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Review

Effects of Hemoglobin Variants and Chemically Modified Derivatives on Assays for Glycohemoglobin

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Background: **Glycohemoglobin (gHb), measured as hemoglobin (Hb) A_{1c} or as total gHb, provides a common means for assessing long-term glycemic control in individuals with diabetes mellitus. Genetic variants and chemically modified derivatives of Hb can profoundly affect the accuracy of these measurements, although effects vary considerably among commercially available methods. The prevalence of genetic variants such as HbS, HbC, and HbE, and chemically modified derivatives such as carbamyl-Hb among patient populations undergoing testing is not insignificant. Clinical laboratories and sites responsible for point-of-care testing of gHb need to be aware of the interferences produced in assays by these Hbs.**

Approach: **We conducted a review of the literature describing the effects of variant Hbs on gHb assay methods commonly used in clinical laboratories.**

Content: **This review summarizes the documented effects of both common and uncommon Hb variants and derivatives on the measurement of gHb. Where known, we discuss mechanisms of interference on specific assays and methodologies. We specifically address effects of commonly encountered Hbs, such as carbamyl-Hb, HbS, HbC, HbE, and HbF, on assays that use cation-exchange chromatography, immunoassays, or boronate affinity methods for measuring gHb.**

Summary: **A variety of patient- and laboratory-related factors can adversely affect the measurement of gHb in patients harboring Hb variants or derivatives. Identification of the variant or derivative Hb before or during testing may allow accurate measurement of gHb by the selection of a method unaffected by the given variant or derivative. However, laboratories should make available alternative, non-Hb-based methods for assessing long-term glycemic control in individuals with HbCC, HbSS, or HbSC disease, or with other underlying disorders where the concentration of gHb does not accurately reflect long-term glycemic control.**

The measurement of glycohemoglobin (gHb)[1] serves as a powerful tool in the evaluation and management of patients with diabetes mellitus. Concentrations of gHb provide a means of assessing long-term glycemic control and correlate well with the risk for the development of chronic complications related to diabetes *(1–3)*. More than 20 methods for the determination of gHb are commercially available to clinical laboratories. These methods measure gHb based on its physical, chemical, or antibody-recognized characteristics. Structural variants and chemical derivatives of hemoglobin (Hb) interfere with many methods. This review surveys the effects of Hb variants and derivatives on commonly used commercial assays.

Hb Variants and Derivatives

More than 700 characterized Hb variants have been reported *(4)*. The majority arise from point mutations in the α, β, γ, or δ Hb chains. The widespread measurement of gHb has identified new variants, many of which produce no phenotypic abnormalities. Of the nearly 16 million diabetic patients in the United States, estimates suggest that >150 000 carry one of these genetic Hb variants *(5, 6)*. HbS and HbC represent those most commonly encountered. In other parts of the world, the prevalence of variant Hbs has been demonstrated to be as high as one-third of all diabetic patients undergoing testing *(7)*.

In addition to genetic variants, the measurement of

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[1] Nonstandard abbreviations: gHb, glycohemoglobin; Hb, hemoglobin; GSP, glycated serum protein; GSA, glycated serum albumin; IEF, isoelectric focusing; and ES-MS, electrospray mass spectrometry.

gHb can be affected by chemical modifications of Hb, which may be chronically present in diabetic patients. These modifications may mimic gHb physically and chemically, leading to inaccurate determinations of gHb, particularly when separation methods based on charge differences are used. Carbamylated Hb, which is increased in uremic patients, represents the most frequently encountered derivative. High concentrations of acetylated Hb occur with uncommon mutations at the NH_2 terminus of the β-globin chain that enhance formation of acetyl-Hb in vivo. Although in vitro exposure of normal Hb to aspirin has been shown to produce acetylated Hb, no effects from in vivo exposure have been detected in patients chronically taking 1 g or less of aspirin a day *(8)*.

Many hemoglobinopathies, including sickle cell disease, homozygous HbC disease, HbSC disease, and β-thalassemia, frequently show increased amounts of minor Hb species, i.e., HbA_2 and HbF, which interfere with some gHb methods. In addition, pathologic conditions affecting red cell half-life, including hemolysis *(9)*, hemorrhage *(9)*, iron deficiency anemia *(10, 11)*, or red cell transfusion *(12)* affect gHb values.

gHbs

In healthy adults, Hb consists of ~97% HbA, 2.5% HbA_2, and 0.5% HbF *(13)*. Fractionation of HbA by chromatography identifies several minor peaks referred to as HbA_1, or fast Hbs, which include the glycated forms HbA_{1a}, HbA_{1b}, and HbA_{1c} *(13)*. These fast Hbs form as the result of a two-step reaction. In the first step, a reversible reaction between the free aldehyde group of glucose or other sugars and nonprotonated free amino groups on the Hb molecule forms a Schiff base. This reversible reaction is followed by an irreversible, nonenzymatic Amadori rearrangement that produces gHb *(13)*. The glycation alters the structure of the Hb molecule and decreases its net positive charge. Many forms of testing use one or both differences to separate gHb from nonglycated Hbs.

The N-terminal valine of the β chain provides the most common site of glycation within the Hb tetramer, accounting for 80% of HbA_1 *(13)*. The IFCC defines HbA_{1c} as Hb that is irreversibly glycated at one or both N-terminal valines of the β chains. The remaining gHbs have glucose, glucose-6-phosphate, fructose-1,6-diphosphate, or pyruvic acid bound to 1 of 44 additional sites occurring at ε-amino groups of lysine residues or at the NH_2 terminus of the α chain *(13)*. Although all commercially available methods include HbA_{1c} in gHb measurements, they vary in their ability to detect non-A_{1c} gHb.

Current clinical recommendations of the American Diabetes Association suggest that gHb be maintained at 7%, consistent with a decreased risk for developing long-term complications from diabetes mellitus. A reevaluation of the treatment regimen should be undertaken in patients with repeated gHb values >8% *(1)*.

In a healthy individual, gHb readings reflect the degree of glycemic control over the preceding 2–3 months, reflecting the average circulating life span of 120 days for red blood cells *(1)*. Pathophysiological conditions affecting red cell turnover, such as sickle cell disease and HbCC and HbSC disease, thus limit the utility of gHb testing in assessing long-term glycemic control. Alternative tests, such as measurement of glycated serum proteins (GSPs) and glycated serum albumin (GSA), should be performed when the interpretation of gHb is confounded by variables affecting red cell turnover or when Hb variants affect the ability of the Hb molecule to be glycated. However, clinicians should be aware of two important points concerning the use of GSPs or GSA as measures of long-term glycemic control: (*a*) these tests assess the degree of glycemic control over a period of ~2 weeks, as opposed to 2–3 months for gHb; and (*b*) neither test has been correlated with the development of long-term complications from diabetes mellitus, as was shown with gHb in the Diabetes Control and Complications Trial or with the United Kingdom Prospective Diabetes Study *(1)*.

