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## Hb Tacoma by seven HbA1c methods – one with significant interference

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### ABSTRACT

Hemoglobin Tacoma is known to potentially interfere HbA1c assays. The variant is common in Finland with prevalence of up to 2% regionally and cases are also reported in areas that have attracted Finnish immigrants, especially in Sweden and North America. Here, we investigated the effect of Hb Tacoma on seven HbA1c methods. 20 non-variant and 20 Hb Tacoma samples were measured with Tina-quant Gen. 3 (immunoassay, considered as reference) and the following point of care instruments: Afinion 2, HbA1c 501 (both utilizing boronate affinity), QuikRead go, cobas b 101, DCA Atellica, and Standard F (all immunoassays). Repeatability was also assessed by measuring both non-variant and Hb Tacoma samples five times each at two different levels. For non-variant samples, the mean relative bias with all methods was  $< \pm 4\%$ , whereas for Hb Tacoma samples Standard F had 38% mean relative bias. In absolute bias, the difference was 17 mmol/mol on average and constant through the measured range. For other methods the mean relative bias for Hb Tacoma samples was  $< \pm 6\%$ . The repeatability with all methods was similar for non-variant and Hb Tacoma samples and at highest 4.1% (mean CV% of two levels). The observed interference by Standard F is likely due to two-antibody assay design as Hb Tacoma has been shown to result in conformational change. This interference is clinically significant and highlight the need for better controlling and better understanding hemoglobin variants in HbA1c testing.

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### Introduction

Glycated hemoglobin (HbA1c) is utilized both in the monitoring and diagnosis of diabetes [1]. HbA1c is formed when glucose attaches to the N-terminus of the beta chain of hemoglobin A (the main form of adult hemoglobin (95–98%)), and the more glucose in the surrounding fluid, the more HbA1c is formed [2]. Furthermore, glycation is an ongoing process throughout the whole life span of an erythrocyte. The reference range for HbA1c is 20–42 mmol/mol in healthy individuals and values above 48 mmol/mol are indicative of diabetes [1]. The appropriate target for good glycemic control in many diabetics is 53 mmol/mol (but may be individually less or more stringent), and 75 mmol/mol is often considered as threshold for poor glycemic control [1].

Hemoglobin variants may cause erroneous HbA1c results by interfering with the measurement of the analyte, or by interference with the diagnostic principle which assumes a red blood cell life span of around 100–115 days on average and a certain glycation rate [3]. While the interference by the most common variants worldwide (namely HbS, HbC, HbD and HbE traits) has usually been carefully evaluated [4], the more infrequent variants have rarely been studied at all. In total, more than 1400 genetic hemoglobin variants

have so far been reported, most of them being single nucleotide point mutations [5].

Hemoglobin Tacoma is a common hemoglobin variant in Finland with prevalence of up to 2% in parts of the country [6]. Occasional cases have also been identified in areas that have attracted Finnish immigrants, especially in Sweden and North America [7–11]. While hemoglobin A consists of two beta and two alpha subunits, Hb Tacoma is a beta variant mutated at position 93, where guanine is replaced with thymine causing arginine to serine change at the amino acid level (NM\_000518.5(HBB):c.93G>T (p.Arg31Ser), HGVS nomenclature) [5]. Clinically, heterozygous Hb Tacoma is usually an incidental finding or may be associated with mild anemia (reviewed in [6]). Also homozygous cases have been reported yet their clinical characterizations are still forthcoming [6]. In addition, the same amino acid change may be due to guanine to cytosine mutation, namely Hb Tacoma II (NM\_000518.5(HBB):c.93G>C (p.Arg31Ser)), but this variant has a different geographic and ethnic pattern [5,12,13].

The aim of this study was to investigate the effect of Hb Tacoma on six different HbA1c point of care instruments (and on considered reference method). Previous studies have shown the potential of Hb Tacoma to interfere with HbA1c assays [6,14] but studies with more methods

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and with the representativeness of our current health care sector are needed.

