RESEARCH ARTICLE

Hemoglobin S monitoring on TOSOH G8 in hemoglobin A1c mode in case of urgent red blood cell exchange

Sophie Van Aelst¹ | Helena Claerhout¹ | Elke Nackers¹ | Koen Desmet^{1,2} | Davy Kieffer^{1,3}

¹Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium

²Department of Cardiovascular Sciences, KU Leuven, Leuven, Belgium

³Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium

Correspondence

Sophie Van Aelst, Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium.

Email: sophie.van.aelst@hotmail.com

Background: Pre- and post-transfusion hemoglobin S (HbS) levels are used to document the efficacy of red blood cell exchange (RCE) in patients with sickle cell disease (SCD). In case of urgent RCE a 24/7 short turn-around time (STAT) analysis, with the ability to identify and quantify HbS, is warranted. The use of TOSOH G8 (Tosoh Europe) is evaluated for this purpose, using the variant HbA1c mode.

Methods: Analytical performance of the HbS analysis on TOSOH G8 in variant HbA1c mode was evaluated, including assessment of imprecision and linearity for HbS. In addition, a comparison study between TOSOH G8 and Minicap Flex Piercing (FP) system CZE (Sebia) using 32 HbS samples (HbS range: 9%-93%) was carried out to evaluate analytical and clinical concordance.

Results: Total HbS imprecision was 1.77% and 0.31% for a sickle cell trait and a sickle cell anemia sample, respectively. An acceptable linearity (HbS range: 6%-88%) was observed ($R^2 > .99$). Passing-Bablok regression analysis showed a significant proportional bias; however, a good analytical concordance ($r > .95$) was found. Our results suggested that TOSOH G8 underestimated HbS results compared with those of Minicap FP system (mean difference: -3.54%), especially in samples with a high HbS concentration.

Conclusion: Hemoglobin S results obtained with TOSOH G8 in variant HbA1c mode are clinically acceptable to monitor urgent RCE. The observed underestimation will not alter clinical decision-making.

KEYWORDS

hemoglobin S, red blood cell exchange, sickle cell disease, short turn-around time analysis, Tosoh Automated Glycohemoglobin Analyzer HLC-723G8

1 | INTRODUCTION

Sickle cell disease (SCD) describes a group of inherited disorders characterized by sickled red blood cells, caused by homozygous carrier state of the sickle hemoglobin mutation or by compound heterozygosity of the sickle hemoglobin mutation and a second β -globin gene mutation. Patients with SCD can suffer from acute and sometimes life-threatening complications, for example, acute stroke, severe acute chest syndrome, and acute multisystem organ failure. Red

blood cell exchange (RCE) is an important therapeutic option in the management of these acute complications. RCE allows a correction of anemia and rapid decrease in the hemoglobin S (HbS) concentration. Pre- and post-transfusion HbS levels are used to document the efficacy of RCE. The goal of RCE is to achieve a post-transfusion HbS level of 30% or less.¹⁻⁴ In the clinical laboratory of the University Hospitals Leuven, samples are batched and routine hemoglobinopathy screening is performed using the Minicap Flex Piercing (FP) system capillary zone electrophoresis CZE-HEMOGLOBIN(E) (Sebia, Lisses, France) in

combination with the cation-exchange high-performance liquid chromatography (CE-HPLC) Bio-Rad Variant II™ instrument β -thalassaemia Short Program (Bio-Rad Laboratories, Munich, Germany). Specifically in case of suspicion of HbS, a sickle solubility test instead of CE-HPLC is used to confirm its presence. This approach is important for initial diagnosis, requires experience, and is quite laborious. Although sickle solubility tests have a good specificity, their sensitivity is quite low and yield qualitative results. Routine hemoglobinopathy screening is performed twice a week in batch by a select group of trained laboratory technicians. Therefore, in case of urgent RCE a 24/7 short turnaround time (STAT) analysis, with the ability to identify and quantify pre- and post-transfusion HbS, is mandatory. RCE is monitored by a decrease in HbS (HbS level, <30%), in combination with the Hb level (close to but not >10 g dL⁻¹).^{4,5} We evaluated the analytical performance of Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 (TOSOH G8) (Tosoh Europe, Amsterdam, the Netherlands) for this purpose, using the variant HbA1c mode. Results were compared with our routine analyzer Minicap FP system to evaluate analytical and clinical concordance.

