# Study of the effect of altitude on the measurement of glycated haemoglobin using point-of-care instruments

Sandra W Veigne, Eugene Sobngwi, Brice E Nouthe, Joelle Sobngwi-Tambekou, Eric V Balti, Serge Limen, Mesmin Y Dehayem, Vicky Ama, Jean-Louis Nguewa, Maimouna Ndour-Mbaye, Alioune Camara, Naby M Balde, Jean-Claude Mbanya

## Abstract

We measured the glycated haemoglobin ($HbA_{1c}$) levels of a total of 24 non-diabetic volunteers and diabetic patients using a point-of-care (POC) analyser in three Cameroonian cities at different altitudes. Although 12 to 25% of duplicates had more than 0.5% (8 mmol/mol) difference across the sites, $HbA_{1c}$ values correlated significantly ($r$ = 0.89–0.96). Further calibration studies against gold-standard measures are warranted.

**Keywords:** glycated haemoglobin, altitude, diabetes

Submitted 7/12/14, accepted 18/12/14
*Cardiovasc J Afr* 2015; **26**: 38–40 www.cvja.co.za

DOI: 10.5830/CVJA-2014-078

$HbA_{1c}$ concentration is used for the appropriate diagnosis and management of diabetes,[1,2] but the standard way of measurement requires an expensive and time-consuming ion-exchange, high-performance liquid chromatography (HPLC) technology. Point-of-care (POC) instruments represent a cheaper alternative to determine $HbA_{1c}$ levels in five to 10 minutes. They can be used by non-laboratory staff to tailor a patient's care and educational messages to $HbA_{1c}$ values and clinical findings in a one-stop-shop approach.[3,4] Their potential shortcomings include cases of haemoglobinopathy or some environmentally linked limitations.[5,6]

While operating temperature and humidity are easily controlled, altitude cannot be standardised for operation. We investigated the performance of one of the most commonly used POC $HbA_{1c}$ instruments in African clinical settings, situated at varying altitudes.

## Methods

In this cross-sectional study, $HbA_{1c}$ concentrations were measured in three cities of Cameroon in blood samples simultaneously collected from the same individuals. The study settings were Douala (13-m altitude), Yaounde (650-m altitude), and Bamenda (1 600-m altitude).

The study was approved by the National Ethics Committee of Cameroon. All participants gave their informed consent.

The study participants were 24 volunteers distributed in four groups: six non-diabetic (healthy) volunteers [no clinical symptoms, fasting glycaemia < 1.26 g/dl (6.99 mmol/l) and $HbA_{1c}$ levels < 6.6% (< 49 mmol/mol)], six patients with diabetes with $HbA_{1c}$ levels < 6.6% (< 49 mmol/mol), six patients with $HbA_{1c}$ levels at 6.6–8.0% (49–64 mmol/mol) and six patients with $HbA_{1c}$ levels > 8.0% (> 64 mmol/mol).

All patients had to have had diabetes for at least one year, with stable treatment and $HbA_{1c}$ values over at least three months preceding the study defined by $HbA_{1c}$ variation < 1% between two measurements. Exclusion criteria included any haemoglobinopathy, recent malaria, haematological disorder or any other acute medical condition in the preceding month, total haemoglobin level > 11 g/dl, and creatinine clearance < 60 ml/min.

**National Obesity Center, Yaoundé Central Hospital and Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Yaoundé, Cameroon**
Sandra W Veigne, MD
Eugene Sobngwi, MD, MPhil, PhD, sobngwieugene@yahoo.fr
Brice E Nouthe, MD
Eric V Balti, MD
Serge Limen, MD
Mesmin Y Dehayem, MD
Vicky Ama, MD
Jean-Louis Nguewa, MD
Jean-Claude Mbanya, MD

**Molecular Medicine and Metabolism Laboratories, Bio-technology Center, University of Yaoundé 1, Yaoundé, Cameroon**
Eugene Sobngwi, MD, MPhil, PhD
Jean-Claude Mbanya, MD

**Department of Medicine, McGill University, Montreal, Quebec, Canada**
Brice E Nouthe, MD

**Centre of Higher Education in Health Sciences, Catholic University of Central Africa, Yaoundé, Cameroon**
Joelle Sobngwi-Tambekou, MD