Laboratory Methods for Determining gHb

Boronate affinity or affinity-binding chromatography, cation-exchange chromatography, and immunoassays form the most common means for measuring gHb. Analysis of clinical laboratory proficiency surveys for gHb measurement, conducted by the College of American Pathologists in 1999,[2] revealed that more than one-half of participating clinical laboratories use boronate affinity chromatography for determining gHb, either as HbA_{1c} or as total gHb *(14)*. Approximately 30% use a cation-exchange or ion-exchange HPLC method. Nearly 15% use immunoassay methods, whereas <5% use electrophoretic methods. Common cation-exchange and HPLC systems include the Tosoh A1c 2.2+ and the Bio-Rad Variant, Diamat, and Diastat systems. Available immunoassays include the Bayer DCA-2000, a staple of gHb testing in physicians' offices, and the Roche TinaQuant II and Unimate assays. Boronate affinity chromatography and boronate affinity-binding methods include the Abbott IMx, the CLC 330 and CLC 385 from Primus Corporation, the Glyco-Tek from Helena Laboratories, and the Bio-Rad Variant GHB and Variant Express. The boronate affinity-binding methods measure total gHb but report results either as total gHb or as a corrected HbA_{1c} equivalent.

[2] The College of American Pathologists 1999 data were used to calculate relative percentages of methods in use as this survey provides a more accurate indication of current trends. During the time of the 2000 GH2-A Survey Set, Abbott had withdrawn their reagents for the IMx from the market. Many laboratories using the IMx as their primary method thus switched to an alternative method for measuring gHb during this period, significantly affecting the ratios of methods in use.

Methods of gHb Determination and Mechanisms by Which Hb Variants and Derivatives May Affect Results

CATION-EXCHANGE CHROMATOGRAPHY

Cation-exchange chromatography separates Hb species based on charge differences. Hb species elute from the cation-exchange column at different times with the application of buffers of increasing ionic strength *(13)*. A spectrophotometer measures the concentration of Hb in each collected fraction, which is then quantified by calculating the area under each peak. Automated HPLC methods use similar principles. The following equation enables determination of the amount of HbA_{1c} in a given sample:

$$\%HbA_{1c} = 100 \times \frac{HbA_{1c}}{HbA + HbA_{1c}}$$

Carriers of variant Hbs or Hb adducts or derivatives that elute separately from HbA and HbA_{1c} generally have little effect on HbA_{1c} measurements because they do not factor into the above equation. Inaccurate HbA_{1c} values occur when the Hb variant, or its glycated derivative, cannot be separated from HbA or HbA_{1c}. Fig. 1 illustrates three circumstances that produce inaccurate determinations of HbA_{1c} (Fig. 1, C–E); variants known to produce these patterns are shown in Tables 1 and 2 and are discussed in the text. Interestingly, the same variant may produce falsely increased or decreased HbA_{1c}, depending on the method used. In individuals homozygous for variant Hbs, such as HbSS or HbCC, modifications to the HPLC protocol, the algorithms used to calculate Hb A_{1c}, and the use of altered reference ranges have been proposed to provide more accurate determinations of in vivo concentrations of HbX_{1c} *(15)*. However, clinicians should consider methods other than gHb for determining long-term glycemic control, given the multiple factors confounding the interpretation of gHb results for these individuals.

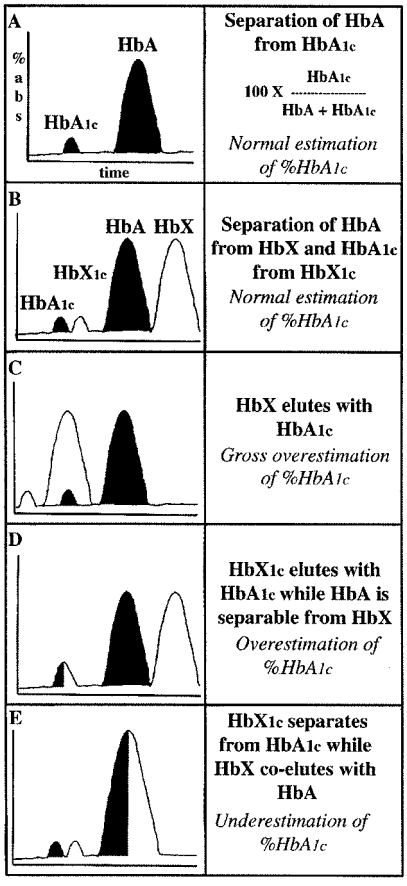

Fig. 1. Sample chromatographs for the determination of percentage of HbA_{1c} and effects on the calculation of $\%HbA_{1c}$.

HbA and HbA_{1c} are indicated by *shaded areas*; HbX and HbX_{1c} by *unshaded areas*. (*A*), subject with no variant Hb forms. (*B*), the variant Hb (HbX) and its glycated form (HbX_{1c}) are fully separable from HbA and HbA_{1c}, respectively. The analytical determination of $\%HbA_{1c}$ is unaffected. (*C*), HbX comigrates with HbA_{1c} producing a gross overestimation of $\%HbA_{1c}$. (*D*), HbX_{1c} and HbA_{1c} comigrate, whereas HbX is separable from HbA, producing mild, spurious increases in $\%HbA_{1c}$. (*E*), HbX_{1c} and HbA_{1c} separate, whereas HbA and HbX co-elute, producing a spurious decrease in the calculated value of $\%HbA_{1c}$.

The native Hb variant co-elutes with HbA_{1c} (Fig. 1C and Table 1). Many native Hb variants substitute a neutral amino acid for a positively charged residue on the α or β chain. The alteration decreases the retention time of the nonglycated variant Hb, causing it to co-elute with HbA_{1c} and leading to a substantial overestimation of HbA_{1c}. Some patients have reported HbA_{1c} values as high as 54% *(16)*. Examples include Hb Raleigh (β1Val→Ala) *(16)*, Hb Graz (β2His→Leu) *(17, 18)*, Hb Sherwood Forest (β104Arg→Thr) *(17, 18)*, Hb South Florida (β1Val→Met) *(19)* and Hb Niigata [βN-Methionyl-1(NA)Val→Leu] *(20)*. In the cases of Hb Raleigh, Hb South Florida, and Hb Niigata, the substitution at the NH_2 terminus enhances the formation of acetyl-Hb in vivo, providing a physiological basis for spuriously increased HbA_{1c}. Carbamyl-HbA has also been found to produce false overestimation of HbA_{1c} in some methods by co-eluting with HbA_{1c} *(8)*.

To confound matters, the co-elution of the variant Hb with HbA_{1c} or its separation from HbA_{1c} often depends on the method used. These inconsistencies arise from the distinct solvent mixture, column, and additional elution conditions, including temperature, pressure, flow rate, and program time, used in each system. The same Hb variant may thus yield very different results for HbA_{1c}, depending on the method used. Furthermore, the algorithms used to calculate HbA_{1c} can lead to inaccurate results if they do not recognize the presence of aberrant peaks and either provide warning flags or allow for corrections to make accurate determinations.