2 | MATERIALS AND METHODS

2.1 | Patient sample selection and storage

The study was performed with full respect for individuals' rights to confidentiality and in accordance with procedures supervised by local authorities responsible for ethical research. The study was conducted with sample leftovers from routine hemoglobinopathy screening. All samples were aliquoted and stored at -80°C until analyses. The used anticoagulant was ethylenediaminetetraacetic acid (EDTA).

2.2 | High-performance liquid chromatography

High-performance liquid chromatography (HPLC) was performed using TOSOH G8 in variant HbA1c mode according to the manufacturer's instructions. TOSOH G8 in variant HbA1c mode measures HbA1c as a percentage of total amounts of Hb. This program is normally used for HbA1c analysis and additional variant detection. Variant HbA1c mode is not the dedicated mode for hemoglobinopathy screening.

The instrument is equipped to lyse whole blood samples collected in EDTA tubes and to separate the different Hb fractions. The separation is obtained by CE-HPLC using 3 positively charged buffers. Hb is separated into 6 different fractions: A1A, A1B, F, LA1C+, SA1C, and A0. In addition, the major Hb variants hemoglobin D (HbD), HbS, and hemoglobin C (HbC) can be identified. HbS is reported as H-V1. Detection of the separated Hb fractions and Hb variants is accomplished by measuring the absorbance at a wavelength of 415 nm. Time of analysis is 1.6 minutes per sample (no centrifugation).

2.3 | Capillary zone electrophoresis

Capillary zone electrophoresis (CZE) was performed using Minicap FP system-HEMOGLOBIN(E) according to the manufacturer's

instructions. The instrument is equipped to suspend and lyse whole blood samples collected in EDTA tubes and to separate Hb variants. The lysed RBCs are electrophoresed in an alkaline buffer (pH 9.4) allowing separation to be directed by pH and endosmosis. Detection of eluting Hb species is accomplished by measuring the absorbance at a wavelength of 415 nm. Time of analysis is 6.5 minutes per sample (after centrifugation and in idle status).

Table 1 compares TOSOH G8 in variant HbA1c mode, Minicap FP system, and sickle solubility test.

2.4 | Analytical performance of TOSOH G8 in variant HbA1c mode

A whole blood sample from a patient with sickle cell trait (heterozygous carrier state of HbS [HbAS]) and a whole blood sample from a patient with sickle cell anemia (homozygous carrier state of HbS [HbSS]) were selected, aliquoted, and stored at -80°C until analysis to prevent degradation. Within-run, between-run and total imprecision were assessed using the daughter aliquots of both selected samples and analyzing them twice a day in duplicate for 10 different days. Prior to each run, an aliquot was thawed and vortexed.

Two linearity studies were carried out by mixing whole blood samples without a detectable HbS concentration and whole blood samples from patients with sickle cell anemia. First, a dilution set of 11 samples with an HbS concentration range from 0% to 88% was analyzed in duplicate on TOSOH G8 in variant HbA1c mode. In addition, to confirm the linearity in the low HbS range, a second linearity study was performed (0% to 19%) also using duplicate measurements of a dilution set of 11 samples. Expected HbS percentages of the dilution sets were calculated, taking into account the Hb concentration in the undiluted samples.

2.5 | Method comparison

After thawing and vortexing, a selection of samples from patients with sickle cell trait ($n = 5$) and patients with sickle cell anemia ($n = 27$) were analyzed both on TOSOH G8 and Minicap FP system on the same day. HbS concentration ranged from 9% to 93% and hemoglobin F (HbF) concentration ranged from 0% to 50% on Minicap FP system.

