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Analytical verification and quality assessment of the Tosoh HLC-723GX HbA_{1c} analyzer

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ABSTRACT

Objectives: Ion-exchange high-performance liquid chromatography (IE-HPLC) has long been used as a reproducible and versatile analytical tool for HbA_{1c} measurement.

In this study, we performed analytical verification and quality assessment of the recently introduced small IE-HPLC Tosoh HLC-723GX HbA_{1c} analyzer, and a comparison of results to immunoassay (IA) and capillary electrophoresis (CE).

Design and methods: The total imprecision of Tosoh HLC-723GX was verified according to CLSI EP15-A2 protocol using commercial control materials (C-QC) and pooled human whole blood samples (HWB). The Sigma metric was used for the evaluation of quality targets. HbA_{1c} results were compared to automated CE (MiniCap Flex Piercing, Sebia, France) and IA (Tina-quant HbA_{1c} Gen 2, Cobas Integra 400+, Roche Diagnostics, USA) procedures.

Results: The total imprecision of Tosoh HLC-723GX-HbA_{1c} for IFCC(mmol/mol) and NGSP(%) units was: 1.91/1.25% (HbA_{1c}=31 mmol/mol/5.0%) and 0.51/0.63% (HbA_{1c}=84 mmol/mol/9.8%) for C-QC, and 0.39/0.2% (HbA_{1c}=47 mmol/mol/6.5%) and 0.77/0.46% (HbA_{1c}=94 mmol/mol/10.8%) in HWB samples, respectively. Bland-Altman analysis did not reveal any deviation of the results between Tosoh HLC-723GX and CE: mean difference 0.0% (95%CI: −0.02927 to 0.02653%), while the mean HbA_{1c} difference against IA was −0.07% (95% CI: −0.1039 to −0.02765). At the selected HbA_{1c} clinical decision level (48 mmol/mol/6,5%), six sigma analysis gave σ value of 3.91, within a desirable classification of performance.

Conclusion: The analytical performance of the Tosoh HLC-723GX complies with the rigorous quality criteria for clinical use of HbA_{1c}, with the results comparable to the CE procedure. Tosoh HLC-723GX provides a plausible analytical choice for reliable HbA_{1c} measurement in low-volume laboratories.

1. Introduction

HbA_{1c} assay has been established as the cornerstone of diabetes care for decades [1,2]. Advances in methodology and global standardization of HbA_{1c} assays have enabled an effective laboratory tool for global implementation of recommended clinical practice based on specific targets for diagnosis and monitoring of diabetes [3]. However, the issue of defining and attaining quality criteria for reliable clinical use remains an important challenge for HbA_{1c} assays [4,5]. The International Federation of Clinical Chemistry (IFCC) Task Force on Implementation on HbA_{1c} Standardization (IFCC-TF-HbA_{1c}) recently proposed the sigma metric

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model for setting quality targets within and between laboratories [6].

Despite its lack of specificity, ion-exchange high-performance liquid chromatography (IE-HPLC) has long been used as a reproducible analytical tool for HbA_{1c} measurement and the reference for setting glycaemic targets traceable to evidence-based studies demonstrating a strong association between HbA_{1c} levels and the risk for adverse outcomes in patients with type 1 [7] and type 2 diabetes [8]. However, the need for dedicated equipment and expertise has limited its use to high-volume clinical laboratory facilities.

A recently introduced user-friendly, fully automated bench top IE-HPLC analyzer (HLC-723GX, Tosoh Bioscience, Tokyo, Japan) may enable laboratories with low workloads to achieve quality standards for reliable use of HbA_{1c}, which may be particularly important for diagnosis and management of diabetes in dispersed primary health care facilities.

In this study, we performed analytical verification and quality assessment of the Tosoh HLC-723GX analyzer, and compared the results to an immunoassay (IA) and the capillary electrophoresis (CE).

2. Materials and methods

2.1. Tosoh HLC-723GX

The Tosoh HLC-723GX is a small (370×525×482 mm), fully automated bench top analyzer. Whole blood samples from primary tripotassium EDTA tubes are automatically diluted and hemolyzed by the hemolyzing reagent (Tosoh Bioscience). A small volume (4 μL) of the sample is then injected onto a cation exchange column where fractions are separated based on differences in ionic interactions between the column's cation exchange groups and the hemoglobin components. The separated hemoglobin components are measured by changes in absorbance at 415 nm, and the relative percentage of each hemoglobin fraction is calculated by the analyzer from integrated and reduced raw data. Total analysis time is 2.2 min per sample, after the first sample for which the total analysis time is 6.6 min, due to an automatic analyzer initialization procedure scheduled at the beginning of each series of samples.