**Diabetes Research Center, Faculty of Medicine and Pharmacy, Brussels Free University-VUB, Brussels, Belgium**
Eric V Balti, MD

**Cheick Anta Diop University, Dakar, Senegal**
Maimouna Ndour-Mbaye, MD

**University Teaching Hospital of Donka, Conakry, Guinea**
Alioune Camara, MD
Naby M Balde, MD

**University of Technology, Kingston, Jamaica**
Jean-Claude Mbanya, MD

Volunteers were invited, and after informed consent, we conducted an interview, clinical examination and biochemical investigations for the ascertainment of eligibility. Collections of venous blood in eligible participants were all done the same day from an antecubital vein in four EDTA tubes stored in refrigerated containers for all three assays.

The blood samples collected on the same day for each participant were immediately transported by car to the target settings in a refrigerated container. The room temperature was standardised for all study sites at 25°C, and humidity was maintained between 45 and 60%.

$HbA_{1c}$ measurements were performed using the In2it POC device (Bio-Rad laboratories, Deeside, UK), which was calibrated prior to the study, with all reagents from the same lot (072T128). The same operator performed the assays in each of the settings within 48 hours of blood collection. All manipulations were done following the operating procedure of the manufacturer in order to reduce the variability of the measurements.

## Statistical analysis

Using SPSS 17.0, data were analysed and expressed as mean ± standard deviation. Comparisons across the groups were done using analysis of variance, and associations were verified by Spearman's correlation. Agreement between methods was assessed using Bland and Altman plots of the difference against the means of the two methods.

## Results

Participants were 12 males and 12 females, aged 54 ± 15 years. Their mean body mass index was 28.9 ± 5.8 kg/m$^2$, mean systolic and diastolic blood pressures were 128 ± 18 and 77 ± 8 mmHg, respectively, and mean haemoglobin was 13.4 ± 1.8 g/dl. The duration of diabetes in all patients was 10 ± 6 years with a pre-inclusion $HbA_{1c}$ value of 7.8 ± 2.3%.

Overall, there was no statistically significant difference between mean $HbA_{1c}$ measurements across the sites (Table 1). The correlation between measurements varied from $r = 0.89$, $p < 0.001$ between the 650-m/1 600-m altitudes, $r = 0.92$, $p < 0.001$ between the 13-m/650-m altitudes, to $r = 0.96$, $p < 0.001$ between 13-m/1 600-m altitudes. The coefficient of variation (CV) was 3.4% for the 650-m/13-m duplicates, 5.1% for 1 600-m/13-m duplicates and 3.2% for 1 600-m/650-m duplicates.

**Table 1. Comparison of mean $HbA_{1c}$ levels by group across the sites**

| | *Point-of-care In2it analyser* | | | |
|---|---|---|---|---|
| *Study group* | *Douala (13 m)* | *Yaounde (650 m)* | *Bamenda (1 600 m)* | p-*value* |
| Healthy controls | 5.0 ± 0.6 | 5.4 ± 0.3 | 5.6 ± 0.5 | 0.15 |
| Patients with diabetes | | | | |
| $HbA_{1c}$ < 6.5% (< 49 mmol/mol) | 5.9 ± 0.6 | 5.7 ± 0.6 | 5.9 ± 0.4 | 0.29 |
| $HbA_{1c}$ 6.5–8.0% (49–64 mmol/mol) | 8.1 ± 3.0 | 7.9 ± 3.1 | 8.0 ± 3.0 | 0.66 |
| $HbA_{1c}$ > 8.0% (> 64 mmol/mol) | 8.4 ± 1.8 | 8.5 ± 1.7 | 9.0 ± 2.2 | 0.84 |
| All study participants | 6.8 ± 2.2 | 6.9 ± 2.2 | 7.1 ± 2.3 | 0.31 |

The mean differences expressed as estimates (95% CI) in percentages between measurements at two different sites were –0.04 (–1.05−0.97%), +0.14 (0.95−1.24%) and +0.13 (–0.45−0.70%), respectively, between the 650-m/13-m (Fig. 1A), 1 600-m/650-m (Fig. 1B), and 1 600-m/13-m altitudes (Fig. 1C).