2.6 | Statistics

To investigate linearity, the measured and expected fractions were compared using linear regression. An acceptable linearity is defined by R -squared (R^2) > .99. Passing-Bablok regression analysis was used to study interchangeability between results. A good analytical concordance is defined by correlation coefficient (r) > .95. Data analysis was performed using Microsoft Excel Analyse-it version 4.90 (Analyse-it software Ltd, Leeds, UK) and the differences were considered as statistically different if the P -value was <.05.

TABLE 1 Comparison between TOSOH G8 in variant HbA1c mode, Minicap FP system, and sickle solubility test

	TOSOH G8 in variant HbA1c mode	Minicap FP system	Sickle solubility test
Measurement principle	CE-HPLC	CZE	Manual solubility assay
Time of analysis	1.6 min	6.5 min (in idle status)	Ca. 25 min ^a
Sample preparation	Whole blood	Packed cells	Packed cells
Advantage	24/7 availability ^b Quantitative detection of HbA1c and HbS (cf. current study), qualitative detection of other common Hb variants Fully automated software peak identification	Qualitative and quantitative detection of Hb variants Semi-automated software peak identification	24/7 availability ^b Easy to perform
Disadvantage	False-negative results possible when HbS <6% (cf. linearity study)	Less suitable for 24/7 availability Batched samples Requires more experience	False-negative results possible when HbS is <20% More prone to operator errors
Linearity	6%-88% HbS (cf. linearity study)	0.0%-87.0% HbS ^c	Qualitative results

CE-HPLC, cation-exchange high-performance liquid chromatography; CZE, capillary zone electrophoresis; FP, Flex Piercing; HbS, hemoglobin S.

^aIncluding the preparation time of the buffer solution.

^bA trained lab technician is available 24/7 to perform the analysis.

^cClaimed by manufacturer.

3 | RESULTS

3.1 | Analytical performance of TOSOH G8 in variant HbA1c mode

Following Clinical and Laboratory Standards Institute guidelines EP5-a, the within-run, between-run and total coefficients of variation (CVs) of HbS analysis were estimated for 1 sickle cell trait sample and 1 sickle cell anemia sample. Within-run, between-run and total CV were <2% as proposed by Stephens et al⁶ (Table 2).

During the linearity study in the low HbS range, TOSOH G8 could not detect HbS in the 2 samples with the highest dilution factor and expected HbS concentrations of 3.84% and 1.92%. Those samples were excluded and an acceptable linearity was observed (HbS range, 6%-19%, $R^2 = .997$ [Figure 1A]). In addition, an acceptable linearity between 8% and 88% HbS was observed ($R^2 = .998$) in the second linearity study (Figure 1B).

TABLE 2 Analytical imprecision of HbS analysis on TOSOH G8 in variant HbA1c mode

	HbAS sample	HbSS sample
Mean HbS (%)	30.31	73.10
HbS range (%)	28.45-30.93	72.58-73.45
Within-run CV (%)	0.51	0.13
Between-run CV (%)	1.69	0.01
Total CV (%)	1.77	0.31

CV, coefficients of variation; HbAS, heterozygous carrier state of HbS; HbS, hemoglobin S; HbSS, homozygous carrier state of HbS.

3.2 | Method comparison

Passing-Bablok regression analysis comparing HbS results obtained with TOSOH G8 and Minicap FP system showed a correlation coefficient of .998 and a slope and intercept of 0.94 (95% CI: 0.92-0.98) and 0.057 (95% CI: -2.2 to 1.3), respectively (Figure 2A). Despite an acceptable correlation coefficient ($r > .95$), a significant proportional bias was found (slope $\neq 1$). In addition, Passing-Bablok regression analysis was also carried out for HbF results showing an acceptable correlation coefficient (.982) and a slope and intercept of 0.79 (95% CI: 0.77-0.83) and 0.59 (95% CI: -0.081 to 1.05 [Figure 2B]), respectively.

