Letter to the Editor

Dominika Szőke*, Assunta Carnevale, Sara Pasqualetti, Federica Braga, Renata Paleari and Mauro Panteghini

More on the accuracy of the Architect enzymatic assay for hemoglobin A_{1c} and its traceability to the IFCC reference system

DOI 10.1515/cclm-2015-0550
Received June 11, 2015; accepted July 23, 2015; previously published online August 18, 2015

Keywords: analytical performance; HbA_{1c}; metrological traceability.

To the Editor,

We read with great interest the article by Teodoro-Morrison et al. [1] that reported the evaluation of the direct whole blood enzymatic assay for hemoglobin A_{1c} (HbA_{1c}) on the Architect *c*8000 platform (Abbott Diagnostics). The authors found the new automated Abbott method quite robust and able to meet the analytical performance requirements to support the HbA_{1c} use for diagnosis and management of diabetes mellitus (DM). As this HbA_{1c} method (ref. #4P52-21) was recently introduced in our clinical laboratory, but on a different platform of the Architect family (*c*4000), here we aimed to provide further data about its analytical performance by reporting the experience accumulated during the first 6-month period of work, with special emphasis on the accuracy of measurement and its quality when compared to established analytical goals [2].

The fully automated measurement was performed according to manufacturer's instructions. The assay calibrators (ref. #4P52-02), which values are traceable to the IFCC HPLC-capillary electrophoresis (CE) reference procedure, have a declared expanded uncertainty (U) (obtained by multiplying the standard uncertainty by a coverage factor of 2) of 1.05% (cal 1, 33.32 μmol/L) and 0.77% (cal 2, 103.28 μmol/L), respectively [3]. The system alignment was checked two times per day (at 07:00 and at 12:00) using BioRad Lyphochek Diabetes Control materials (two levels, ref. 740) by verifying that results fell inside the established acceptable ranges (target value ±20%). Assay imprecision was obtained from daily measurements of aliquots of a pool of human EDTA blood samples stored at –20 °C, with a HbA_{1c} concentration around the diagnostic cut-off for DM. We previously demonstrated that fresh-frozen pooled whole blood is a suitable and cheap material for intra-laboratory evaluation of imprecision of HbA_{1c} measurements [4]. Regular participation to a regional external quality assessment (EQA) program (one-level control material measured in singlicate – 8 exercises/year) provided periodic information on the assay's accuracy [5]. Finally, we participated in a pilot national EQA scheme, organized by the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC) in November 2014, with materials (two concentration levels) value-assigned by the IFCC HPLC-CE reference procedure performed in the reference laboratory of the Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, accredited as calibration laboratory by the national calibration service (Accredia) (accreditation no. 217). Stability and commutability of the employed EQA materials had been previously demonstrated [6, 7]. Limits for clinical acceptability of HbA_{1c} measurement of patient samples were defined according to Braga and

***Corresponding author: Dominika Szőke,** UOC Patologia Clinica, Azienda Ospedaliera "Luigi Sacco", Via GB Grassi 74, 20157 Milan, Italy, Phone: +39 02 39042683, Fax: +39 02 39042364, E-mail: szoke.dominika@hsacco.it; and Clinical Pathology Unit, "Luigi Sacco" University Hospital, Milan, Italy
Assunta Carnevale and Sara Pasqualetti: Clinical Pathology Unit, "Luigi Sacco" University Hospital, Milan, Italy
Federica Braga and Renata Paleari: Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milan, Italy
Mauro Panteghini: Clinical Pathology Unit, "Luigi Sacco" University Hospital, Milan, Italy; and Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milan, Italy

Panteghini, i.e. an U budget ≤2.0% and an allowable maximum total error (TE) of ±6% [2].