The $HbA_{1c}$ differences were > 0.5% (8 mmol/mol) in 3/24 (12%) between the 1 600-m/13-m measurements, 4/24 (17%) between the 650-m/13-m measurements and in 6/24 (25%) between the 1 600-m/650-m measurements. In only one case associated with more than one percentage difference across sites was a patient with one of the readings at 4.2% (22 mmol/mol) in one site, which normally would have prompted a second check. We did not find any differences in the percentage variation of $HbA_{1c}$ levels at the low ($n = 12$), medium ($n = 6$) and high ($n = 6$) values for the different study sites, namely 650-m/13-m ($p = 0.453$), 1 600-m/650-m ($p = 0.111$) and 1 600-m/13-m altitudes ($p = 0.344$).

## Discussion

This study indicates that the POC analyser showed no significant differences across Cameroonian sites located at altitudes varying from 13 to 1 600 m (≤ 0.5% in 75% of comparisons). Although measurements were not repeated in each site to reflect clinical practice, our results suggest a test reliability of the In2it POC instrument below 1 600 m.

Interestingly, previous studies in which the device calibration was performed with HPLC, had suggested satisfactory external validity.[7] This was however not investigated in our study and therefore represents a major limitation with the sample size.

However, considering our findings and the cut-off value of 3.5% of CV for optimal performance between laboratories (between study sites in our case), one could say that although

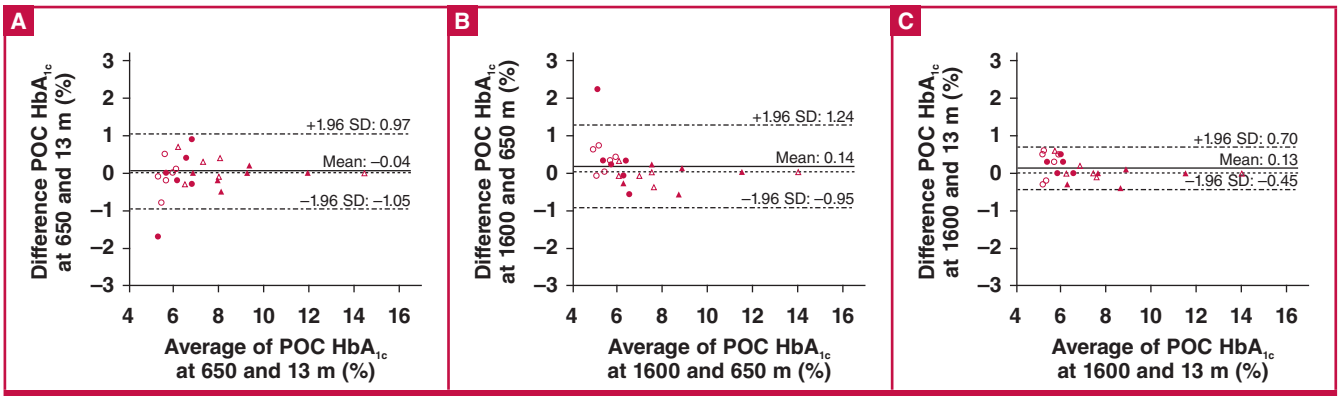


**Fig. 1.** Plots of the differences against averages of POC $HbA_{1c}$ levels at 13-m and 650-m altitudes (A), 1 600-m and 650-m altitudes (B), and a1 600-m and 13-m altitudes (C), with mean difference (bias) and 95% agreement limits.

no significant difference was observed between $HbA_{1c}$ levels at the three altitudes, the POC apparatus had a relatively high variability between 13 and 1 600 m.[8] As expected, this variability was higher in low and normal $HbA_{1c}$ levels (not shown).

In this regard, the use of the POC $HbA_{1c}$ analyser could be more indicated for the monitoring of patients with a view to comparing before- and after-treatment glucose control, especially in the lower values, even in the absence of calibration with an HPLC machine.