Differences in HbS results between TOSOH G8 and Minicap FP ranged from -8.76% to +0.36% (mean difference: -3.54% [95% CI: -4.29% to -2.78%] Table 3; Figure 3A). More specifically, for samples with an HbS concentration of <25%, HbS results on TOSOH G8 differed between -0.34% and +0.36% (mean difference: +0.08%) compared with those of Minicap FP system. For samples with an HbS concentration >25%, differences in HbS results ranged from -8.76% to -0.43% (mean difference: -4.20%). Differences in HbF results between TOSOH G8 and Minicap FP ranged from -10.26% to +3.62% (mean difference: -2.01% [95% CI: -3.04% to -0.98%] Table 3; Figure 3B). Chromatograms obtained with each system for an HbSS sample are shown in Figure 4.

4 | DISCUSSION

The aim of this study was to investigate the suitability of TOSOH G8 in variant HbA1c mode to identify and quantify HbS as a 24/7 STAT

FIGURE 1 Linearity study hemoglobin S (HbS) measurement on TOSOH G8: A, HbS range, 6%-19%; B, HbS range, 8%-88%

FIGURE 2 Passing-Bablok regression analysis comparing hemoglobin S (HbS) results (A) and hemoglobin F (HbF) results (B) obtained with TOSOH G8 in variant HbA1c mode and Minicap Flex Piercing (FP) system

analysis to monitor pre- and post-transfusion HbS levels in case of urgent RCE.

TOSOH G8 can be used in 3 modes: standard HbA1c mode for rapid HbA1c analysis (1.0 minute), variant HbA1c mode for HbA1c analysis, and additional variant detection (1.6 minutes) and β -thalassemia mode for hemoglobinopathy screening and Hb variant detection (6.0 minutes). In the clinical laboratory of the University Hospitals Leuven, TOSOH G8 is used in variant HbA1c mode to quantify HbA1c. The manufacturer claims no interference from HbC, HbD, or HbS with HbA1c measurements; however, the study of Rohlfing et al⁷ showed interference of HbC trait, HbD Punjab trait, hemoglobin E trait, and HbS trait (software version 5.21). The interference of common Hb variants were corrected by a software update to version 5.24.⁸ During our study, we used an earlier software version 5.23 on TOSOH G8. Previous studies demonstrated reliable HbS, HbC, and HbD identification using TOSOH G8 in variant HbA1c mode.^{9,10} However, these studies focused only on effective detection of Hb variants and not on Hb variant quantification. Analytical performance of variant Hb quantification on TOSOH G8 in the variant HbA1c mode was not assessed. Quantitative data from our study were generated using leftover samples from patients with sickle cell trait and sickle cell anemia.

Our study showed a good analytical performance for HbS quantification using TOSOH G8 in variant HbA1c mode, as evidenced by

the low within-run, between-run and total CV and the acceptable linearity (HbS range, 6%-88%).

In the clinical laboratory of the University Hospitals Leuven, a sickle solubility test is used to confirm HbS when an Hb variant with the electrophoretic characteristics of HbS is detected on CZE. The sickle solubility test can be performed using the necessary in-house reagents or a commercial kit. Sickle solubility test can generate false-positive results (eg, increased plasma proteins or large amounts of Heinz bodies) and false-negative results when the proportion of HbS is <20% (eg, early neonatal period). Therefore, we were interested in the linearity of the HbS analysis on TOSOH G8 in variant HbA1c mode in the low HbS range (HbS range: 0%-19%). During the linearity study, it was noticed that TOSOH G8 in variant HbA1c mode could not detect HbS in 2 samples with an expected HbS concentration of 3.84% and 1.92%. However, the expected HbS concentration of 5.66% was correctly detected and quantified on TOSOH G8. In addition, this sample was analyzed 10 times on TOSOH G8 (mean value: 5.87% HbS) and an acceptable CV (1.64%) was obtained. Based on these findings, the detection limit of HbS analysis on TOSOH G8 in variant HbA1c mode can be assumed to be approximately 6%. Therefore, HbS analysis on TOSOH G8 in variant HbA1c mode can replace the sickle solubility test during routine hemoglobinopathy screening. The sickle solubility test can still be used to confirm the positive results on TOSOH G8 in variant HbA1c mode.

