Abbott

TECHNICAL BRIEF

Effect of Hemoglobin Variants on HbA1c Measurement

Hemoglobinopathies are a group of disorders passed down through families (inherited) in which there is abnormal production (thalassemia) or structure of the hemoglobin molecule. Structural changes to the hemoglobin molecules are caused by mutations and these modified molecules are called hemoglobin (Hb) variants.[1,2,3]

More than 1,000 naturally occurring hemoglobin variants have been discovered. Most of these hemoglobin variants are however rare and also clinically silent.[2] The most common Hb variants worldwide, in descending order of prevalence, are HbS, HbE, HbC and HbD. In the United States the order of prevalence is HbS, HbC, HbE and HbD.

Individuals who are heterozygous for the variant hemoglobin are carriers of the Hb variant trait (e.g. HbAS). They are usually asymptomatic and have a normal red cell survival.[4,5,6] Individuals who are homozygous for these Hb variant (e.g. HbSS), will have no, mild or severe clinical signs of disease. In the case of HbSS the individuals suffers from sickle cell anemia.[5,7]

Fetal hemoglobin (HbF) is produced in high concentrations in the fetal period. At birth the production of HbF decreases, to adult levels around 1 year of age. The adult hemoglobin is usually made up of less than 1% HbF.[8,9,10] Some genetic conditions are known to influence production of HbF during adulthood, including hereditary persistence of fetal hemoglobin (HPFH) and δβ-thalassemia.[8] HbF levels may vary considerably in these individuals, with HbF levels up to 30%. These individuals are generally asymptomatic.[4]

HEMOGLOBIN VARIANTS AND HbA1c TESTING METHODS

Currently, there are five major methods for HbA1c measurement: borate affinity, immunoassay, enzymatic, ion-exchange high-performance liquid chromatography, and capillary electrophoresis. Most patients have hemoglobin A and the choice of method for HbA1c measurement will not drastically affect interpretation of their results. However, for individuals with an Hb variant, the choice of testing method can result in falsely high or low HbA1c values, ultimately leading to over-treatment and under-treatment of diabetes.[4,18,23]

ION-EXCHANGE

Ion-exchange separates hemoglobin species based on differences in charge and elution time between HbA1c and other hemoglobins. The presence of a hemoglobin variant causes an alteration in retention. Co-elution of the hemoglobin variant with HbA1c will cause gross overestimation of HbA1c, while co-elution of the hemoglobin variant with hemoglobin A, will underestimate the HbA1c results.

When the glycated derivatives of the hemoglobin variant co-elute with HbA1c, and the non-glycated hemoglobin variant is resolved from hemoglobin A, overestimation of HbA1c will occur.[4,17,19,20]

IMMUNOASSAY

Immunoassays measure HbA1c specifically. The antibodies used recognize the N-terminal glycated amino acids (usually the first 4–10 amino acids) of the hemoglobin β-chain. Hemoglobin variants with mutations in this region will affect HbA1c measurements by immunoassay.[4,20]

CAPILLARY ELECTROPHORESIS

Capillary electrophoresis is performed in thin capillaries that separates HbA1c and other hemoglobin fractions via charge difference at high voltage using electro-osmotic flow.[19,21] This method can be useful in patients who possess variant hemoglobins because it has a longer runtime, leading to better resolution.[21] The interpretation of HbA1c performed by capillary electrophoreses should be carried out with knowledge of type of Hb variants as certain variants can co-migrate with normal Hb fractions.[18]

