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Narrative Review

Effect of Hemoglobin Variants on HbA1c Estimation: Analytical Challenges and Clinical Implications

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ABSTRACT

Background: Glycated haemoglobin (HbA1c) occupies an unchallenged position as the premier biomarker for the diagnosis and longitudinal monitoring of diabetes mellitus. Its clinical utility, however, depends entirely on the analytical fidelity of the measurement platform in use. Haemoglobin variants—encompassing both structural (qualitative) and quantitative abnormalities of the haemoglobin molecule—constitute a significant, yet frequently un recognised, source of analytical interference, capable of generating both falsely elevated and falsely depressed HbA1c values. Given that approximately 7% of the global population carries an abnormal haemoglobin gene, this interference represents a clinical concern of escalating global relevance.

Objective: This narrative review critically examines the biochemical mechanisms by which common haemoglobin variants—including HbS, HbC, HbE, HbD, and elevated fetal haemoglobin (HbF)—interfere with established HbA1c measurement methodologies, evaluates method-specific susceptibilities, and delineates the consequent clinical implications for diabetes diagnosis and glycaemic management. Practical laboratory recommendations for managing HbA1c testing in the presence of haemoglobin variants are also provided, with particular attention to the Indian clinical context.

Methods: A comprehensive, structured search of peer-reviewed literature was performed using PubMed, Medline, Cochrane Library, and ClinicalKey databases. Clinical biochemistry guidelines from the American Diabetes Association (ADA), International Federation of Clinical Chemistry (IFCC), and National Glycohemoglobin Standardization Program (NGSP) were systematically reviewed. Preference was accorded to articles published between 2014 and 2024, supplemented by foundational and landmark studies of enduring methodological relevance.

Results: Interference mechanisms vary by variant type and measurement platform. Ion-exchange high-performance liquid chromatography (IE-HPLC) and turbidimetric immunoassay are most susceptible, with co-elution of variant peaks and antibody epitope disruption being the dominant mechanisms, respectively. Capillary electrophoresis demonstrates superior discriminatory capacity for most common variants, while enzymatic assays exhibit the least susceptibility. False-low HbA1c predominates in patients with HbSS, HbCC, and HbE-beta-thalassaemia, attributable to haemolysis-mediated shortening of erythrocyte lifespan. HbD-Punjab may produce false elevation via co-elution in certain HPLC platforms. Alternative biomarkers—fructosamine and glycated albumin—offer validated monitoring surrogates in affected individuals.

Conclusion: Awareness of haemoglobin variant interference is indispensable for laboratorians and clinicians in high-prevalence regions. A systematic laboratory response—encompassing trained chromatogram review, instrument flag recognition, confirmatory variant analysis, alternative marker deployment, and transparent clinician communication—is essential to mitigate diagnostic misclassification and safeguard individual patient outcomes. This imperative is particularly acute in India, where the concurrent burden of haemoglobin variants and diabetes mellitus creates a large population at risk of HbA1c misinterpretation.

Keywords: *HbA1c; haemoglobin variants; HPLC interference; glycated haemoglobin; fructosamine; HbS; HbE; HbF; diabetes mellitus; capillary electrophoresis; analytical interference; NGSP; glycated albumin*

1. Introduction

Haemoglobin A1c (HbA1c) represents the fraction of haemoglobin that undergoes non-enzymatic glycation at the N-terminal valine residue of the beta-globin chain following sustained exposure to ambient plasma glucose concentrations. Since its clinical relevance was first characterised in the early 1970s through the pioneering work of Rahbar and subsequently extended by Koenig and colleagues, HbA1c has undergone a remarkable evolution—from an arcane biochemical curiosity to the single most widely deployed biomarker in the global management of diabetes mellitus. The American Diabetes Association (ADA) recommends an HbA1c threshold of ≥6.5% (≥48 mmol/mol) for the diagnosis of diabetes mellitus and a target of <7.0% (53 mmol/mol) for most non-pregnant adults with established disease.[1]

International standardisation of HbA1c measurement has been advanced through two complementary frameworks. The National Glycohemoglobin Standardization Program (NGSP) in the United States reports results as a percentage traceable to the landmark Diabetes Control and Complications Trial (DCCT) reference method. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference system, adopted more widely in Europe and internationally, expresses results in millimoles per mole (mmol/mol). The inter-conversion formula HbA1c (NGSP %) = 0.0915 × HbA1c (IFCC mmol/mol) + 2.15 enables arithmetic harmonisation across reporting systems, though analytical equivalence at the measurement level remains contingent upon the absence of interfering conditions.[2]