Seven different reagent lots were used during the study period. The monthly CV (on average, n=20) was between 0.6% and 1.9% and the cumulative CV during the whole examined period (November 2014 to April 2015) (n=122) was 1.1% (mean HbA_{1c} concentration, 47.8 mmol/mol). During the evaluation period five exercises of the regional EQA program were carried out. Target values of EQA materials (from 49.3 to 82.6 mmol/mol) were calculated as the mean of all method results after outliers exclusion (no. of participating laboratories, 311). In this EQA scheme, participating laboratories test control samples in the same manner they test biological samples, i.e. in a single measurement. When only a single measurement is performed, it is impossible to separate the different sources of measurement's error and TE is useful in defining the inaccuracy of the measurement [8]. A mean TE of −0.22% (range −3.90% to 1.84%) was obtained. As the commutability of the employed materials was not definitively proved and control values were not assigned with the reference procedure, results could not prove the traceability of the assay. However, they were useful in proving the lack of concentration-dependent trends in TE shown in a previous study [9]. The evaluation of assay traceability was obtained by analyzing two fresh EDTA blood samples with target HbA_{1c} values (±U) assigned by the IFCC HPLC-CE reference procedure (level 1, 37.4 [±0.57] mmol/mol; level 2, 62.0 [±0.91] mmol/mol). For both levels, our laboratory values (37 and 63 mmol/mol) were within the range of U of the IFCC true value, indicating no bias and negligible TE (−1.1% and 1.6% vs. an allowable maximum TE of ±6%). This suggests a quite perfect alignment of Architect enzymatic assay to the higher order metrological references [2].

Figure 1 shows in detail the traceability chain of the HbA_{1c} measurement and the related combined standard uncertainty associated with our experimental situation. In the scheme, the values of uncertainty attributable to the secondary calibrator is that obtained in the pilot SIBioC EQA study by the CIRME reference laboratory for sample 1 and the uncertainty attributable to the manufacturer's product calibrator is that declared by Abbott for the HbA_{1c} calibrator 1 employed on the Architect systems for the enzymatic method [3]. Finally, the uncertainty estimate of the measurement obtained with the routine procedure (Architect *c*4000 platform) derives from the average imprecision (expressed as CV) of the system (1.1%) used in our clinical laboratory of the 'Luigi Sacco' hospital in Milan. Using these data the relative combined standard uncertainty associated with the measurement of a biological sample by the Abbott enzymatic HbA_{1c} method on the Architect *c*4000 platform is 1.44%, which corresponds to an U equal to 2.88%. This is still >2.0%, i.e. the quality target, but it represents a significant improvement when