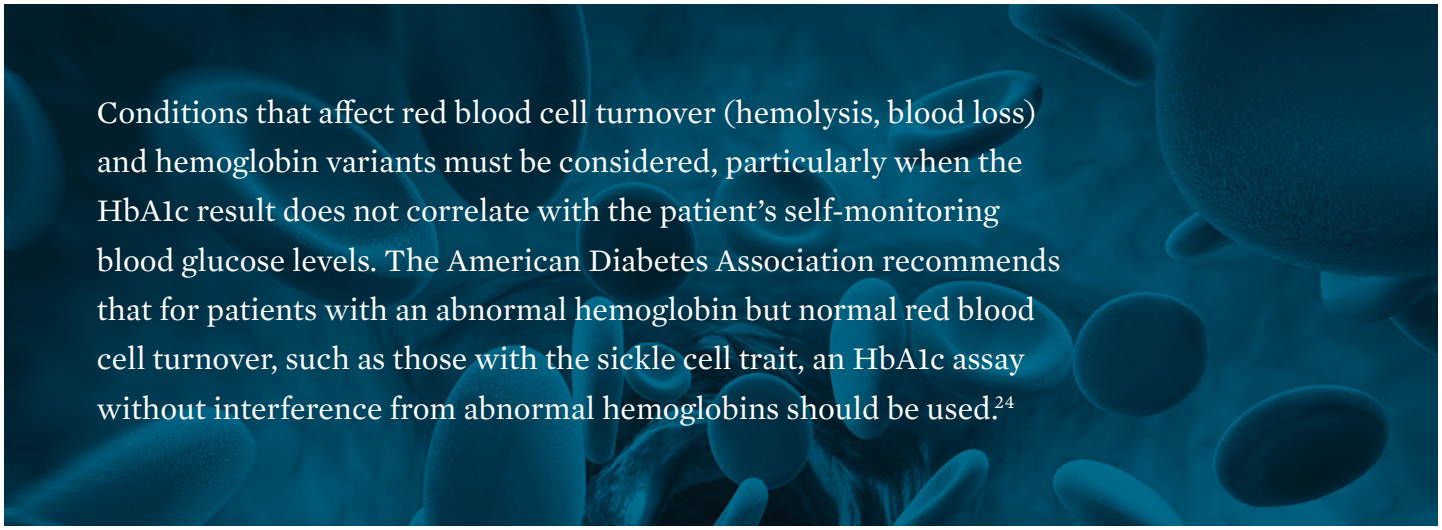
ENZYMATIC METHODS

The enzymatic method currently available measures HbA1c by using an enzyme that specifically cleaves the N-terminal valine.[4]

BORONATE AFFINITY

Borate affinity shows the least interference by Hb variants in HbA1c measurement through separation and quantitation of both glycated and non-glycated hemoglobin, regardless of structural differences.[18]

THE AFINION™ HbA1c ASSAY uses boronate affinity and has been shown to have no interference from the common hemoglobin variants HbAC, HbAS, HbAE and HbAD.[13,23]



Conditions that affect red blood cell turnover (hemolysis, blood loss) and hemoglobin variants must be considered, particularly when the HbA1c result does not correlate with the patient's self-monitoring blood glucose levels. The American Diabetes Association recommends that for patients with an abnormal hemoglobin but normal red blood cell turnover, such as those with the sickle cell trait, an HbA1c assay without interference from abnormal hemoglobins should be used.[24]

The table below references some of the more common methods and the Hb variants that may cause interference with the measurements. Some interferences for some methods are highlighted in gray indicating that they have been tested using a new stricter criterion of >7% difference at 6 and 9% HbA1c to define clinical significance. The other methods were tested using either criteria of >10% at 6 and 9% HbA1c or some other criteria.[23]