TABLE 3 HbF and HbS results from the comparison study between TOSOH G8 in variant HbA1c mode and Minicap FP system

Carrier state of HbS	HbF (%) Minicap FP system	HbF (%) TOSOH G8	Δ HbF(%) TOSOH G8—Minicap FP system	HbS (%) Minicap FP system	HbS (%) TOSOH G8	Δ HbS (%) TOSOH G8—Minicap FP system
HbSS	0.6	1.47	0.87	9.0	9.36	0.36
HbSS	0.3	1.37	1.07	15.1	15.27	0.17
HbSS	0	1.11	1.11	15.8	15.91	0.11
HbAS	6.4	8.43	2.03	21.3	21.41	0.11
HbAS	0	1.09	1.09	24.5	24.16	-0.34
HbAS	0.9	0.71	-0.19	38.8	37.04	-1.76
HbAS	0.9	1.32	0.42	37.7	37.27	-0.43
HbAS	16.7	12.56	-4.14	40.7	37.43	-3.27
HbSS	6.8	8.64	1.84	46.1	42.15	-3.95
HbSS	49.5	39.24	-10.26	49.7	46.82	-2.88
HbSS	22.8	19.6	-3.2	63.1	58.7	-4.4
HbSS	29.6	24.13	-5.47	68.4	63.22	-5.18
HbSS	30.2	24.24	-5.96	67.7	63.54	-4.16
HbSS	29.1	32.72	3.62	68.9	66.02	-2.88
HbSS	26.1	21.25	-4.85	71.2	67.29	-3.91
HbSS	24.8	20.13	-4.67	72.1	67.49	-4.61
HbSS	24.1	19.06	-5.04	73.4	67.56	-5.84
HbSS	23.7	19.83	-3.87	74.0	69.59	-4.41
HbSS	22.8	18.51	-4.29	75.0	70.76	-4.24
HbSS	19.5	17.11	-2.39	77.9	73.81	-4.09
HbSS	16.1	13.46	-2.64	81.7	76.6	-5.1
HbSS	14.7	12.2	-2.5	82.7	77.88	-4.82
HbSS	13.9	11.38	-2.52	82.7	78.03	-4.67
HbSS	8.9	8.1	-0.8	87.2	78.44	-8.76
HbSS	12.7	9.38	-3.32	84.5	79.92	-4.58
HbSS	9.6	7.97	-1.63	86.2	80.96	-5.24
HbSS	8.8	6.48	-2.32	86.4	81.04	-5.36
HbSS	5.9	4.27	-1.63	90.0	85.95	-4.05
HbSS	5.3	4.79	-0.51	91.1	86.13	-4.97
HbSS	6.9	5.19	-1.71	89.7	86.53	-3.17
HbSS	5.8	4.33	-1.47	90.5	87.76	-2.74
HbSS	3.3	2.29	-1.01	92.7	88.64	-4.06

FP, Flex Piercing; HbAS, heterozygous carrier state of HbS; HbF, hemoglobin F; HbS, hemoglobin S; HbSS, homozygous carrier state of HbS.

Good correlation with Minicap FP system was found for both HbS and HbF analyses, although the results were not statistically interchangeable. Our results suggested that TOSOH G8 in variant HbA1c mode underestimates the HbS fraction compared to Minicap FP system, especially in samples with a HbS concentration >25%. These observed differences may simply be explained by the different separation technique of HPLC compared with that of CZE. However, observed differences (partly) caused by our pre-analytical sample conditions (storage at -80°C and 1 freeze-thaw cycle) cannot be excluded, which could introduce degradation. One hypothesis may be that these degradation products overlap with the HbS fraction using CZE causing a higher HbS

concentration and do not overlap with the HbS fraction using the HPLC separation technique.

