td>Variant ITM</td>
<td>Variant ITM</td>
</tr>
<tr>
<td><strong>In vitro</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary tube</td>
<td>6.9</td>
<td>7.5</td>
</tr>
<tr>
<td>Incubation with 2.5 M of glucose</td>
<td>6.8</td>
<td>7.4</td>
</tr>
<tr>
<td>Control</td>
<td>6.6</td>
<td>7.4</td>
</tr>
<tr>
<td>30 min</td>
<td>6.8</td>
<td>7.4</td>
</tr>
<tr>
<td>60 min</td>
<td>6.8</td>
<td>7.4</td>
</tr>
<tr>
<td>90 min</td>
<td>8.0</td>
<td>8.3</td>
</tr>
<tr>
<td>After elimination</td>
<td>7.9</td>
<td>8.4</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary tube</td>
<td>7.9</td>
<td>8.4</td>
</tr>
</tbody>
</table>

* As described under Materials and Methods.

Table 2

Effects of carbamylated haemoglobin (CHb) formed in vitro on HbA1c assay.

<table>
<thead>
<tr>
<th>CHb (%)</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In2it®</td>
</tr>
<tr>
<td>Sample 1</td>
<td>5.4</td>
</tr>
<tr>
<td>Sample 2</td>
<td>7.0</td>
</tr>
<tr>
<td>Sample 3</td>
<td>9.3</td>
</tr>
</tbody>
</table>

* As described under Materials and Methods.
2.43 to 3.86%), but were in the higher range of values obtained with other analyzers. However, this feature is often observed with affinity-based techniques [15,16].

As for the results obtained with the In2it® and other two field methods, correlation with the Variant II® HPLC method was good ($r^2 = 0.974$) throughout the entire measurement range. The slope of the correlation curve was 0.945 and the origin ordinate was close to zero, indicating that the results obtained with the In2it® analyzer were 5% lower overall than those obtained with the Variant II® analyzer. However, this difference may be explained by the different analytical principles used by the two techniques [17]. Their different procedures of calibration may also have been responsible for a systematic difference, as has been reported in the literature for other methods [15].

On comparing the In2it® results with those obtained with the DCA 2000® analyzer, a compact device used for HbA1c determination based on an immunological method [3], due to the time constraints of the present study, a more limited number of fingertip blood samples were used. Although the correlation was acceptable ($r^2 = 0.794$), some unexplained random discrepancies were observed between the two methods. However, the correlation between the In2it® and Variant II® results was comparable to that previously reported with the DCA® analyzer [3]. Nevertheless, none of these three methods can be considered a reference method. This means that users need to be aware of the possibility of discrepancies in any individual sample when different methods are used for measuring HbA1c in a same patient.

The observed linearity of the method was highly satisfactory, and covered most of the values found in patients. However, no results are given when values are greater than 14% (triggering the “Hi” alarm) or less than 4% (triggering the “Lo” alarm) and, at the time of the present evaluation, the analyzer would then print an HbA1c value of 0% and keep this value in its memory. This made it impossible to retrospectively distinguish between a very low and very high HbA1c value. However, this feature was corrected by software modifications in October 2009. Since then, the device prints out the values as either “greater than 14%” or “less than 4%” and stores them in its memory.

No interferences were observed from LA1c and CHb, which is of particular importance in patients with renal insufficiency [18,19]. In addition, it was confirmed that bilirubin did not interfere in the range indicated by the manufacturer (no interference < 350 µmol/L). On the other hand, the method was sensitive to the interference of high triglyceride concentrations, although results up to 6 mmol/L of triglycerides can be delivered with confidence (Fig. 3). An awareness of such limited interference is important in the interpretation of routine HbA1c results, especially in outpatients testing, as any discrepancy between HbA1c results and other clinical or biological parameters of diabetic control should evoke the suspicion of such interference.

During the present evaluation, discordant values were noted in samples with total haemoglobin concentrations less than 72 g/L. The manufacturer made the same observation and has therefore announced that the device will be modified to delete HbA1c results when the total haemoglobin concentration is outside the range of 80–200 g/L.

