The never ending story of Hb-variants interferences on the measurement of HbA1c

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Rohlfing *et al* (2021) published a paper on the interference of hemoglobin C, D, E and S traits on measurements of hemoglobin A1c by fifteen different methods [1]. Clinically significant effects were observed for the Tosoh G11 variant mode for HbAD, Roche b101 for HbAC and HbAE, and Siemens DCA Vantage for HbAE and HbAS. As European Reference Laboratory for Glycated Hemoglobin (ERL) we have evaluated many different HbA1c methods, including the three mentioned methods and we did not find clinically significant interference other than with HbAE on the Roche b101 [2, 3]. However, some previous studies have shown that interferences from Hb-variants may occur when the software changes on the instrument are implemented [4]. In this study we investigate if the findings in the paper of Rohlfing *et al* could be confirmed.

Forty frozen whole blood samples from individuals homozygous for HbA and 20 frozen samples heterozygous for HbC, HbD, HbE and HbS with HbA1c values covering the clinical relevant range, were analyzed in singleton on the Premier Hb9210, the Tosoh G11 and the Siemens DCA. The Roche b101 was not tested in this study. Hemoglobin variants were identified in as previously described [2].

To be able to compare the results presented in the previous paper with the current results, we used both our own criteria and those used in the study by Rohlfing *et al*:

1) A mean relative difference exceeding ±10% in SI units compared to the assigned value but corrected for bias in HbAA samples, was defined as clinical significant [2].

2) Deming regression was used to determine if the bias for each variant vs HbAA was clinically significant at 42 mmol/mol (6%) or 75 mmol/mol (9%) HbA1c. Clinical significance was defined as difference exceeding ± 6%, so 0.36% at 6% NGSP units and 0.54% at 9% NGSP units [1].

Fig. 1A shows the results from the DCA Vantage and Fig. 1B shows the results from the Tosoh G11. The mean relative difference for the DCA Vantage for HbAS, HbAC, HbAD and HbAE was 7.5, 4.3, 2.5 and 3.7% and for the Tosoh G11 4.8, 3.2, 7.1 and 0.8% in SI units. Neither method exceeded the bias limit of 0.35% at 6% NGSP units or 0.54% at 9% in NGSP units for all investigated Hb-variants. When both the ERL criteria and the criteria used by Rohlfing *et al,* are applied the interference of HbAD for the Tosoh G11 and HbAE and HbAS for the DCA Vantage could not be confirmed. It should be noted however, that the positive bias for HbAS on the DCA Vantage and for HbAD on the Tosoh G11 were the largest seen in our study (mean bias >7% in SI units) which is similar to the findings of Rolhfing *et al.* but not clinically significant. Also 3 individual data points of the HbAS samples on the DCA Vantage and 2 HbAD on the Tosoh G11 fell outside of the 10% cutoff for bias, however this did not affect the overall correlation of results. Differences in findings between this study and that of Rolhfing *et al* can be partly explained by differences in distributions of HbA1c values for each variant but also lot-to-lot variations in cartridges/reagents/columns and updated software may have contributed.

These studies show that it is important to continue to critically assess the interferences of Hb-variants on different HbA1c methods as there is potential for significant impact on the patient results when these methods are being used for the diagnosis of diabetes and these differences are dynamic, changing with improvements/changes in methods over time.

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References

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Legends

Figure 1A-B
Interference from common Hb-variants on DCA Vantage (Figure 1A) and Tosoh G11 (Figure 1B) in SI units and bias calculated at 6 and 9% in NGSP units corrected for bias in HbAA samples.