The glycated Hb variant co-elutes with HbA_{1c}, whereas the nonglycated Hb variant is resolved from HbA (Fig. 1D). This pattern overestimates the concentration of HbA_{1c}. The glycated Hb variant becomes incorporated in the HbA_{1c} peak, whereas the denominator consists only of the area under the native HbA peak. This effect occurs in the presence of Hb $G_{Philadelphia}$ on the Tosoh A1c 2.2+ system. Conversely, Hb $G_{Philadelphia}$ produces a falsely decreased

Table 1. Uncommon Hb variants that migrate with HbA_{1c}, producing spuriously increased HbA_{1c} values by ion-exchange chromatography.

Hb variant	Affected method(s)	% HbA_{1c}	Unaffected method(s)	Corrected HbA_{1c}/gHb	Reference(s)
Carbamyl-Hb	Bio-Rad Diamat	14.3% (nondiabetic)	Immunoassay Pierce Glycotest II affinity chromatography	3.0% 4.8%	*8*
Graz β2His→Leu	Bio-Rad Diamat and Variant	49.1–49.5% (nondiabetics)	Roche immunoassay	3.55–5.59%	*17, 18*
Hope β136Gly→Asp	Helena Labs Hb Quick Column	35% (53 F,[a] diabetic)	TBA[b] colorimetric assay	6%	*52*
	Bio-Rad Variant	Not indicated	Bayer DCA-2000 Roche TinaQuant Tosoh A1c 2.2+ Primus CLC 385	Not indicated	*21*
Kanagawa α40Lys→Met	Tosoh HPLC-723GHb	15% (70 M,[a] diabetic)	Not assessed	Not assessed	*53*
Long Island/Marseille βN-Methionyl-2(NA2)His→Pro	Bio-Rad Diamat	56% (29 M,[a] nondiabetic)	Not assessed	Not assessed	*28*
Niigata βN-Methionyl-1(NA)Val→Leu	Tosoh A1c 2.2+	13.1% (58 M,[a] nondiabetic)	Latex agglutination Olympus Au-600[c]	Not indicated	*20*
	Hitachi Hi-AUTOA1c HA-8150	13.2–13.8% (nondiabetics)	ES-MS Bayer DCA-2000[c]	3.7–3.8% by ES-MS or Bayer DCA-2000	*34*
Okayama β2His→Gln	Hitachi Hi-AUTOA1c HA-8150	21.9% (nondiabetic)	ES-MS Bayer DCA-2000	5.1% by ES-MS 5.5% by DCA-2000	*34*
Old Dominion β143His→Tyr	Hi-Auto HPLC (Biomen) Glycomat HPLC-(Ciba-Corning)	44.2% (33 M,[a] diabetic)	Boronate affinity chromatography	7.3%	*54*
Raleigh β1Val→Ala	Bio-Rad Variant	46% (65 M,[a] diabetic, ESRD)	Boronate affinity chromatography Bayer DCA-2000	4–8% by affinity chromatography 3.8% by Bayer DCA-2000[b]	*16*
	Bio-Rad Variant Tosoh A1c 2.2+	Not indicated	Bayer DCA-2000 Primus CLC 385	Not indicated	*21*
Sherwood Forest β104Arg→Thr	Bio-Rad Diamat and Variant Menarini Hi-AUTO Aic	51.8% (88 F,[a] type II DM) 39.3–53% (nondiabetics)	Roche immunoassay	4.64%	*17, 18*
South Florida β1Val→Met	Bio Rex 70 ion exchange	14.8% (8 M,[a] nondiabetic)	TBA colorimetric assay Boronate affinity chromatography	6.4%	*19*
Turriff α99Lys→Gln	Bio-Rad Diamat	22% (29 F,[a] diabetic)	Not assessed	Not assessed	*55*

[a] Indicates age (in years) and gender of the single patient discussed in the reference study.

[b] TBA, thiobarbituric acid; ESRD, end stage renal disease; DM, diabetes mellitus.

[c] Reported results by immunoassay believed to be falsely low because of enhanced acetylation at the NH_2 terminus of Hbs Raleigh and Niigata.

value on the Bio-Rad Variant system *(21)*. The Bayer DCA-2000, Roche TinaQuant, and Primus CLC 385 methods are not affected by this variant *(21)*.

The Hb variant co-elutes with HbA, whereas the glycated Hb variant is resolved from HbA_{1c} (Fig. 1E and Table 2). This elution pattern underestimates HbA_{1c} as the denominator includes both HbA and the variant Hb, thus falsely decreasing the percentage of HbA_{1c}. Examples producing this pattern include HbD, $G_{Philadelphia}$, $J_{Baltimore}$, and O_{Padova} on the Bio-Rad Variant system *(21, 22)* and Hb Sherwood Forest and O_{Padova} on the Hitachi L-9100 *(22)*. Different platforms and systems may produce spurious increases or decreases with the same Hb variants. On the Tosoh A1c 2.2+, HbE and $J_{Baltimore}$ generate this pattern, but not D or $G_{Philadelphia}$ *(20, 21)*.

electrophoresis

Agar gel electrophoresis is used infrequently to determine gHb in clinical laboratories in the US. The method separates Hb species based on charge differences *(13)*. Scanning densitometry of the gel allows quantification of Hb species in each sample. This method has patterns of interference from Hb variants similar to those observed with ion-exchange chromatography. However, minor variations in pH, ionic strength, or temperature have little effect on the migration pattern. In contrast to procedures involving cation-exchange chromatography, interferences from individual Hb variants are expected to be more consistent among different systems and platforms using gel electrophoresis. Comigration of Hb variants or derivatives with either HbA or HbA_{1c} interferes with HbA_{1c} determinations. Comigration of HbF or carbamylated Hb with HbA_{1c} produces spuriously increased HbA_{1c} values *(23)*.

isoelectric focusing

Isoelectric focusing (IEF) is also used infrequently for determining gHb. IEF uses a pH gradient gel to separate Hb species based on their charge *(13)*. After fixation of the gel, quantification is performed by a high-resolution integrating microdensitometer. Variants such as Hb Pavie (α135Val→Glu) *(24)*, Hb Hafnia (β116His→Gln) *(25)*, and Hb Fontainebleau (α21Ala→Pro) *(26)* comigrate with HbA_{1c} on IEF gels and can cause spuriously increased readings.

immunoassays

Several commercial methods quantify HbA_{1c} using antibody-mediated inhibition of latex agglutination or immunoturbidimetric assays. Antibodies recognize the N-terminal glycated amino acid in the context of the first 4–10 amino acids of the Hb β chain *(27)*. These antibodies do not recognize the reversible Schiff base or other gHb species, including chemically modified derivatives. Of note, the most commonly encountered Hb mutations, HbS and HbC, fall within this susceptible region, and will be discussed subsequently.

