Glycated Haemoglobin A1c Measurement in Stored Whole Blood Sample is Reliable for Clinical Use
CE Ezenwaka¹, D Seales¹, R Surujlal², RP Mathura²

ABSTRACT
Glycated haemoglobin A1c (HbA1c) gives an integrated plasma glycaemia for the previous 2–3 months and its measurement is central in the management of diabetic patients. However, in many developing countries because kits/regents or expertise for HbA1c measurement are not always available and the test must be conducted on fresh whole blood samples, HbA1c tests are not routinely performed. Thus, this study aimed to determine if the degradation products from whole blood sample storage are significant enough to compromise the diagnostic value of HbA1c measurements. Two hundred and thirty-one fresh whole blood samples with pre-determined HbA1c values were stored at between 2–8°C and using boronate affinity immunoassay technique, HbA1c values were then measured in the same whole blood samples after 20 days of storage. The results showed that there were no significant differences in the mean values of the initial HbA1c measurement and the values obtained after storage (7.5 ± 2.0 vs. 7.5 ± 2.1, \(p > 0.05\)) and this was irrespective of gender. Furthermore, irrespective of gender, there were significant correlations between the HbA1c values measured in fresh whole blood samples and values obtained after storage (\(r = 0.83, p < 0.01\)). Therefore, based on these findings and other previous reports, the effect of storage degradation product was not significant enough to compromise the clinical or research use of HbA1c test results from stored whole blood samples. However, we recommend that diagnostic laboratories should evaluate their HbA1c measurement techniques for HbA1c determination in stored whole blood samples. Any persistent upward or downward bias in stored whole blood samples should be reported to guide the physician in interpreting HbA1c results from stored whole blood samples from that laboratory and/or technique.

La Medición de la Hemoglobina Glicada en Muestras de Sangre Entera Almacenadas es Confiable para el uso Clínico
CE Ezenwaka¹, D Seales¹, R Surujlal², RP Mathura²

RESUMEN
La hemoglobina glicada o glicosilada A1c (HbA1c) produce una glicemia plasmática integrada en los últimos 2–3 meses y su medición es fundamental para el tratamiento de pacientes diabéticos. Sin embargo, en muchos países en vías de desarrollo – debido a que no siempre hay kits/reactivos o conocimiento experto para la medición de HbA1c, y la prueba tiene que realizarse con muestras de sangre entera fresca – no se realizan tests de HbA1c de forma rutinaria. Así, este estudio apuntó a determinar si los productos de degradación del almacenamiento de la muestra de sangre entera son suficientemente significativos como para comprometer el valor del diagnóstico de las mediciones de las dimensiones de HbA1c. Doscientos treinta y una muestras de sangre entera fresca con valores HbA1c pre-determinados, fueron almacenadas entre 2–8°C y usando la técnica de inmunoensayo de afinidad al boronato, los valores de HbA1c fueron entonces medidos en las mismas muestras de sangre entera después de 20 días de almacenamiento. Los resultados mostraron que no había ninguna diferencia significativa en los valores promedios de la medición inicial de HbA1c y los valores obtenidos después del almacenamiento (7.5 ± 2.0 vs. 7.5 ± 2.1, \(p > 0.05\)), independientemente del género. Además, con independencia del género, hubo correlaciones significativas entre los valores de HbA1c, medidos en las
Haemoglobin A1c samples hence HbA 1c is typically conducted on fresh whole blood samples. Delays in procurement of assay kits can take up to 21 days to arrive, we decided to store the samples for twice the manufacturer’s recommendation to determine if longer storage could significantly affect diagnostic values of the results. Thus, the initial HbA1c levels and date of measurements were recorded in the computer spreadsheet. After 20 days of the initial test, the HbA1c measurements were repeated on the stored whole blood samples using the same boronate affinity immunoassay technique and the values recorded. The two hundred and thirty-one blood samples were determined in 14 batches based on the dates blood samples were drawn. The repeat laboratory tests for HbA1c in the stored whole blood samples were performed by the same technician that performed the initial analysis.

The results are expressed as mean ± SD. The Statistical Package for the Social Sciences (SPSS Inc, Chicago, USA) software was used in all analyses. The difference between the initial HbA1c values and the values after storage were determined using paired student’s t-tests while the correlation between the initial HbA1c values and the values after storage were determined by Pearson correlation technique. A p-value < 0.05 was considered statistically significant on two-tailed testing for all analysis.