The system is calibrated automatically at bimonthly intervals with IFCC traceable calibrators (Tosoh Bioscience) and the results are reported in both IFCC (mmol/mol) and National Glycohemoglobin Standardization Program (NGSP; %) aligned reporting units. A built-in dedicated master-equation is used for the conversion of the results between the reporting units.

The maintenance of the analyzer is simple and automated, requiring minimal user engagement.

2.2. Comparison methods

Cobas Integra turbidimetric inhibition immunoassay (Roche Diagnostics, Indianapolis, IN, USA) and capillary electrophoresis (Capillarys Minicap, Sebia, Lisses, France) [9] were carried out according to the manufacturer's instructions and established standard operating procedures for accredited (ISO15189) medical laboratories.

2.3. Validation

Verification of total imprecision of the Tosoh HLC-723GX analyzer was performed over five consecutive days using two levels of commercial control materials and two levels of pooled human whole blood samples, tested in triplicate in accordance with CLSI EP15-A2 protocol [10].

Two levels of commercial control materials (C-QC; Tosoh Bioscience) manufactured from human blood cells and preserved by lyophilization were prepared according to the manufacturer's instructions, while two fresh whole blood samples were randomly selected from the routine workload and used after anonymization. Between the individual measurements, C-QC and whole blood samples were stored at +4 °C.

Over 8 days, a total of 146 whole blood samples were analyzed in the between-method comparison study. EDTA whole blood samples were selected after routine analysis from remaining samples obtained from patients (all Caucasian) referred to our laboratory for HbA_{1c} measurements. Samples with HbA_{1c} values covering the clinically relevant range (12–134 mmol/mol; 3.2–14.4%) were selected in the following ranges: < 37 mmol/mol (< 5.5%; n=26), 38–48 mmol/mol (5.6–6.5%; n=20), 49–58 mmol/mol (6.6–7.5%; n=30), 59–69 mmol/mol (7.6–8.5%; n=30), 70–86 mmol/mol (8.6–10.0%; n=20) and > 86 mmol/mol (> 10.0%; n=20). Measurements on selected samples were performed within 24 h on all three systems.

The Sigma metric was used for the evaluation of quality targets, as recommended by the IFCC-TF-HbA_{1c}[6]. Lyophilized samples (n=5) from the European Reference Laboratory for the Glycohemoglobin Standardization (ERL) scheme were used for the assessment of bias. ERLsamples, used in our laboratory for the external quality assessment, were reconstituted and analyzed on the Tosoh HLC-723GX analyzer and the bias calculated according to the assigned IFCC- and NGSP-reference method based target values. We used ERL-samples with the assigned values nearest to the clinical decision limit (6.5%/48 mmol/mol) recommended for quality assessment [6]. The mean bias from the selected samples was used for further calculations.

Statistical analyses were carried out with MedCalc for Windows, version 12.7.8 (MedCalc Software, Ostend, Belgium).

The study was approved by the hospital's Ethics Committee. Since the remaining routine samples were selected after collection for routine analysis, based on HbA_{1c} results, and used for the study after anonymization, written consent from the patients was not sought.

Table 1
Repeatability and total imprecision of the HbA_{1c} results on Tosoh HLC-723GX in commercial control material samples (QC-L, QC-H) and tripotassium EDTA-whole blood samples (WB-1, WB-2) obtained from subjects with diabetes.

	QC-L	QC-H	PWB-1	PWB-2
	HbA_{1c} (mmol/mol)			
$HbA_{1c\text{-mean}}$	30.8	84.1	47.2	93.9
CV-repeatability (%)	1.19	0.53	0.95	0.48
CV-total imprecision (%)	1.91	0.63	0.39	0.77
	HbA_{1c} (%)			
$HbA_{1c\text{-mean}}$	4.97	9.83	6.50	10.80
CV-repeatability (%)	0.73	0.37	0.56	0.24
CV-total imprecision (%)	1.25	0.51	0.20	0.46

3. Results

In the imprecision study, the calculated total imprecision of HbA_{1c} in the commercial control materials for Tosoh HLC-723GX assay was 1.91%/1.25% (HbA_{1c}=31 mmol/mol/5.0%) and 0.51%/0.63% (HbA_{1c}=84 mmol/mol/9.8%), while for the whole blood samples total imprecision was 0.39%/0.2%/(HbA_{1c}=47 mmol/mol/6.5%) and 0.77%/0.46% (HbA_{1c}=94 mmol/mol/10.8%), respectively (Table 1).