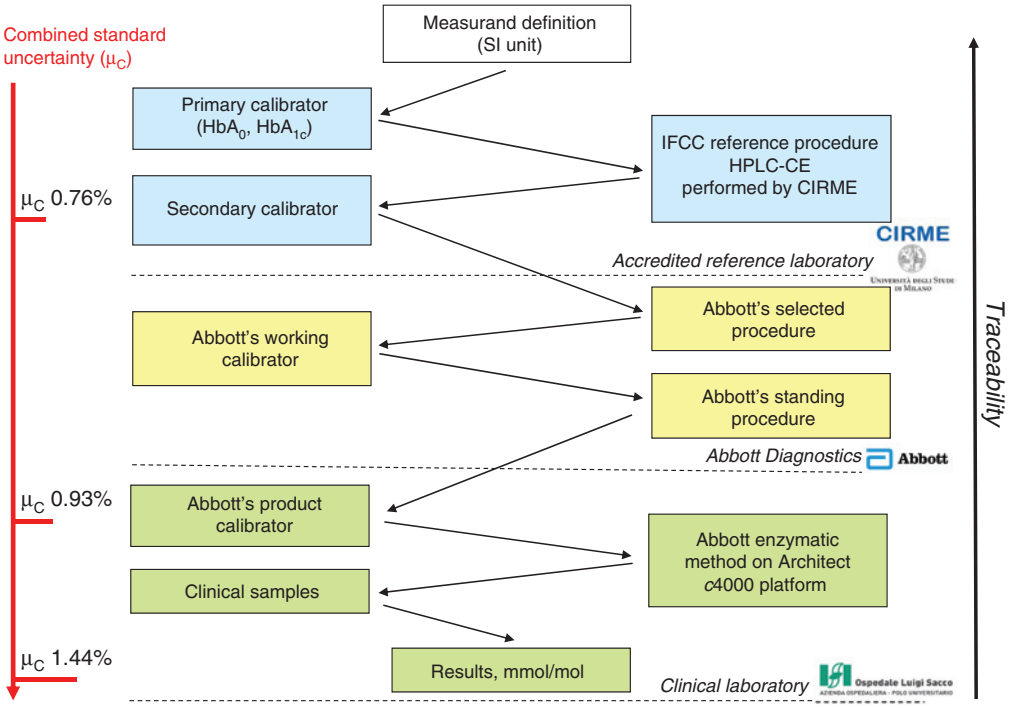

Figure 1: Traceability chain of the HbA_{1c} measurement associated with the conditions described in the study.
In the scheme, the displayed values of uncertainties are those related to HbA_{1c} concentrations in the range between 30 and 50 mmol/mol.

compared with the estimated U of approximately 4.0%, previously obtained in standardized conditions [2]. These results highlight that the important considerations on the needed advances in the quality of HbA_{1c} measurement we did in the conclusions of a previous paper (i.e. to reduce uncertainty associated with higher-order metrological references [reference procedures and materials] and to increase the precision of commercial HbA_{1c} assays) have been actively considered both by the reference laboratory, for which accreditation is evidently a factor of improved performance, and the assay manufacturers, for which stricter requirements for certification of HbA_{1c} methods will likely to become mandatory [2].

In conclusion, our experience with the Architect enzymatic HbA_{1c} method confirms its good analytical performance. The full automation of the method, including on-board red blood cell lysis, provides relatively low imprecision that significantly contributes to reduce the measurement uncertainty. The alignment of the analytical system to the IFCC reference measurement system is quite perfect, resulting in virtually unbiased HbA_{1c} results on patient samples.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Teodoro-Morrison T, Janssen MJ, Mols J, Hendrickx BH, Velmans MH, Lotz J, et al. Evaluation of a next generation direct whole blood enzymatic assay for hemoglobin A_{1c} on the ARCHITECT *c*8000 chemistry system. Clin Chem Lab Med 2015;53:125–32.
2. Braga F, Panteghini M. Standardization and analytical goals for glycated hemoglobin measurement. Clin Chem Lab Med 2013;51:1719–26. Erratum in Clin Chem Lab Med 2013;5:2064.
3. Abbott ARCHITECT traceability and uncertainty of measurement document 306354/R02, 2013.
4. Braga F, Dolci A, Scapellato L, Mosca A, Panteghini M. Fresh-frozen human whole blood for intralaboratory evaluation of imprecision of glycated hemoglobin determination. Biochim Clin 2010;34:203–6.
5. Mosca A. Some practical advices on how to implement the international standardization of glycated hemoglobin measurement in Italy. Biochim Clin 2011;35:36–41.
6. Mosca A, Weykamp C. Feasibility of an EQAS for HbA1c in Italy using fresh blood samples. Clin Chem Lab Med 2014;52: e151–3.
7. Ponzo P, Dolci A, Scapellato L, Milano M, Paleari R, Mosca A, et al. Evaluation of commutability for Cobas Integra analytical system of the EQA materials for hemoglobin A1c. Biochim Clin 2008;32:44–7.
8. Panteghini M. Application of traceability concepts to analytical quality control may reconcile total error with uncertainty of measurement. Clin Chem Lab Med 2010;48:7–10.
9. Jaisson S, Desmons A, Renard B, Chevelle B, Leroy N, Gillery P. Analytical performances of a new enzymatic assay for hemoglobin A_{1c}. Clin Chim Acta 2014;434:48–52.