## Materials and methods

The study included 20 non-variant and 20 heterozygous Hb Tacoma samples (whole blood K<sub>2</sub>EDTA, ranges (according to chosen reference method) 25–83 mmol/mol and 30–77 mmol/mol, respectively). The samples were identified by capillary electrophoresis (Capillarys 3 Tera, Sebia) based on characteristic electropherograms [6] from routine HbA1c analytics, and stored for further analysis under temperature control (2–8°C) for a maximum of 3 days. As capillary electrophoresis is interfered by Hb Tacoma, it was only used for characterization, and Tina-quant Gen. 3 was used as reference in all comparisons. The analyses with point of care devices and reference method were performed in four batches (i.e. on four separate days). All the methods were checked daily before the analyses using controls provided by the manufacturers and each sample was analysed with all the methods consecutively. A variant sample and a paired non-variant sample with an equal HbA1c and the same sampling date were also always analysed in parallel. Repeatability was assessed by measuring one non-variant and one Hb Tacoma sample five times at two different levels. The study was performed in the FINAS accredited (SFS-EN ISO 15189) Clinical Chemistry Laboratory of Seinäjoki Central Hospital (South Ostrobothnia, Finland). The genotype of Hb Tacoma samples originating from the performing laboratory has been confirmed in two previous studies [6,14].

The HbA1c methods listed below were compared. A difference of 5 mmol/mol at an HbA1c level of 50 mmol/mol was considered significant, according to the IFCC Task Force on Implementation of HbA1c Standardization, and corresponding to a mean relative difference of 10% [15].

- Tina-quant HbA1c Gen. 3 on cobas c 502 analyzer, one-site immunoassay, turbidometry inhibition (Roche Diagnostics)
- Afinion 2 HbA1c, point of care testing (POCT), boronate affinity (Abbott Diagnostics)
- HbA1c 501, POCT, boronate affinity (HemoCue)
- QuikRead go HbA1c, POCT, one-site immunoassay, turbidometry (Aidian)
- cobas b 101 HbA1c, POCT, one-site immunoassay, agglutination inhibition (Roche Diagnostics)
- DCA Atellica HbA1c, POCT, one-site immunoassay, agglutination inhibition (Siemens Healthineers)
- Standard F HbA1c on Standard F200 reader, POCT, two-site immunoassay, lateral flow (SD Biosensor), currently not used in Finland

The study utilized anonymized left-over samples of routine diagnostics, and therefore no informed consent from the patients was obtained. No data were collected about the patients. The study was approved according to the institutional protocol of the Wellbeing Services County of South Ostrobothnia, Finland.

**Table 1.** Relative differences and repeatability of study methods for non-variant and Hb Tacoma samples.

Method	Bias % (mean) non-variant samples	Bias % (mean) Hb Tacoma samples	Repeatability (mean CV%) non-variant samples	Repeatability (mean CV%) Hb Tacoma samples
Tina-quant Gen. 3	reference	reference	2.5	3.5
Afinion 2	2.1	1.6	1.3	1.5
HbA1c 501	0.3	−4.4	4.1	2.4
QuikRead go	−2.1	−5.9	3.5	3.4
cobas b 101	3.8	3.9	0.9	1.0
DCA Atellica	0.8	2.6	2.2	2.9
Standard F	−2.8	38.2	2.0	2.7