However as mentioned before, pre- and post-transfusion HbS levels are primarily used to document the efficacy of RCE by detecting a decrease in HbS concentration. The goal of RCE is to achieve a post-transfusion HbS level of 30% or less. Despite the observed differences (mean difference: -3.54%), it is concluded that HbS results on TOSOH G8 in variant HbA1c mode and Minicap FP system are clinical concordant and TOSOH G8 in variant HbA1c mode is sufficiently suitable to monitor HbS levels following urgent RCE. Importantly, HbS on TOSOH G8 can only be requested in case of urgent RCE. Our routine hemoglobinopathy screening will still be

FIGURE 3 Bland-Altman plot comparing hemoglobin S (HbS) results (A) and hemoglobin F (HbF) results (B) obtained with TOSOH G8 in variant HbA1c mode and Minicap Flex Piercing (FP) system

FIGURE 4 Chromatogram with TOSOH G8 in variant HbA1c mode and Minicap Flex Piercing (FP) system. TOSOH G8 in variant HbA1c mode: hemoglobin S (HbS) is reported as H-V1. Minicap FP system: Hemoglobin F (HbF), HbS and hemoglobin A2 (HbA2) peak identification is done by an experienced laboratory technician

performed by using CZE Minicap FP in combination with CE-HPLC Variant II™.

In conclusion, HbS quantification on TOSOH G8 in variant HbA1c mode is suitable to monitor urgent RCE. The observed underestimation will not alter clinical decision-making. In addition, our study shows the possibility to replace the sickle solubility test as a

screening tool to detect HbS using HbS measurements on TOSOH G8 in variant HbA1c mode.

ORCID

Sophie Van Aelst <http://orcid.org/0000-0003-0967-5461>

REFERENCES

1. Estcourt LJ, Fortin PM, Hopewell S, Trivella M. Red blood cell transfusion to treat or prevent complications in sickle cell disease: an overview of Cochrane reviews. *Cochrane Database Syst Rev*. 2016;(2):pii: CD012082.
2. Lovett PB, Sule HP, Lopez BL. Sickle cell disease in the emergency department. *Emerg Med Clin North Am*. 2014;32:629-647.
3. Glassberg J. Evidence-based management of sickle cell disease in the emergency department. *Emerg Med Pract*. 2011;13:1-20.
4. Kim HC. Red cell exchange: special focus on sickle cell disease. *Hematology Am Soc Hematol Educ Program*. 2014;2014:450-456.
5. Swerdlow PS. Red cell exchange in sickle cell disease. *Hematology Am Soc Hematol Educ Program*. 2006;463:48-53.
6. Stephens AD, Colah R, Fucharoen S, et al. ICSH recommendations for assessing automated high-performance liquid chromatography and capillary electrophoresis equipment for the quantitation of HbA2. *Int J Lab Hematol*. 2015;37:577-582.
7. Rohlfing C, Hanson S, Weykamp C, et al. Effect of hemoglobin C, D, E and S traits on measurements of hemoglobin A1c by twelve methods. *Clin Chim Acta*. 2016;455:80-83.
8. Lenters-Westra E. Common Hb-variants show no longer interference on the Tosoh G8 after an update of the software. *Clin Chim Acta*. 2016;455:73-74.
9. Froom P, Henig C, Zalman L, Barak M. Incidental findings of variant hemoglobin during hemoglobin A(1c) testing. *Am J Clin Pathol*. 2012;138:425-428.
10. Badiou S, Guillot J, Kuster N, et al. Comparison of Arkray/ELITech ADAMS HA-8180V with Bio-Rad Variant, II Turbo2.0 and Tosoh Bioscience HLC-723G8 for HbA1c determination. *J Clin Lab Anal*. 2014;28:428-434.

How to cite this article: Van Aelst S, Claerhout H, Nackers E, Desmet K, Kieffer D. Hemoglobin S monitoring on TOSOH G8 in hemoglobin A1c mode in case of urgent red blood cell exchange. *J Clin Lab Anal*. 2018;32:e22453. <https://doi.org/10.1002/jcla.22453>