RESULTS
The assay has a measuring range of 3–18% HbA1c while the measuring interval is 0.1% HbA1c. The repeat tests for the 231 blood samples (14 batches) had overall inter-assay coeffi-
ficient of variation of 3.9% and 7.3% for the normal and the abnormal quality control samples respectively. Table 1 shows the initial mean ± SD values of the HbA1c obtained from fresh whole blood samples and values obtained after 20 days of storage at 2–8°C. There was no significant difference in the mean value of the initial HbA1c measurement and the value obtained after 20 days of storage irrespective of gender ($p > 0.05$). Although the mean of the differences range from -0.04% to 0.02% in female and male subjects respectively, the overall mean of the differences and the standard deviation (measure of upward or downward bias) obtained were -0.01% and 1.2% (Table 1). Figures 1 and 2 show the cor-

<table>
<thead>
<tr>
<th>Characteristic of samples</th>
<th>All</th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td>Fresh blood sample mean (±SD) (%)</td>
<td>7.5 (2.0)</td>
<td>7.3 (2.1)</td>
<td>7.5 (2.0)</td>
</tr>
<tr>
<td>Stored blood sample mean (±SD) (%)</td>
<td>7.5 (1.6)</td>
<td>7.4 (1.8)</td>
<td>7.5 (2.1)</td>
</tr>
<tr>
<td>Mean of the difference (stored – fresh sample) %</td>
<td>-0.01</td>
<td>0.02</td>
<td>-0.04</td>
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<tr>
<td>±SD of the mean of the differences (measure of upward or downward bias) %</td>
<td>1.2</td>
<td>1.1</td>
<td>1.3</td>
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DISCUSSION

The present study attempted to determine the reproducibility of HbA1c measurements in stored whole blood samples and the results have shown that reliable HbA1c results could still be obtained from whole blood samples stored for up to twice the kit manufacturer’s storage recommendation. This is consistent with other reports on frozen whole blood samples stored for even longer periods (10). This has important implications in the management and treatment of diabetic patients and for diabetes research.

The present observation is potentially useful for physicians and researchers in developing countries where HbA1c measurement is typically conducted on fresh blood samples and yet the reagents/kits for HbA1c measurements are often in short supply. The apparent limitations (resources, expertise etc) in measuring HbA1c on a routine and/or regular basis in many developing countries might have contributed, in part, to several research reports of poor glycaemic control in many developing countries (11–14). In Trinidad and Tobago, reports of poor glycaemic control is particularly worse at the primary care setting where we have previously reported on the cardiovascular risk implications of
poor glycaemic control (14, 15). Of concern is the fact that in most primary care clinics in Trinidad and Tobago, the mean HbA1c value for each clinic exceeded the 7.0% cut-off point recommended from the landmark UKPDS report (5). In our experience, laboratory requests for HbA1c tests for diabetic patients are not routine and regular given that physicians, especially at the primary care settings, rely on the routine fasting blood glucose measurement in the clinic for the review of the patient’s medical prescription. We are of the view that HbA1c measurement should be routine and regular to guide prescription review especially as diabetes health educators and dieticians are in short supply in many developing countries (16). Furthermore, measurement and use of fasting blood glucose results, as a guide in determining the patient’s plasma glycaemia, is not technically reliable given that most patients do not always conform to the requirements for determining fasting blood glucose concentration as stipulated in the new diagnostic criteria (17). Therefore, for effective management of diabetic patients, it is important that the physician know the true integrated long-term glycaemic level of his/her patient as estimated by HbA1c tests. In many developing countries, this is largely dependent on the availability of the HbA1c test kits given that non-availability of the test kit/reagents or even technical and laboratory expertise, as is often the case at primary care settings, means that the physician would have missed this important laboratory information that would better guide prescription review. This is essentially the general uptake in many primary care settings in the developing countries and since there is no research documentation to suggest or recommend blood sample storage for HbA1c measurement, HbA1c tests are often omitted when the reagents/kits are not readily available. For instance, in the present study, the leaflet in the kit used (Axis Shield PoC AS, N-0504, Oslo, Norway) recommended storage duration of less than 10 days. Therefore, the results of the present study, which found a strong correlation between the fresh HbA1c values and storage values, should encourage researchers and laboratory managers currently using the above manufacturers’ HbA1c kits to carry out tests on whole blood samples stored longer than the manufacturer’s recommendation. Indeed, the present study has demonstrated that the effect of storage degradation product was not significant enough to compromise the clinical or research use of HbA1c test result from stored whole blood samples. The finding of 1.2% bias in this study for stored samples is further strengthened by a previous report that showed only 0.35% upward bias in frozen whole blood samples stored for over a decade (10). Therefore, based on the present findings and other previous reports (10), researchers and clinical diagnostic laboratories should evaluate their HbA1c measurement techniques for HbA1c determination in stored whole blood samples. Any persistent upward or downward bias in stored whole blood samples should be reported to guide physicians in interpreting HbA1c results from stored whole blood samples from that particular laboratory and/or technique.

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REFERENCES