Bland-Altman analysis of the inter-method comparison study between Tosoh HLC-723GX and CE results did not reveal any difference in HbA_{1c} results between the two methods: mean difference 0.0% (95%CI: −0.02927 to 0.02653%; P=0.978). When compared to IA, Bland-Altman analysis detected an average Tosoh HLC-723GX HbA_{1c} difference of 0.07% (95%CI: 0.1039 to 0.02765; $P < 0.001$) (Fig. 1), with a more pronounced bias at very high HbA_{1c} levels (0.31%, 95% CI: 0.2128–0.4172; HbA_{1c} > 86 mmol/mol/ > 10.0%).

At the selected HbA_{1c} clinical decision level (48 mmol/mol/6.5%), and an average bias of 0.40 mmol/mol/0.04% and imprecision of 0.46 mmol/mol/0.04% six sigma analysis gave σ value of 3.91, within the desirable classification of performance.

4. Discussion

Our results show that HbA_{1c} results obtained by the Tosoh HLC-723GX, a fully automated bench top HPLC analyzer, are highly reproducible and comparable to the CE procedure. A small, but significant mean difference in comparison to IA became more prominent at very high HbA_{1c} levels (> 86 mmol/mol/ > 10.0%), but this difference (0.31%) is unlikely to influence clinical outcomes, as intensive treatment for establishing glucose control is immediately warranted in such severe chronic hyperglycaemia [1]. Previous reports on the Tosoh HPLC HbA_{1c} instruments indicated excellent precision [11] with a relatively high bias [6,12], which resulted into unfavorable outcome in sigma-scores. The results of our study clearly demonstrate that Tosoh HLC-723GX, with a very low bias at the recommended HbA_{1c} clinical decision level (48 mmol/mol/6.5%) and excellent precision, meets the recently recommended sigma-based quality criteria for reliable clinical use of HbA_{1c} in routine laboratories. Thus, compliance to the quality targets, as evidenced in this study, together with a user-friendly interface, compact size and total automation, support the Tosoh HLC-723GX analyzer as a reliable analytical choice for HbA_{1c} measurement in low-volume laboratories.

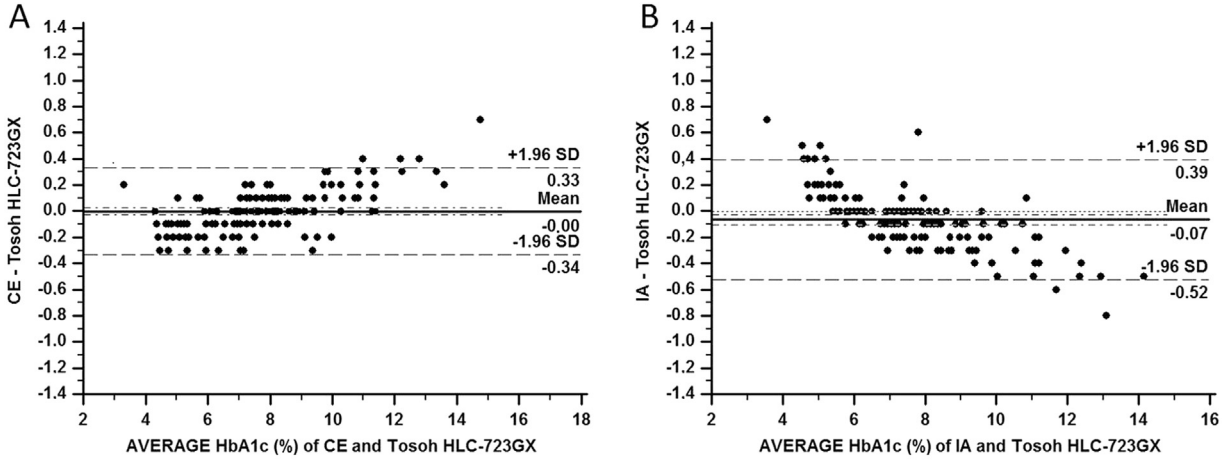

Fig. 1. Bland-Altman plots of between-method comparison of the Tosoh HLC-723GX HbA_{1c} with capillary electrophoresis (CE; A) and Immunoassay (IA; B).

Conflict of interest statement

Author's conflict of interest disclosure

The authors state that there are no conflicts of interest associated to the publication of the manuscript. The study design, collection, analysis and interpretation of data, as well as the preparation of this manuscript and the decision to submit the article for publication and publication choice were not influenced by the research funding source.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.plabm.2016.12.001.

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