HbF, Hb Graz, and Hb Raleigh are among those shown to cause decreased HbA_{1c} values by immunoassay *(16, 17)*. Other Hb variants with alterations in the first 4–10 N-terminal amino acids could produce similar results. In the case of Hb Raleigh, the Val→Ala substitution produces substantial acetylation at the NH_2 terminus, preventing the formation of the gHb at this position and further decreasing results obtained by immunoassay *(16)*. gHb determinations in this and other N-terminal variants, namely Hb Long Island *(28)*, Hb South Florida *(19)*, and Hb Niigata *(20)*, are also of limited utility in assessing long-term diabetic control because of the extensive acetylation of Hb in vivo.

boronate affinity chromatography

Among commercially available methods, boronate affinity chromatography tends to demonstrate the least interference from the presence of Hb variants and derivatives *(5, 29, 30)*. The method determines total gHb, including HbA_{1c} and ketoamine structures formed on lysines and N-terminal valine residues of both the α and β chains. *m*-Aminophenylboronic acid, cross-linked to agarose or glass beads, reacts specifically with *cis*-diol groups of glucose bound to Hb to form a reversible five-member ring complex, thus immobilizing gHb to the column. Addition of sorbitol dissociates the complex and elutes the gHb *(13)*. The gHb may then be measured spectrophotometrically or by quenching of Hb fluorescence with an added fluorophore.

Studies using the CLC 330 and CLC 385 (Primus Corporation) have reported no interference from common Hb variants or derivatives. Significant spurious increases in gHb values have been reported with the Quick Column method (Helena) in HbAC and HbCC samples *(31)*. One study reported a positive bias in gHb readings from HbAC samples with the Abbott IMx® Glycated Hemoglobin assay, which uses a boronate/ion-capture method. Frank et al. *(5)* hypothesized that the bias may be attributable to the polyanion capture reagent, which may react with the Lys substitution at position 6 of nonglycated HbC.

Of the less common Hb variants that have been studied, only Hb Himeji (β140Ala→Asp) has demonstrated falsely increased gHb results, which were attributed to excessive glycation of Hb Himeji in vivo *(32)*. Accurate HbA_{1c} values were ultimately obtained by performing cation-exchange chromatography on the eluate with Iso Glyc-affin GHb columns. This fractionation allowed determination of HbA_{1c} relative to HbA (Table 2). The authors hypothesized that the β140Ala→Asp substitution in Hb Himeji may interact with the NH_2 terminus of the β chain, enhancing the in vivo formation of $Hb\ Himeji_{1c}$ *(32)*.

Table 2. Uncommon Hb variants producing falsely low values for HbA_{1c}.

HB variant	Affected method(s)	% HbA_{1c}	Unaffected method(s)	Corrected HbA_{1c}/gHb	Reference(s)
Camden β131 Gln→Glu	Bio-Rad Variant	Not indicated	Bayer DCA-2000 Primus CLC 385	Not indicated	*21*
D β121Glu→Val	BioRad Variant and Diamat Merck Hitachi L-9100	2.9–3.9% (nondiabetics)	Affinity chromatography (IMx)	5.3%	*22*
E β26Glu→Lys	Tosoh A1c 2.2+	Not indicated	DCA-2000 Roche TinaQuant Primus CLC 385	Not indicated	*21*
$G_{Philadelphia}$	BioRad Variant	Not indicated	DCA-2000 Roche TinaQuant Primus CLC 385	Not indicated	*21*
Hamadan β56Gly→Arg	Unspecified HPLC method	0% and 1.2% (36 F,[a] gestational diabetes)	Not assessed	Not assessed	*56*
	Hitachi Hi-AUTOA1c HA-8150	1.0–2.9% (nondiabetics)	ES-MS Bayer DCA-2000	2.7–5.3% by ES-MS 4.5–5.3 by DCA-2000	*34*
Himeji β140Ala→Asp	Daiichi Auto A1c cation-exchange HPLC	5.1% (52 M,[a] type 2 diabetes)	Glyc-affin GHb; (Iso Laboratories) Boronate affinity chromatography[b]	10.3%[c]	*32*
Hoshida β43Glu→Gln	Hitachi Hi-AUTOA1c HA-8150	2.5% (nondiabetic)	ES-MS Bayer DCA-2000	4.1% by ES-MS 5.2% by DCA-2000	*34*
$J_{Baltimore}$	Bio-Rad Variant Tosoh A1c 2.2+	Not indicated	Bayer DCA-2000 Roche TinaQuant Primus CLC 385	Not indicated	*21*
Moriguchi β97His→Tyr	Daiichi Auto A1c cation-exchange HPLC	2.9% (69 F,[a] diabetic)	Not assessed	Not assessed	*57*
North Manchester β51His→Pro	HPIEC[d] (Daiichi HA-B121; Biomen)	2.7% (88 M,[a] diabetic)	Boronate affinity chromatography (Helena)	5.7%	*58*
Riyadh β120Lys→Asn	Tosoh LC-723 GHb III HPLC	Not indicated	Not assessed	Not assessed	*59*
	Hitachi Hi-AUTOA1c HA-8150	3.1% (74 M,[a] diabetic) 2.7–4.3% (nondiabetics)	ES-MS Bayer DCA-2000	4.2–6.1% by ES-MS 4.5–6.6 by DCA-2000	*34*
Sagami β139Asn→Thr	Hitachi HI-AUTOA1c HA-8150	1.1% (nondiabetic)	ES-MS	3.2%	*34*
Takematsu β120Lys→Gln	Hitachi Hi-AUTOA1c HA-8150	2.5% (nondiabetic)	ES-MS Bayer DCA-2000	3.7% by ES-MS 4.5% by DCA-2000	*34*

[a] Indicates age (in years) and gender of the single subject discussed in the referenced study.

[b] Value of 10.3% required further fractionation of eluted gHb by cation-exchange chromatography to determine HbA_{1c} relative to HbA because of excessive glycation of Hb Himeji in vivo: 17.6% of HB Himeji was estimated to have been glycated, as opposed to 10.3% of HbA.

[c] Total gHb values measured by affinity chromatography were 13.4% prior to normalizing the results to HbA_{1c} and HbA.

[d] HPIEC, high-performance IEF.

electrospray mass spectrometry

Electrospray mass spectrometry (ES-MS) appears to provide a means of measuring total gHb that is unaffected by the presence of genetic or chemical modifications to the Hb molecule. The IFCC has proposed using ES-MS and capillary electrophoresis as candidate reference methods for the determination of gHb. ES-MS is also frequently used to characterize variant Hbs, including those identified by gHb testing *(33, 34)*. Hb prepared from samples of whole blood is denatured and injected into the ES-MS instrument. Multiple positive ions are generated for each protein in the sample, including individual Hb chains, glycated forms, or chains containing other chemical modifications. A mass spectrometer separates the ions based on their mass-to-charge ratio *(35)*. The profile gives an accurate indication of total gHb as well as proportions of carbamylated and variant forms. In patients with Hb variants, the same profile can also provide information concerning the nature of the variants *(35, 36)*. Despite these attributes, the prohibitive cost of ES-MS and the complicated nature of its installation and operation make it unlikely that it will be used in most clinical laboratories in the near future *(36)*.