Consistent with our results, a recent study of $HbA_{1c}$ variations in Chinese populations living at different altitudes did not find meaningful variations in the $HbA_{1c}$ levels and the estimated average glucose levels of patients living in different sites.[9]

However, on the one hand, Ju *et al.*[9] in their study used the immunoturbidimetric method for the measurement of $HbA_{1c}$ levels (also without validation against the gold standard for $HbA_{1c}$ measurement), while we used a baronate affinity chromatography to separate glycated from non-glycated haemoglobin for photometry.[4,9] On the other hand, we sought to evaluate the possible effect of altitude on the accuracy of a POC $HbA_{1c}$ analyser in patients with diabetes, while they aimed to evaluate whether altitude could modify the glycation of $HbA_{1c}$.

In our study, we observed that 12–25% of duplicates had more than a 0.5% (8 mmol/mol) difference across the sites. The performance of POC apparatus in general and the In2it in particular has (independent of altitude) been assessed before. These investigations constituted a body of evidence showing the need for improvement in the performance of devices for optimal care.[10-12]

The recent performance of these devices has given promising results. This also was the case where the In2it apparatus is concerned, despite the between-batch variability of results, which still needs to be addressed.[7,13] To circumvent this in our study, we used reagents from the same lot number at all study sites. However, in daily clinical practice, this could indeed be a concern for patients' follow up.

With the generalisation of $HbA_{1c}$ use, especially in developing countries that have limited access to an HPLC and have a wide variety of physical environments, it is important to know which parameters should be taken into account when validating POC $HbA_{1c}$ devices, which are commonly presented as the adequate alternative to estimate glycaemic control of patients.

## Conclusion

Our results reinforce the need for calibration of POC instruments against the HPLC in each setting used, to ensure validity of the readings. We did not find any significant differences when measuring $HbA_{1c}$ levels at different altitudes on the same samples. However this requires validation with further studies, using larger sample sizes and addressing situations with higher proportions of patients with haematological disorders.

This project was supported by BRIDGES. BRIDGES is an International Diabetes Federation programme supported by an educational grant from Lilly Diabetes.

## References

1. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care* 2009; **32**: 1327–1334.
2. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2014; **37**(Suppl 1): S81–S90.
3. Cagliero E, Levina EV, Nathan DM. Immediate feedback of $HbA_{1c}$ levels improves glycemic control in type 1 and insulin-treated type 2 diabetic patients. *Diabetes Care* 1999; **22**: 1785–1789.
4. Petersen JR, Omoruyi FO, Mohammad AA, Shea TJ, Okorodudu AO, Ju H. Hemoglobin $A_{1c}$: assessment of three POC analyzers relative to a central laboratory method. *Clin Chim Acta* 2010; **411**: 2062–2066.
5. De Mol P, Krabbe HG, de Vries ST, *et al*. Accuracy of handheld blood glucose meters at high altitude. *PLoS One* 2010; **5**: e15485.
6. Gautier JF, Bigard AX, Douce P, Duvallet A, Cathelineau G. Influence of simulated altitude on the performance of five blood glucose meters. *Diabetes Care* 1996; **19**: 1430–1433.
7. Martin M, Leroy N, Sulmont V, Gillery P. Evaluation of the In2it analyzer for $HbA_{1c}$ determination. *Diabetes Metab* 2010; **36**: 158–164.
8. Sacks DB, Arnold M, Bakris GL, *et al*. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Diabetes Care* 2011; **34**: e61–e99.
9. Ju H, Yang L, Fan J, Shu Z. Comparison of blood sugar and glycosylated hemoglobin in type 2 diabetic patients of Chinese provinces at different altitudes. *Biomed Res* 2014; **25**: 311–316.
10. Al-Ansary L, Farmer A, Hirst J, *et al*. Point-of-care testing for Hb A1c in the management of diabetes: a systematic review and metaanalysis. *Clin Chem* 2011; **57**: 568–576.
11. Lenters-Westra E, Slingerland RJ. Six of eight hemoglobin A1c point-of-care instruments do not meet the general accepted analytical performance criteria. *Clin Chem* 2010; **56**: 44–52.
12. Lenters-Westra E, Slingerland RJ. Three of 7 hemoglobin A1c point-of-care instruments do not meet generally accepted analytical performance criteria. *Clin Chem* 2014; **60**: 1062–1072.
13. Little RR, Lenters-Westra E, Rohlfing CL, Slingerland R. Point-of-care assays for hemoglobin A(1c): is performance adequate? *Clin Chem* 2011; **57**: 1333–1334.