

Not all HbA1c Assays are Equal

“Effects of Different Rare Hb Variants on HbA1c Measurement in Eight Methods” by Randie R. Little et al. (2015) Journal of Diabetes Science and Technology

This overview summarizes the pivotal findings in the Randie R. Little et al. study (Fig. 1) that highlights the value of using an HbA1c testing method to reveal the presence of rare hemoglobin variants which can affect accuracy. This study was funded by Roche.



Figure 1. Little RR et al. (2015).

Introduction

The study examined analytical interference from 49 different less common variants comparing seven different HbA1c methods. HbA1c accuracy was assessed as well as the likelihood for the systems to report inaccurate results. The authors noted that previous studies with some assay methods have shown interference with HbA1c measurement from the four most common heterozygous Hb variants (HbAS, HbAE, HbAC, and HbAD).

Methods

Samples were screened for Hb variants using Bio-Rad ion-exchange HPLC, confirmed with alkaline and acid electrophoresis, and sequenced. Some samples were excluded due to limitations of the comparative methods

including samples with HbF >10% and samples with HbA1c results <4% or >12%. Five samples were excluded due to discordant results between two instruments used as comparative methods.

Instruments evaluated: Four ion-exchange HPLC methods (2 Bio-Rad, 2 Tosoh), one enzymatic method (Diazyme), and one capillary electrophoresis method (Sebia) following the manufacturers’ instructions, with exceptions. The immunoassay, Tinaquant Gen. 2 (Roche) method, was used as the comparative method.

The methods were evaluated for reporting inaccurate results and classified as “reportable” and/or “accurate” per the manufacturers’ instructions.

Study Results

Total samples	% Total samples with accurate results	Instrument	Method	# Variants tested	# Variants that interfered with A1c results	% Variants that interfered with A1c results
75	100%	Bio-Rad D-10 System	HPLC	39	0	0%
84	97.7%	Bio-Rad VARIANT II TURBO System	HPLC	43	2	4.6%
87	96.6%	Tosoh G7	HPLC	43	3	6.9%
88	92.1%	Roche Tinaquant	Immunoassay	44	5	11.3%
33	87.9%	Sebia Capillars 2	Capillary electrophoresis	18	2	11.1%
88	87.5%	Diazyme Direct Enzymatic	Enzymatic	44	8	18.1%
87	67.7%	Tosoh G8	HPLC	44	9	20.4%

Conclusion

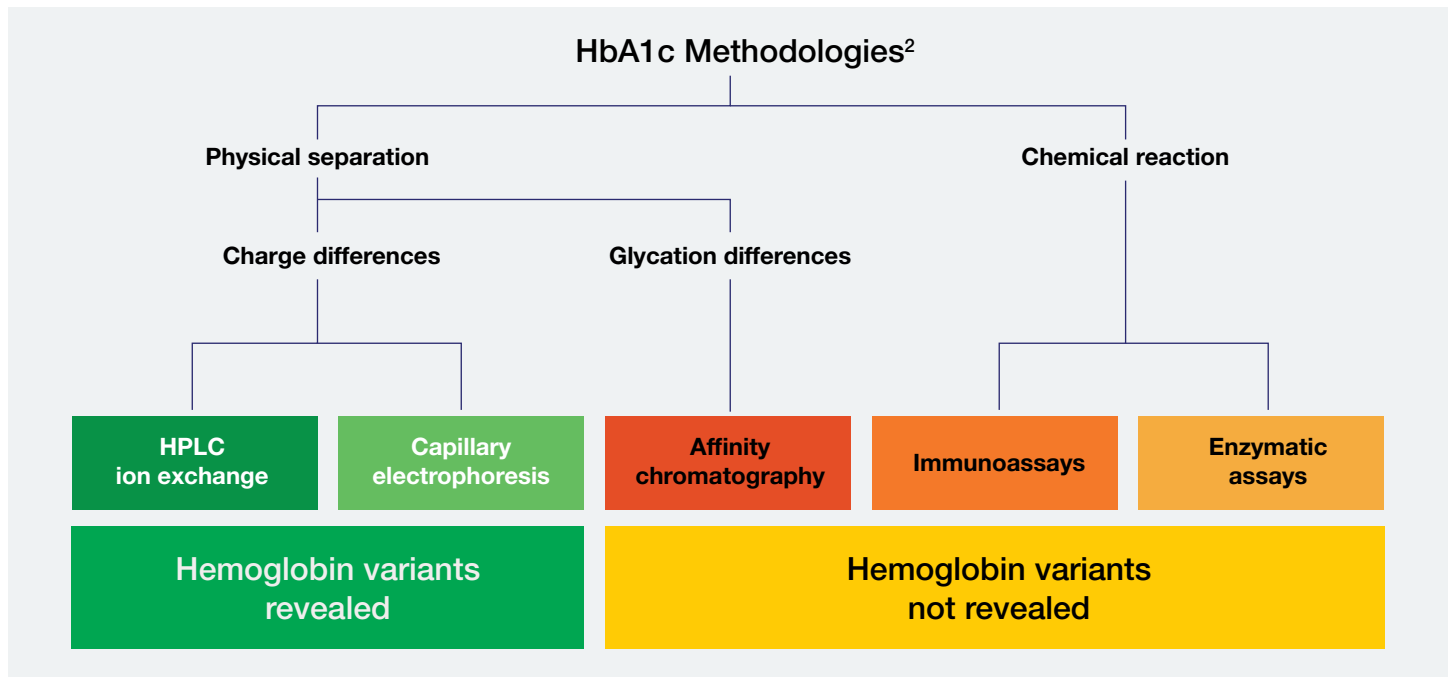
The authors concluded that in order to ensure accurate HbA1c results, it’s important to know if patients have a hemoglobin variant and if so, how that variant affects their HbA1c results. Laboratories should take care when reporting results if a variant is suspected



Not all HbA1c methods reveal hemoglobin variants

Hemoglobin variants may interfere with HbA1c determination through glycation rate, erythrocyte lifespan, or changes in net charge.

“Subjects who are heterozygous for any of these Hb variants are usually asymptomatic and have normal red cell survival. Thus, a physician may be unaware that his or her patient with diabetes has one of these variants in the heterozygous form. Results from some methods [HPLC and capillary electrophoresis] can alert the clinician that the Hb variant is present but may or may not give accurate HbA1c results. Other methods [immunoassay and enzymatic] do not show the presence of the variant and may or may not provide accurate results. The worst-case scenario, of course, is the case where the variant is not indicated and the HbA1c result is inaccurate. The effect of each variant must be examined with each specific HbA1c method.” *Little RR et al.*



“When reporting HbA1c results, it’s important for clinicians to know if a hemoglobin variant is present.” *Little RR et al.*

REFERENCES

1. Little RR et al. (2015). Effects of 49 Different Rare Hb Variants on HbA1c Measurement in Eight Methods. *Journal of Diabetes Science and Technology* 1-8. <https://pubmed.ncbi.nlm.nih.gov/25691657>.
2. Chart adapted from: Weykamp C (2013). HbA1c: A Review of Analytical and Clinical Aspects. *Anal of Laboratory Medicine*. <http://dx.doi.org/10.3343/alm.2013.33.6.393>.