Effect of Commonly Encountered Chemical Derivatives and Genetic Variants on gHb Methods

The findings of several studies concerning the effects of carbamyl-Hb, HbS, HbC, HbE, and HbF on common methods for determining gHb are summarized in Table 3. We have opted to present data from studies where (*a*) unless indicated, multiple samples with the given variant were studied, (*b*) measurements were performed on samples covering a range of expected gHb values, and (*c*) the comparison method is known to be unaffected by the variants under study.

carbamyl-Hb

Urea spontaneously dissociates in vivo to form ammonia and cyanate. Protonation of cyanate leads to the formation of isocyanic acid, which reacts with the α and ϵ amino groups on proteins, forming a carbamyl moiety *(37)*. The N-terminal valine of the Hb β chain is particularly reactive with isocyanic acid, leading to the stable formation of carbamyl-Hb. Previous studies have demonstrated that 1 mmol/L urea is associated with the formation of 0.063% carbamyl-Hb in vivo *(8)*. Uremic patients may have carbamyl-Hb concentrations as high as 3% of total Hb. In addition, clinicians should be aware of additional factors in uremic patients that impact red cell turnover, such as shortened red cell life span in hemodialysis patients, which in turn affect the accuracy of gHb readings.

Carbamyl-Hb has an isoelectric point similar to HbA_{1c} and can thus interfere with charge-based methods of measuring gHb. In vitro carbamylation of Hb, to concentrations as high as 5.4% carbamyl-Hb, has been shown to produce significant spurious increases in HbA_{1c} values in multiple cation-exchange methods, including the Bio-Rad Variant and Diamat, and the Tosoh A1c 2.2+ *(38)*. However, studies assessing in vivo effects of carbamyl-Hb have shown differences that range from insignificant to significant. For example, effects of carbamyl-Hb on the Bio-Rad Diamat system demonstrated discrepancies ranging from 0.02% *(39)* and 0.47% increases in HbA_{1c} values *(39)* to spurious increases of 1.1% *(40)*. Immunoassay and boronate affinity methods, including the Roche TinaQuant II, Bayer DCA-2000, and Primus CLC 385, have been shown to be unaffected by concentrations of carbamyl-Hb encountered in uremic patients *(38, 40)*.

sickle trait

HbS (β6Glu→Val) is the most commonly encountered variant Hb in the United States; 7.8% of African Americans carry the sickle trait, whereas homozygous sickle cell disease afflicts nearly 50 000 Americans *(41)*. In parts of sub-Saharan Africa, the prevalence of HbAS has been shown to be as high as one-third of all patients undergoing HbA_{1c} testing *(7)*.

Commonly used boronate affinity methods have demonstrated accurate gHb readings in the presence of HbS *(5, 15)*. Effects of HbS on cation-exchange chromatographic assays vary, depending on the method and platform used. A recent study found overestimation of HbA_{1c} in the Bio-Rad Diamat or Variant HPLC systems, especially in the lower HbA_{1c} range. The interference from HbS varied in the Diamat system, depending on the lot of columns used *(5)*. Studies with the Tosoh gHb 2.2Ac+ and Menarini 8140 platforms showed no effect as HbS and HbS_{1c} were readily separable from HbA and HbA_{1c} *(42, 43)*. The epitopes recognized by the antibodies used in the DCA-2000 and TinaQuant fall within the first four and six amino acids and are not affected by the mutation in HbS *(8, 27)*. However, a significant spurious increase has been noted with the Roche Unimate immunoassay, which uses the same antibody as the Bayer DCA-2000 *(44)*. The Unimate assay differs from the DCA-2000 in that it uses pepsin to cleave the β chain near the NH_2 terminus, and thus measures glycation on the peptide fragments. One study hypothesized that the antibody may have a higher affinity for the peptide fragments of HbS_{1c} and HbC_{1c}, leading to the spurious increases in gHb *(44)*.

HbC trait

HbC trait (β6Glu→Lys) has a prevalence of 2.3% among African Americans and a prevalence as high as 30% in parts of sub-Saharan Africa *(41)*. HbC trait behaves similarly to HbS trait with regard to many, but not all, assay systems. As discussed above, false increases have been reported with the boronate affinity/cation-capture method used in the Abbott IMx Glycated Hemoglobin method *(5)*, with the Quick Column method from Helena Laboratories *(31)* and with the Roche Unimate immunoassay *(44)*. Use of the 3-min elution program on the Tosoh A1c 2.2+ has been recommended to fully separate HbC from HbA and allow for accurate readings *(43, 45)*. The

Table 3. Effects of frequently encountered Hb derivatives and variants on analytical measurement of glycated Hb.

Method	Carbamyl-Hb	HbAS	HbAC	HbAE	HbSS[a]	HbCC[a]	HbSC[a]	Increased HbF
Cation-exchange chromatography								
Bio-Rad Variant	Spurious increase at 2% carbamyl-Hb *(8)*	Spurious increase at low percentage of HbA_{1c} Unaffected at increased HbA_{1c} *(5)*	Unaffected *(5)*	Suitable if adjustments made in calculations *(21, 29)*	Falsely low *(22)*[a,b]	No published data	No published data	No published data
Bio-Rad Diamat	Spurious increase at 2% carbamyl-Hb *(8, 40)* 0.02% increase in CRF[c] patients with BUN of 27–101 mg/dL *(39)*	Spurious increase at low percentages of HbA_{1c} Unaffected at increased HbA_{1c} *(5)*[d] Suitable if adjustments made to calculations *(8)*	Unaffected *(5)*	Suitable if adjustments made in calculations *(8)*	Unsuitable method *(8)*	Unsuitable method *(8)* Falsely low *(43)*[a,e]	Falsely low *(43)*	Falsely low; requires mathematical corrections be made before interpretation[f] *(8)* Uninterpretable for HbF >60% *(60)*
Tosoh gHb 2.2Ac+	Unaffected *(38, 40)*	Unaffected *(5, 50)*	Unaffected *(5, 50)*	Falsely decreased values *(21)*[g]	Unable to determine a value *(50)*	Unable to determine a value *(50)*	No published data	Unaffected *(46, 50)*
Menarini 8140	Spurious increase at 2% carbamyl-Hb *(40)* 0.29% increase in HbA_{1c} at >60 μg carbamyl-Hb/gHb Unaffected at lower concentrations *(38)*	Unaffected *(42, 43)*	Unaffected *(42, 43)*	Falsely decreased values *(42)*	No results; flagged abnormal Hb *(42,43)*	No results; flagged abnormal Hb *(42,43)*	No results; flagged abnormal Hb *(42,43)*	Unaffected *(42)*
Immunoassay								
Bayer DCA-2000	Unaffected *(8, 40)*	Unaffected *(5, 8, 45)*	Unaffected *(5, 8, 45)*	Unaffected *(8, 21, 45)*	Requires use of different reference range *(8)*[a]	Requires use of different reference range *(8)*[a]	No published data	Positive bias over HbF 5–15% Falsely low with HbF >30% *(51)* Unaffected *(8)*[f]
Roche TinaQuant II	Unaffected *(40)*	Unaffected *(5, 27)*	Unaffected *(5, 27)*	Unaffected *(27)*	No published data	No published data	Unaffected *(27)*	Falsely low with HbF >30% Unaffected for HbF ≤30% *(27)*
Roche Unimate	Unaffected *(61)*	Overestimation *(44)*	Overestimation *(44)*	No published data	No published data	No published data	No published data	Unaffected *(61)*
Boronate affinity chromatography								
Primus CLC 330 and CLC 385	Unaffected *(30, 39)*	Unaffected *(5)*	Unaffected *(5)*	Unaffected *(21)*	Unaffected (Noffsinger)[a,h]	Unaffected (Noffsinger)[a,h]	Unaffected (Noffsinger)[a,h]	Unaffected (Noffsinger)[h]
Abbott IMx gHb	Unaffected *(40)*	Unaffected *(5, 60)*	Falsely low *(5)* Unaffected *(60)*[i]	No published data	Unaffected *(22)*[a,i]	No published data	No published data	Unaffected for HbF <15% *(60)*
Pierce Glycotest II	Unaffected *(8)*	Unaffected *(8)*	Unaffected *(8)*	Unaffected *(8)*	Requires use of different reference range *(8)*[a]	Requires use of different reference range *(8)*[a,e]	No published data	Unaffected *(8)*

