## 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.

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Randie R. Little, PhD <sup>1</sup> , Sonia L. La'ulu, BS <sup>1</sup> , Steven E. Hanson, MS <sup>1</sup> , Curt L. Robling, BES <sup>1</sup> and Robert L. Schmidt, MD, PhD, MBA <sup>1</sup>		
Abstract		
Background: Previous studies have shown interference with I- Hb variants (HbAS, HbAE, HbAC, and HbAD) with some assig- different law common variant with 7 different lables method	bAlc measurement from the - methods. Here we examine a	I most common hetercoy nalytical interference fro
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Conclusions: Laboratories must be cautious about reporting	results when the presence of a	variant is suspected.
Keywords accuracy. HbA.Lc. hemoelobin variants, interference		
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Grouth hemosphetic (GHB), reported as HMAs; is a bis- chemical matter that is contrady used in the management of the strengthese methods in guitter gappenetic control and a strengthese methods are not strengthese control and galatine that have an investedly matching by addition of glu- ghtein that have an investedly matching by addition of galaxies that have an investedly matching by addition of galaxies that have an investedly matching by addition of a strength of the strength strength strength strength strength one of strength a 1 month strength strength strength strength states matching strength strength strength strength strength strength strength strength	accuracy of some HbAlc variant. <sup>1,4</sup> The most common hemogl in descending order of provak HbD. In the United States Ho <sup>1</sup> Department of Pathlog & Accura- University of Pathlogs & Accura- University of Pathlogs & Accura- University of Pathlogs, Christen II 54027 Instance for Clinical and Euro UT, USA. <sup>1</sup> Department of Pathlogs, University Ba Liab Chy, UAA	methods depending on obin (HD) variants world mee are HDS, HDE, HDE, S is the most prevalent va- ted latences and Odd Healt, and al Methon, Calanda, HD, resented Patholog, Sols Late D of Club Health Tomore Com-

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 Capillarys 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

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