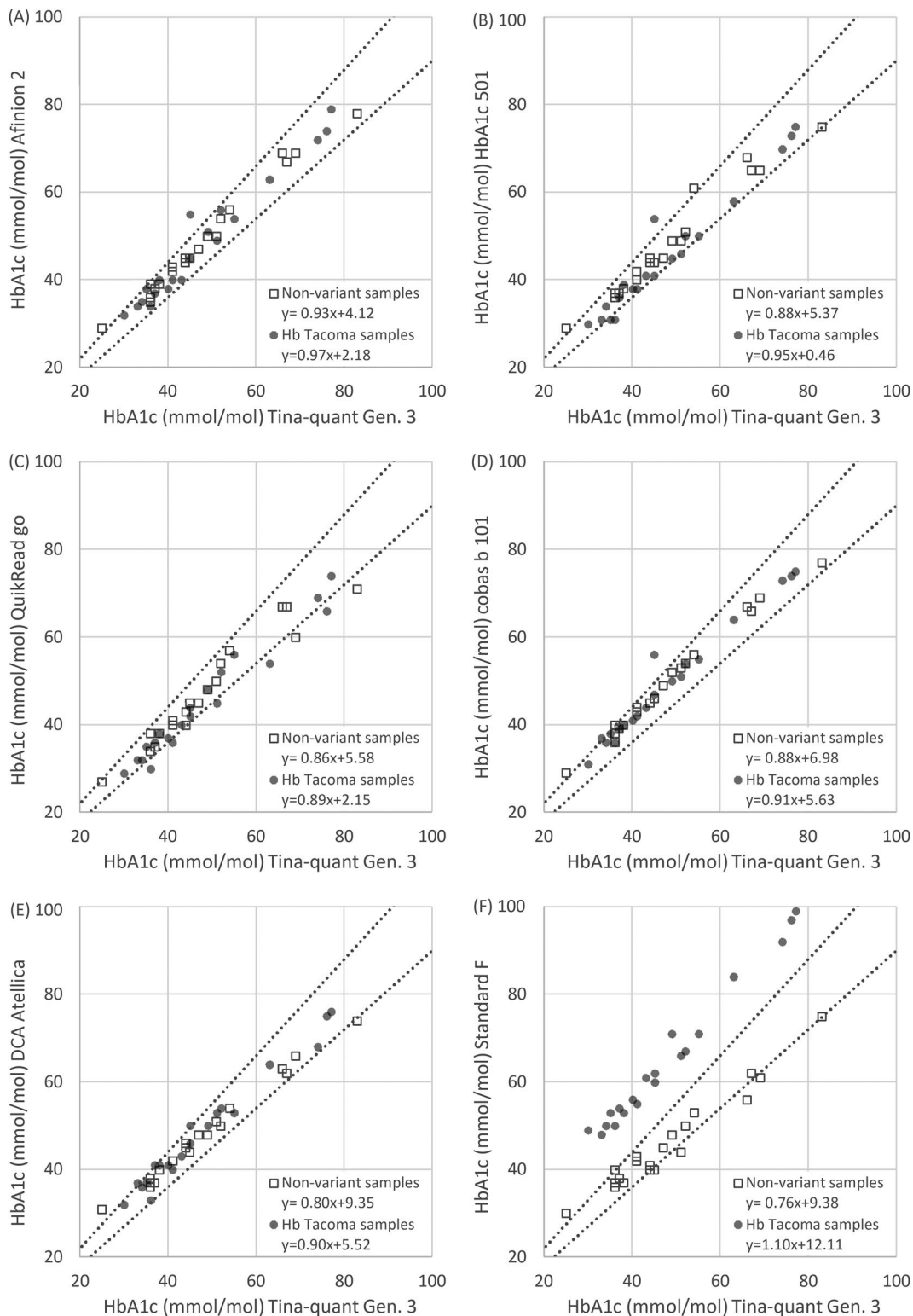
## Results

Table 1 and Figure 1(A–F) summarize the findings of this study. For non-variant samples, the results with all the studied methods were in accordance (mean relative bias ranging from −2.8% to 3.8%, criteria  $< \pm 10\%$  [15]). For Hb Tacoma samples, all the other methods gave similar results (relative bias range from −5.9% to 3.9%), except for Standard F which had a mean bias as high as 38.2% (17.2 mmol/mol on average, with a range 14–22 mmol/mol). In general, Figure 1(A–F) shows most results within the allowable difference (indicated by the dotted lines) with both sample types, although numerous results also occur at the borderline and outside the allowable limits. The repeatability with all the methods was essentially similar for non-variant and Hb Tacoma samples, the mean CV% being 4.1 at highest (Table 1).

## Discussion

The present study shows a 17 mmol/mol error caused by Hb Tacoma on SD Biosensor's immunological Standard F HbA1c method. This observation is clinically highly significant – as a comparison, the difference of 5 mmol/mol between two consecutive HbA1c samples is generally considered as a true change in the patient's glycaemic control [16]. Yet, however, the erroneous results remain clinically possible and make the interference therefore difficult to notice. All of this calls for individual clinical assessment and careful consideration of also other parameters reflecting glycaemic status. In fortunate cases, hemoglobin variant would give results that are virtually impossible and make it necessary to look at the patient in more detail [17]. Unfortunately, this is not often the case and variants are more hidden and may eventually lead to unnecessary treatments or delay diagnosis [18–21].

The reason for the discrepant results with Standard F can be sought in its sandwich-type assay design. Briefly, the sample is mixed with extraction buffer and dyed latex-microparticles conjugated to specific antibodies, and this solution migrates past the anti-HbA1c antibody coated line and total hemoglobin zone [22]. Since the anti-HbA1c antibody is against the few first amino acids of the N-terminus of the beta chain [22] and the Tacoma mutation at the 30th amino acid is far from the recognition site both in primary and secondary structure [23,24], interference by the anti-HbA1c antibody is not anticipated. However, interference due to specific (i.e. hemoglobin) antibodies may be



**Figure 1.** HbA1c results of non-variant and Hb Tacoma samples of (A) Afinion 2, (B) HbA1c 501, (C) QuikRead go, (D) cobas b 101, (E) DCA Atellica, and (F) Standard F as compared to Tina-quant Gen. 3. The dotted lines indicate  $\pm 10\%$  allowable error from the line of identity  $y = x$ .

hypothesized as Hb Tacoma is known to result in conformational change [24]. Also total hemoglobin quantitation may be interfered and influence the outcome, but unfortunately its measurement principle is not described. Another less likely scenario behind interference could be conformational changes resulting from Hb Tacoma's instability (reviewed in [6]), and (or) their exacerbation by the assay solutions.