[a] Forms of testing other than gHb should be considered in patients with HbSS, HbCC, and HbSC because of processes that affect the turnover of Hb in vivo, and that limit the utility of gHb testing in these individuals.
[b] Study measured HbA_{1c} in one diabetic patient with HbSS.
[c] CRF, chronic renal failure; BUN, blood urea nitrogen.
[d] Different column lots were noted to have varying effects on the determination of HbA_{1c} with the Diamat system in patients with HbAS.
[e] Study measured HbA_{1c} values in samples from three nondiabetic individuals with HbCC.
[f] Effects evaluated for samples with 22% HbF.
[g] Study measured HbA_{1c} in two nondiabetic individuals with HbAE.
[h] Personal communication, Dr. James Noffsinger, Primus Corporation, Kansas City, MO.
[i] Study measured gHb in three nondiabetic individuals with HbAC.

shorter Tosoh 2.2 program is available only on instruments in use in Japan and in Europe.

HbE trait

HbE (β26Glu→Lys) is most commonly encountered in regions of Southeast Asia where the prevalence can be as high as 30% of the indigenous population *(41)*. HbE_{1c} frequently elutes as a shoulder to HbA_{1c} in most HPLC or cation-exchange chromatographic methods *(46)*. Unless corrected, these methods lead to inaccurate determinations of HbA_{1c} that may be spuriously increased or decreased, depending on the method used *(15, 21, 32)*. As the mutation falls outside regions of recognition of most antibodies, the mutation has little effect on immunoassay methods *(8, 46)*, although one study reported that a correction was required to determine accurate gHb values with the Dako Novoclone immunoassay *(8)*. Boronate affinity methods are also unaffected; results incorporate gHb on both HbA and HbE.

HbSS disease

gHb readings from patients with sickle cell disease must be interpreted with caution given the pathological processes, including anemia, increased red cell turnover, transfusion requirements, and increased HbF, that adversely impact gHb as a marker of long-term glycemic control. Corrections to some HPLC and cation-exchange methods allow accurate, analytical determination of HbS_{1c} *(8)*, although the values have limited utility in assessing long-term glycemic control. Other platforms, including the Menarini 8140 and Tosoh gHb 2.2Ac+, do not report results, although the Menarini 8140 flags the abnormal peaks *(42, 43)*. Although Weykamp et al. *(15)* have advocated the use of alternative reference ranges to account for the shortened half-life of red cells in these patients, additional measures of glycemic control, such as GSA and GSPs, should be considered *(1)*.

HbCC and HbSC disease

Both HbCC and HbSC disease share the same confounding factors in the determination of gHb as HbSS disease, although both entities produce a less severe anemia than sickle cell disease. The prevalence of HbCC disease in the US is approximately 1 in 1800, whereas that of HbSC disease is 1 in 1100 *(41)*. Alternative forms of testing, as described above, should be considered for the determination of long-term glycemic control in these individuals.

HbF

At birth, HbF comprises 70% of all Hb, and falls to <5% by 6 months of age *(47)*. Individuals with hereditary persistence of HbF may have concentrations up to 30% of total Hb, whereas β-thalassemia and sickle cell patients commonly demonstrate concentrations ranging from 2% to 20% of total Hb *(48)*. Slight increases occur during pregnancy, with severe anemias, and in certain leukemias. Approximately 1.5% of the US population has HbF concentrations >2%, although the number of patients hospitalized in tertiary medical centers with HbF >2% has been found to be as high as 12% *(49)*. As indicated in Table 3, HbF concentrations <5% of total Hb have no significant effect on the majority of gHb methods. Most cation-exchange chromatographic techniques separate HbF and gHbF from HbA and HbA_{1c}, allowing for accurate determinations of HbA_{1c} *(50)*. The γ chain in the $\alpha_2\gamma_2$ tetramer shares only 4 of the first 10 amino acids with the β chain of HbA and has little to no immunoreactivity with most antibodies used in gHb assays. However, if the immunoassay result is calculated relative to total Hb in the sample, the calculation produces falsely decreased HbA_{1c} values. Recalculating HbA_{1c} relative to HbA gives more accurate results, provided that the correction can be made *(51)*.

Summary

A variety of patient-related factors and laboratory-related processes can lead to inaccurate determinations of gHb in the setting of variant Hbs. Samples should be evaluated for the presence of a Hb variant with any gHb reading >15% *(16)*. In addition, any patient with a significant change in gHb coinciding with a change in laboratory gHb methods should be evaluated for the presence of variant or derivative Hb. Appropriate evaluation includes obtaining pertinent clinical history regarding hemoglobinopathies, alterations in red cell turnover, or conditions favoring the chemical modification of Hb. Manual review of cation-exchange chromatographs may identify the presence of aberrant peaks produced by variants. The gHb measurement should be repeated with a different assay method, and a Hb analysis by chromatography or electrophoresis should be performed to identify more common Hb variants. In some cases, variants may be identified only by ES-MS or by sequencing the expressed globin genes.

Although most modern chromatographic and immunoassay methods are either unaffected by common heterozygous variants such as HbAS, HbAC, and HbAE or give warning flags concerning the likelihood of an underlying variant, less common variants may give no such warnings. Furthermore, all gHb methods are inadequate for the assessment of long-term glycemic control in patients homozygous for HbS, HbC, or with HbSC disease. Although technologies such as boronate affinity chromatography and ES-MS provide a means of accurately determining gHb in these individuals, results are unlikely to accurately reflect long-term glycemic control due to pathological conditions that affect the formation and turnover of gHb in vivo.