The haemoglobin variants tested in the present study did not interfere with HbA1c evaluation using the In2it® analyzer. Furthermore, In2it® was able to provide a result in a sample including 33.9% HbF and 59.5% HbS, unlike the Variant II® HPLC analyzer. This was due to the underlying principle of the method, which evaluates the total glycation rate of haemoglobin from which it then derives the estimated HbA1c value. We assume that a comparable performance would probably be obtained from samples of homozygous haemoglobinopathies, although we had no opportunity to verify this hypothesis. However, as with immunoassays, the presence of a haemoglobin variant cannot be detected by this method [3]. This suggests that HbA1c results have to be interpreted with caution in such circumstances with the use of a well-controlled approach [20].
As for the practicability of the In2it® analyzer, its small size makes it easy to handle and manipulate. Cartridge identification and all the information necessary for calibration are given by the codes affixed along the edge of the cartridge, which are automatically read by the machine during the analysis. The analyzer is also supplied with a system-control cartridge, which checks that the optical and operating systems are running correctly, although such verification is not mandatory. As the device can run on batteries, the analyzer can also be used at the bedside. This setting, however, was not tested during the present evaluation. The In2it® device requires no maintenance; its life cycle, according to the manufacturer, is 10,000 analyses, but this could not be verified, given the time frame of our present evaluation.

An important feature is the volume of blood required for the analysis. The In2it® needs 10 µL of whole blood, which is significantly more than other comparable analyzers [3]. This issue may prove difficult to achieve without training, especially in young children, as reported during its evaluation in a clinical paediatric unit. Another feature is the duration of the analysis (10 min), which is longer than that of other compact analyzers routinely used for POCT in our hospital.

On evaluating some of the features of the device’s software, we found that the traceability of results could be improved. In the current version of the software, identification of the patient and operator is not mandatory for releasing HbA1c results. However, some results may be lost, as the memory of the analyzer is limited to 200 results, which are automatically erased without an alarm and replaced by incoming values. Also, some of the alarm messages can be confusing; for example, when the analyzer displays the ‘E19’ alarm, which indicates an excess absorbance of a sample (too-large quantity of blood, too-high haematocrit or too-high concentration of total haemoglobin), the alarm code is printed in place of the patient’s identity, whereas it would be better if the alarm codes appeared in the results area while maintaining the patient’s identity.

The quality-control software could also be improved. The system only accepts control materials specifically designed by the manufacturer, which is a limiting feature. Moreover, the systematic assay of HbA1c in control samples is not mandatory. Accordingly, the patient’s results are not retained in cases of incorrect results according to quality control. However, these improvements should be available by 2010.

Finally, results can be transferred to a laboratory computer via a standard USB cable. The transfer software is easy to use, but is not secure; for this reason, we recommend that the data be stored in a protected Word or Excel file.

5. Conclusion

In general, the analytical performance of the In2it® analyzer proved to be satisfactory, especially in terms of precision, linearity and interference. Special attention should, however, be paid to the interference of high hypertriglyceridaemia, which results in underestimation of the HbA1c value. Because of the underlying principle of the method, the precision of the measurements was found in the highest levels of the commonly accepted range.

Although the analyzer is easy to use, the duration of the analysis and the volume of blood required should be decreased, if possible, to better meet the expectations of clinicians and patients. Also, several software specifications need to be improved for its optimal use according to good laboratory practices (management of quality control, traceability of results, data safety), but these are expected to be included in the upgraded versions of the software.

This new analyzer may be used either as a POCT analyzer or as a laboratory analyzer for small series of assays. However, as is the case with every laboratory analyzer, users need to carefully analyze the analytical performance of the device, and weigh the advantages and drawbacks before making this choice, and then keep these characteristics in mind during its daily use. Furthermore, users need to be aware that the use of different analyzers, even when traceable to reference methods, increases the risk of discrepancies and random errors.

Conflicts of interest

This study was supported by Bio-Rad Laboratories in France, which provided the In2it® device and its reagents.

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