METHOD (LISTED IN ALPHABETICAL ORDER BY MANUFACTURER)	INTERFERENCE (YES/NO)				
	HbC trait	HbS trait	HbE trait	HbD trait	Elevated HbF
Abbott Architect c Enzymatic	No	No	No	No	-
Abbott Architect (Seradyn immunoassay reagents)	Yes	Yes	@	@	$
Abbott Architect (Abbott immunoassay reagents)	-	-	Yes	Yes	No <9%
Afinion	No	No	No	No	$
NycoCard	No	No	@	@	$
Arkray ADAMS A1c HA-8180V Variant Mode (Menarini) (*software version EU 1.41)	No	No	HbA1c not quantified / No*	**HbA1c not quantified** / Yes/**No***	No ≤30%
Arkray ADAMS A1c HA-8180T (software version EU 1.41)	No	No	No	No	-
Beckman AU System (reagent lot OSR6192)	Yes	Yes	No	No	No <10%
Beckman Synchron	No	No	No	No	$
Bio-Rad D-100	No	No	No	No	-
Bio-Rad D-10 (short Program)	Yes/**No**	No	No	No	No <10%
Bio-Rad D-10 (extended program)	No	No	No	No	-
Bio-Rad Variant II A1c (NU)	No	No	No	No	No <10%
Bio-Rad Variant II Turbo (270-2415/2417)	No	No	Yes	Yes	No <5%
Bio-Rad Variant II Turbo 2.0	No	No	**No**/Yes	No	No <25%
JEOL BM Test HbA1c on JCA-BM 6010/C	No	No	No	No	No<15%
Menarini HA-8160 (Diabetes Mode)	No	No	Yes	Yes	-
Menarini HA-8160 (Thalassemia Mode)	-	-	No	HbA1c not quantified	-
Ortho-Clinical Vitros	No	No	No	No	$
Polymer Tech Systems A1cNow	Yes	Yes	No	No	$
Roche Cobas Integra Gen2	No	No	No	No	$
Roche Tina-quant II	No	No	No	No	$
Sebia Capillarys 2 Flex Piercing	No	No	No	No	No ≤15%
Sebia Capillarys 3 Tera	No	No	No	No	-
Siemens Advia HbA1c (original version)	Yes	Yes	@	@	$
Siemens Advia A1c (new version)	No	No	@	@	$
Siemens DCA 2000/DCA Vantage	**No**/Yes	No	No	No	No<10%
Siemens Dimension	No	No	No	No	$
Tosoh G7 Variant Mode	**Yes**/No	No	Yes	No	No ≤30%
Tosoh G8 Variant Mode (*software version 5.24)	No/**Yes** ***No**	No/**Yes** ***No**	Yes ***No**	No/**Yes** ***No**	No ≤30%
Tosoh GX SW 1.22 (*SW 1.24)	Yes/*No	Yes/*No	No	Yes/*No	-
Trinity (Primus) Boronate Affinity HPLC	No	No	No	No	No <15%

-Not yet evaluated
@ In the absence of specific method data, it can generally be assumed that immunoassay methods do not have clinically significant interference from HbE and HbD because the E and D substitution are distant from the N-terminus of the hemoglobin beta chain.
$ In the absence of specific method data, it can generally be assumed that both immunoassay and boronate affinity methods show interference from HbF levels above ~10-15%.
Yes/No indicates that there is conflicting data in the literature. The indicator in bold is the opinion of the NGSP based on review of the literature cited.
http://www.ngsp.org/factors.asp

US PREVALENCE OF HEMOGLOBIN VARIANTS AND DIABETES

Hemoglobinopathies are among the most common inherited diseases around the world. It is estimated that around 7% of the world's population are carriers of hemoglobinopathies. Originally they were mainly found in tropical areas of sub-Sahara Africa, Asia and in the Mediterranean area, but with migration these diseases are now found globally.[7,11,12]

The number of foreign-born residents in the United States has increased over the last five decades from 5.4% in 1960 to nearly 13% in 2010. In 2010, 53% and 28% of foreign-born United States residents had immigrated from Latin America and Asia, respectively.[14]

The table summarizes the prevalence/incidence of diabetes and of common hemoglobinopathies in US populations.

RACE/ ETHNICITY	PREVALENCE OF DIABETES (%)[15]	INCIDENCE OF SICKLE CELL TRAIT IN NEWBORNS[16]	PREVALENCE OF HbC TRAIT (%)[17]	PREVALENCE OF HbE TRAIT (%)[17]	PREVALENCE OF HbF[17]
Asian, non-Hispanic	8.0%			About 30% of Southeast Asians	
Black, non-Hispanic, African Americans	12.7%	7.3%	2.3%		
Hispanic	12.1%	0.7%			
White, non-Hispanic	7.4%	0.3%			
All	9.4%	1.6%			1.5% will have HbF concentrations ranging from 2%-12%