In regions where populations have a high prevalence of variant Hbs, methods for the determination of gHb must be carefully selected to allow accurate determination of gHb in these individuals. When dealing with populations in which HbSS, HbCC, or HbSC disease are common and in which gHb determinations have limited utility, laboratories should offer alternative forms of testing, such

as GSPs or GSA, to assist physicians with the determination of glycemic control in these individuals.

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References

1. Goldstein D, Lorenz RA, Malone J, Mathan DM, Peterson CM. Tests of glycemia in diabetes. Diabetes Care 2000;23(Suppl 1):S80–2.
2. Nuttall FQ. Comparison of percent total GHb with percent HbA_{1c} in people with and without known diabetes. Diabetes Care 1998;21: 1475–80.
3. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–86.
4. Anonymous. International Hemoglobin Information Center variant list. Hemoglobin 1994;18:77–183.
5. Frank EL, Moulton L, Little RR, Wiedmeyer HM, Rohlfing C, Roberts WL. Effects of hemoglobin C and S traits on seven glycohemoglobin methods. Clin Chem 2000;46:864–7.
6. Schnedl WJ, Trinker M, Lipp RW. HbA_{1c} determinations in patients with hemoglobinopathies. Diabetes Care 1999;22:368–9.
7. Reid H, Famodu AA, Photiades DP, Osamo ON. Glycosylated haemoglobin HbA_{1c} and HbS_{1c} in non-diabetic Nigerians. Trop Geogr Med 1992;44:131–4.
8. Weykamp CW, Penders TJ, Siebelder CW, Muskiet FA, van der Slik W. Interference of carbamylated and acetylated hemoglobins in assays of glycohemoglobin by HPLC, electrophoresis, affinity chromatography, and enzyme immunoassay. Clin Chem 1993;39: 138–42.
9. Panzer S, Kronik G, Lechner K, Bettelheim P, Neumann E, Dudczak RJ. Glycosylated hemoglobins (GHb): an index of red cell survival. Blood 1982;59:1348–50.
10. Tarim O, Kucukerdogan A, Gunay U, Eralp O, Ercan I. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. Pediatr Int 1999;41:357–62.
11. Gram-Hansen P, Eriksen J, Mourits-Andersen T, Olesen L. Glycosylated haemoglobin (HbA_{1c}) in iron- and vitamin B_{12} deficiency. J Intern Med 1990;227:133–6.
12. Weinblatt ME, Kochen JA, Scimeca PG. Chronically transfused patients with increased hemoglobin A_{Ic} secondary to donor blood. Ann Clin Lab Sci 1986;16:34–7.
13. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry, 3rd ed. Philadelphia: WB Saunders, 1999:790–6.
14. College of American Pathologists. CAP survey set GH2-B. Northfield, IL: CAP, 1999.
15. Weykamp CW, Penders TJ, Muskiet FA, van der Slik W. Influence of hemoglobin variants and derivatives on glycohemoglobin determination, as investigated by 102 laboratories using 16 methods. Clin Chem 1993;39:1717–23.
16. Chen D, Crimmins DL, Hsu FF, Lindberg FP, Scott MG. Hemoglobin Raleigh as the cause of a falsely increased hemoglobin A_{1c} in an automated ion-exchange HPLC method. Clin Chem 1998;44: 1296–301.
17. Schnedl WJ, Reisinger EC, Pieberm TR, Lipp RW, Schreiber F, Hompeier P, Krejs G. Hemoglobin Sherwood Forest detected by high performance liquid chromatography for hemoglobin A_{1c}. Hematopathology 1995;104:444–6.
18. Halwachs-Baumann G, Katzensteiner S, Schedl W, Purstner P, Pieber T, Wilders-Trushnig M. Comparative evaluation of three assay systems for automated determination of hemoglobin A_{1c}. Clin Chem 1997;43:511–7.
19. Shah SC, Malone JI, Boissel JP, Kasper TJ. Hemoglobin South Florida. New variant with normal electrophoretic pattern mistaken for glycosylated hemoglobin. Diabetes 1986;35:1073–6.
20. Watanabe T, Kato K, Yamada D, Midorikawa S, Sato W, Shiga M, et al. A nondiabetic case of hemoglobin variant (Hb Niigata) with inappropriately high and low HbA_{1c} titers detected by different methods. Clin Chem 1998;44:1562–4.
21. Roberts WL, Frank EL, Moulton L, Papadea C, Noffsinger J, Ou C. Effects of nine hemoglobin variants on five glycohemoglobin methods. Clin Chem 2000;46:569–72.
22. Schnedl WJ, Krause R, Halwaches-Baumann G, Trinker M, Lipp RW, Krejs GJ. Evaluation of HbA_{1c} determination methods in patients with hemoglobinopathies. Diabetes Care 2000;23:339–44.
23. Bisse E, Wieland H. HPLC separation of human haemoglobins: simultaneous quantitation of foetal and glycated haemoglobin. J Chromatogr 1988;434:95–110.
24. Wajcman H, Blouquit Y, Riou J, Kister J, Poyart C, Soria J, Galacteros F. A new hemoglobin variant found during investigations of diabetes mellitus: Hb Pavie [α135(H18)Val→Glu]. Clin Chim Acta 1990;188:39–48.
25. Blanke S, Johnsen A, Wimberly PD, Mortensen HB. Hemoglobin Hafnia: α2 (β116(G18)His→Gln)2; a new hemoglobin variant mistaken for glycated hemoglobin. Biochim Biophys Acta 1988; 955:214–9.
26. Wajcman H, Blouquit Y, Gombaud-Saintonge G, Riou J, Galacteros F. Hb Fontainbleau [α21(β2)Ala→Pro]: a new charge silent mutant hemoglobin. Hemoglobin 1989;13:421–8.
27. Chang J, Hoke C, Ettinger B, Penerian G. Evaluation and interference study of hemoglobin A_{1c} measured by turbidimetric inhibition immunoassay. Am J Clin Pathol 1998;109:274–8.
28. Campo S, Sardo M, Raimondo G. Identification and genomic analysis of hemoglobin Long Island-Marseille in a nondiabetic subject of Italian origin. Acta Haematol 1994;91:211–2.
29. Roberts WL, McCraw M, Cook CB. Effects of sickle cell trait and hemoglobin C on determinations of HbA_{1c} by an immunoassay method. Diabetes Care 1998;21:983–6.
30. Philipov G, Charles P, Beng C, Philips P. Alternate site testing for HbA_{1c} using the Primus CLC 330 GHb analyzer. Diabetes Care 1997;20:607–9.
31. Weykamp CW, Martina WV, van der Dijs F, Penders TJ, van der Slik W, Muskiet F. Hemoglobins S and C: reference values for glycohemoglobin in heterozygous, double-heterozygotes and homozygous subjects, as established by 13 methods. Clin Chim Acta 1994;231:161–71.
32. Ohba Y, Miyaji T, Murakami M, Kadowaki S, Fujita T, Oimomi M, et al. Hb Himeji or β140 (H18) Ala–Asp. A slightly unstable hemoglobin with increased β N-terminal glycation. Hemoglobin 1986; 10:109–25.
33. Shackleton CH, Witkowska HE. Mass spectroscopy in the characterisation of variant hemoglobins. In: Desiderio DM, ed. Mass spectroscopy: clinical and biomedical applications, Vol. 2. New York: Plenum, 1994:135–99.
34. Nakanishi T, Miyazaki A, Iguchi H, Shimizu A. The effect of hemoglobin variants on routine HbA_{1c} measurements: an assessment by a mass spectrometric method. Clin Chem 2000;46: 1689–92.
35. Roberts N, Green BN, Morris M. Potential of electrospray mass spectrometry for quantifying glycohemoglobin. Clin Chem 1997; 43:771–8.
36. Miedema K. Electrospray mass spectroscopy for measurement of glycohemoglobin. Clin Chem 1997;43:705–7.
37. Stim J, Mashouf S, Anwar F, Ansari A, Arruda J, Dunea G. Factors