There is only one previous study comparing Hb Tacoma on different HbA1c methods. Lenters-Westra et al. [14] studied four methods of which two (Afinion AS100 and Tina-quant Gen. 2) were precursors of the methods presented here. In both studies, the two methods were at the same level comparing to each other. Interestingly, the third method, Premier Hb9210 (Trinity Biotech), which utilizes

boronate affinity HPLC, showed approximately 20% lower results than Tina-quant Gen. 2 for Tacoma samples. It may be speculated that Hb Tacoma perhaps undergoes degradation in HPLC conditions, similarly to what can be seen in HbA1c capillary electrophoresis [6,11]. Lastly, the fourth method in the aforementioned study, Tosoh G8 (cation exchange HPLC, Tosoh Bioscience) showed a weak correlation with other methods among Hb Tacoma cases and the same has also been observed with capillary electrophoresis (personal experience). The benefit of these two latter methods however is their visibility and hence the possibility to observe and comment on patient's status, and reroute analysis on another platforms if needed.

Each study has its limitations. Regarding this study's setting, it must be emphasized that although Tina-quant Gen. 3 was chosen as reference, conclusions can so far not be made as to which method is analytically correct in case of Hb Tacoma. It is plausible, however, since the results of Hb Tacoma samples obtained with Tina-quant Gen. 3 were shared by five other methods, that they likely represent close to the analytical truth. Assessing in comparison to clinical data could further confirm the matter, but such comparisons were not made in this study. In addition, the sample storage time (maximum of three days) exceeded the instructions of some of the methods. The allowable storage time with the methods in this study ranged from two hours to two weeks, which is quite incoherent, as similar methods and/or same analyte are to be expected to have quite similar requirements. For reassurance, few samples were reanalysed several days apart and differences were not observed (data not shown). Furthermore, the results of this study cannot be explained by the prolonged storage time.

In general, mutations at the very first amino acids of the N-terminus and close to the C-terminus are known to cause analytical errors in immunological methods [3] but reports on mutations at other sites specifically pointing to immunological interference are limited. In one such report, Hb Agenogi ( $\beta 90\text{Glu} > \text{Lys}$ ) was described with differences between antibody methods, some with expected results and others with results higher than expected [25]. The secondary structure was considered to bring the mutation site close enough to the N-terminus to increase its antigenicity. Hb Providence ( $\beta 82\text{Lys} > \text{Asn}$ ) in turn has shown lower than expected immunological HbA1c [20], also plausibly due to close proximity to the N-terminus in secondary structure [23]. In a study by Bissé et al. [26], Hb Rambam ( $\beta 69\text{Gly} > \text{Asp}$ ) was reported with discordance between glycaemic state and immunological HbA1c, whereas total glycohemoglobin concentration was representative of clinical status. The mutation was hypothesized to shift the balance towards the more reactive glycation of amino acids other than the N-terminal valine, i.e. the interference not being antibody-related after all. Taken together, it is evident that every variant, method and manufacturer needs to be tested carefully for interference without any prior assumptions based on the position of the mutation or otherwise.

This study highlights the need for better controlling and better understanding hemoglobinopathies in HbA1c testing. Our results are significant in analytical means, but results

may also become diagnostically misleading if red blood cell life span or glycation kinetics are affected [3]. In addition to hemoglobin variants, thalassemias, which are defects in the quantity of hemoglobin subunits, are very common and may also co-occur with variants. In fact, around 7% of the global population carries an abnormal hemoglobin gene [27]. Today, different HbA1c methods are carefully evaluated for hematologically normal individuals, but in the future more studies are needed among hemoglobin abnormalities. In addition, considering the relatively large allowable error utilized here (10%), the results also show the need to continue developing point of care methods to improve their analytical performance. For comparison, the criteria for method performance in NGSP units (%) is 5% [28], which corresponds to around 7,5% in SI units (mmol/mol) at level 50mmol/mol. While these are the current minimum requirements, the total desirable error for HbA1c derived from data on biological variation has been calculated to be approximately 3% only [29].

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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