1. Association of Public Health Laboratories. Hemoglobinopathies: Current Practices for Screening, Confirmation and Follow-up. 2015. https://www.cdc.gov/ncbddd/sicklecell/documents/ nbs_ hemoglobinopathy-testing_122015.pdf. Accessed 8 Nov 2017.
2. Thom CS *et al*. Hemoglobin Variants: Biochemical Properties and Clinical Correlates. Cold Spring Harb Perspect Med. 2013;3:a011858.
3. Forget BG *et al*. Classification of the Disorders of Hemoglobin. Cold Spring Harb Perspect Med 2013;3:a011684.
4. Little R *et al*. A Review of Variant Hemoglobins Interfering with Hemoglobin A1c Measurements. J Diabet Sci Technol 2009;3(3):446-451.
5. Piel FB *et al*. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. Lancet 2013;381:142-151.
6. Little R *et al*. Effects of 49 Different Rare Hb Variants on HbA1c Measurement in Eight Methods. J Diabet Sci Technol 2015;9(4):849-856.
7. Kohne E. Hemoglobinopathies. Clinical Manifestations, Diagnosis and Treatment. Dtsch Artztebl Int 2011;108(31-32):532-540.
8. de Souza Carrocini *et al*. What influences Hb Fetal production in adulthood? Rev Bras Hematol Hemoter. 2011;33(3):231-236.
9. Steinberg MH *et al*. Disorders of Hemoglobin. Genetics, Pathophysiology, and Clinical Management. Second edition. 2009. ISBN 978-0-521-87519-6.
10. Mosca A *et al*. The relevance of hemoglobin F measurement in the diagnosis of thalassemias an related hemoglobinopathies. Clin Biochem. 2009;42:1797-1801.
11. Weatherall DJ. Hemoglobinopathies Worldwide: present and future. Curr Mol Med 2008;8(7):592-599.
12. Modell B *et al*. Global epidemiology of haemoglobin disorders and derived service indicators. WHO Bulletin 2008;86(6):480-487.
13. Little RR *et al*. Effects of hemoglobin E and D traits on glycated hemoglobin (HbA1c) Measurements by twenty-three methods. Clin Chem 2008;54:1277-1282.
14. Sayani FA *et al*. Increasing prevalence of thalassemia in America: Implications for primary care. Annals Med 2015; 47(7): 592-604.
15. CDC – NCCDPHP. National Diabetes Statistics Report, 20t 17. https://www.cdc.gov/diabetes/ data/statistics/ statistics-report.html. Accessed 3 Nov 2017.
16. Ojodu J *et al*. Incidence of Sickle Cell Trait - United States, 2010. CDC-MMWR 2014;63(49):1155-1158.
17. Bry L *et al*. Effects of Hemoglobin Variants and Chemically Modified Derivates on Assays for Glycohemoglobin. Clin Chem 2001; 47(2):153-163.
18. Wilburn CR *et al*. The Prevalence and Role of Hemoglobin Variants in Biometric Screening of a Multiethnic Population. One Large Health System's Experience. Am J Clin Pathol 2017; 147:589-595.
19. Christenson RH *et al*. Appleton & Lange's Outline Review Clinical Chemistry. 2001. ISBN 0-07-031847-6.
20. Nasir NM *et al*. Hemoglobin variants detected by hemoglobin A1c (HbA1c) analysis and the effect on HbA1c measurements. Int J Diabet Ctries 2010;30(2):86-90.
21. Warade J. Comparison of glycated hemoglobin with HPLC and capillary electrophoresis. Int J Res Med Sci 2017; 5(5):1976-1979.
22. Mongia SK *et al*. Effects of Hemoglobin C and S Traits on Fourteen Commercial Glycated Hemoglobin Assays. Am J Clin Path 2008;130:136-140.
23. NGSP. Factors that Interfere with HbA1c Test Results. www.ngsp.org/interf.asp. Accessed 14 Nov 2017.
24. ADA. Standards of Medical Care. Glycemic Targets. Diabetes Care 2017; 40(Suppl 1):S48-S56.

CVD153410 10001566-03 1/19

Abbott