determining hemoglobin carbamylation in renal failure. Kid Int 1995;48:1605–10.
38. Chachou A, Randoux C, Millart H, Chanard J, Gillery P. Influence of in vivo hemoglobin carbamylation on HbA_{1c} measurements by various methods. Clin Chem Lab Med 2000;38:321–6.
39. Little R, Mathew AS, Tennill AL, Rohlfing CL, Goldstein DG. Measurement of glycohemoglobin (GHB) in patients with chronic renal failure (CRF): are ion-exchange HPLC results really invalid? [Abstract]. Clin Chem 1997;43(Suppl 1):S136.
40. Weykamp CW, Miedema K, de Haan T, Doleman C. Carbamylated hemoglobin interference in glycohemoglobin assays [Letter]. Clin Chem 1999;45:438–40.
41. Beutler E. Disorders of hemoglobin. In: Fauci A, Braunwald E, Isselbacher K, Wilson J, Martin J, Kasper D, Longo D, eds. Harrison's principles of internal medicine, 14th ed. New York: McGraw-Hill, 1996:645–52.
42. John WG, Braconnier F, Miedema K, Aulesa C, Piras G. Evaluation of the Menarini-Arkay HA 8140 hemoglobin A1c analyzer. Clin Chem 1997;43:968–75.
43. Carta M, Dall'Olio G, Soffiati G. Determination of HbA_{1c} in the presence of haemoglobin variants: comparison of three HPLC techniques. Eur J Clin Chem Biochem 1997;35:923–5.
44. Roberts WL, Chiasera JM, Ward-Cook K. Glycohemoglobin results in samples with hemoglobin C or S trait: a comparison of four test systems [Technical Brief]. Clin Chem 1999;45:906–9.
45. Blakney G, Higgins TN, Holmes DJ. Comparison of hemoglobin A_{1c} results by two different methods on patients with structural hemoglobin variants. Clin Biochem 1998;31:619–26.
46. Gibb I, Parnham A, Fonfrede M, Lecock F. Multicenter evaluation of Tosoh glycohemoglobin analyzer. Clin Chem 1999;45:1833–41.
47. Ohls RK, Christensen RD. Development of the hematopoietic system. In: Behrman RE, Kliegman R, Jenson HB, eds. Nelson's textbook of pediatrics, 16th ed. Philadelphia: WB Saunders, 2000:1456–60.
48. Forget BG, Pearson HA. Hemoglobin synthesis and the thalassemias. In: Handin R, LuxR, Stossel T, eds. Blood, principles and practice of hematology. Philadelphia: JB Lippincott, 1995:1525–90.
49. Cox T, Hess PP, Thompson GD, Levinson SS. Interference with glycated hemoglobin by hemoglobin F may be greater than is generally assumed. Am J Clin Pathol 1999;99:137–41.
50. Khuu HM, Robinson CA, Goolsby K, Hardy RW, Konrad RJ. Evaluation of a fully automated high-performance liquid chromatography assay for hemoglobin A_{1c}. Arch Pathol Lab Med 1999;123:763–7.
51. Puukka R, Puukka M. Effect of hemoglobin F on measurements of hemoglobin A_{1c} with physician's office analyzers [Technical Brief]. Clin Chem 1994;40:342–3.
52. King ME, Rifai N, Malekpour, A. Hemoglobin "Hope" interferes with measurement of glycated hemoglobin by ion-exchange chromatography and electrophoresis [Technical Brief]. Clin Chem 1984;30:1106–7.
53. Miyashita H, Hashimoto K, Mohri H, Ohokubo T, Harano T, Harano K, Imai K. Hb Kanagawa [α40(C5)Lys→Met]: a new α chain variant with an increased oxygen affinity. Hemoglobin 1992;16:1–10.
54. Elder GE, Lappin TR, Horne AB, Fairbanks VF, Jones RT, Winter PC, et al. Hemoglobin Old Dominion/Burton-upon-Trent, β143(H21)His→Tyr, codon 143 CAC→TAC—a variant with altered oxygen affinity that compromises measurement of glycated hemoglobin in diabetes mellitus: structure function, and DNA sequence. Mayo Clin Proc 1998;73:321–8.
55. Langdown JV, Davidson RJ, Williamson D. A new α chain variant, Hb Turniff [α99(G6)Lys→Glu]: the interference of abnormal hemoglobin in HbA_{1c} determination. Hemoglobin 1992;16:11–7.
56. Gunton JE, McElduff A. Heterozygous hemoglobin Hamadan affects HbA_{1c} assay. Diabetes Care 1999;22:177.
57. Ohba Y, Imai K, Kumada I, Ohsawa, A, Hiyaji T. Hb Moriguchi or $\alpha 2\beta 2$(FG4)His→Tyr substitution at the $\alpha 1$-$\beta 2$ interface. Hemoglobin 1989;13:367–76.
58. Wiener K, Roberts NB, Green BN. The effect of an unusual haemoglobin variant (β51Pro→His) on haemoglobin A_{1c} measurement. Ann Clin Biochem 1998;35:321–3.
59. Maezawa Y, Yamauchi M, Nakabayashi T, Toshima K, Ikeda, K, Toda G. A diabetic case of Hb Riyadh with a low HbA_{1c} value. Intern Med 1993;32:128–31.
60. Bon C, Revenant MC, Sotta C, Mailliavin A, Bannier E, Goujon R, et al. Multicenter evaluation of the Abbott glycosylated hemoglobin assay on the IMX. Ann Biol Clin 1996;54:151–7.
61. Metus P, Ruzzante N, Bonvicini P, Meneghetti M, Zaninotto M, Plebani M. Immunoturbidimetric assay of glycated hemoglobin. J Clin Lab Anal 1999;13:5–8.