Despite the robustness of these standardisation frameworks, the clinical accuracy of HbA1c is fundamentally contingent upon the absence of conditions that alter haemoglobin structure, erythrocyte survival, or the glycation process itself. Haemoglobin variants—inherited structural abnormalities of the globin chains—present a particularly insidious challenge because they may interact with measurement platforms in ways that produce either spuriously elevated or falsely low HbA1c values without triggering obvious clinical suspicion at the point of care. Critically, the direction and magnitude of the analytical error depend not only on the specific variant but also on the assay platform in use—creating a context-dependent vulnerability that demands methodological literacy from both the laboratory scientist and the clinician.[3,4]

The global burden of haemoglobin variants is substantial. Approximately 7% of the world's population carries a structurally abnormal haemoglobin gene, with the highest concentrations in sub-Saharan Africa, the Indian subcontinent, Southeast Asia, and the Mediterranean basin.[5] In India, the epidemiological mosaic is particularly complex: HbE predominates in the northeastern states and West Bengal, with carrier frequencies reaching 25–50% in certain tribal communities; HbS is found across tribal populations in Odisha, Jharkhand, Chhattisgarh, and Maharashtra; HbD-Punjab is concentrated in the northwestern states; and beta-thalassaemia trait affects approximately 3–4% of the general population, with pockets of substantially higher prevalence in Sindhi, Punjabi, Bengali, and Gujarati communities.[6] With an estimated 77 million persons living with diabetes in India as of 2023—the second-largest diabetic population globally—the co-existence of these variant states with the diabetes epidemic creates a population of considerable size in whom HbA1c results may be systematically unreliable.[7]

This narrative review provides a comprehensive, practically oriented analysis of the mechanisms by which the most clinically relevant haemoglobin variants interfere with established HbA1c measurement methodologies, the clinical consequences of such interference, the utility of alternative glycaemic biomarkers, and a structured laboratory approach to managing these challenges. The review is directed at clinical biochemists, haematologists, diabetologists, and general physicians practising in regions where haemoglobin variants are prevalent.

2. Biochemistry and Principles of HbA1c Measurement

2.1 Mechanism of Haemoglobin Glycation

Glycation of haemoglobin is an irreversible, non-enzymatic, concentration-dependent chemical modification initiated by the nucleophilic attack of the epsilon-amino group of lysine residues or, of greater quantitative significance, the alpha-amino group of the N-terminal valine of the beta-globin chain on the carbonyl group of glucose. This reaction proceeds through a kinetically unstable intermediate—the Schiff base or aldimine—which undergoes an Amadori rearrangement via a 1,2-enaminol transition state to yield the stable ketoamine product quantified analytically as HbA1c.[8] The overall rate of glycation is directly proportional to the time-averaged ambient glucose concentration, rendering HbA1c an integrated measure of glycaemic exposure over the lifespan of the circulating erythrocyte.

Because erythrocyte lifespan averages 90–120 days under physiological conditions, HbA1c reflects glucose exposure over approximately the preceding three months, with a disproportionate contribution—estimated at approximately 50%—from the preceding four to six weeks due to the non-linear kinetics of glycation accumulation over an erythrocyte cohort that includes cells of all ages. This biochemical principle carries a central implication for analytical interference: any condition that truncates or extends erythrocyte survival, modifies the beta-globin N-terminal valine residue, or introduces a competing glycation substrate will predictably distort the HbA1c result, irrespective of the assay platform employed.[9]

The clinical relevance of this principle extends beyond haemoglobin variants per se. Haemolytic anaemias of any aetiology—including those induced by HbE-beta-thalassaemia, immune-mediated haemolysis, or glucose-6-phosphate dehydrogenase deficiency—shorten erythrocyte survival and generate false-low HbA1c values that do not reflect prevailing glycaemia. Conversely, iron deficiency anaemia, by prolonging erythrocyte survival through suppressed erythropoiesis and retention of older, more-glycated cells in circulation, may paradoxically elevate HbA1c independent of glycaemic status.

Renal failure complicates matters further through the accumulation of carbamylated haemoglobin, a chemically distinct haemoglobin derivative that may co-elute with HbA1c in chromatographic assays or cross-react with anti-HbA1c antibodies.[8]

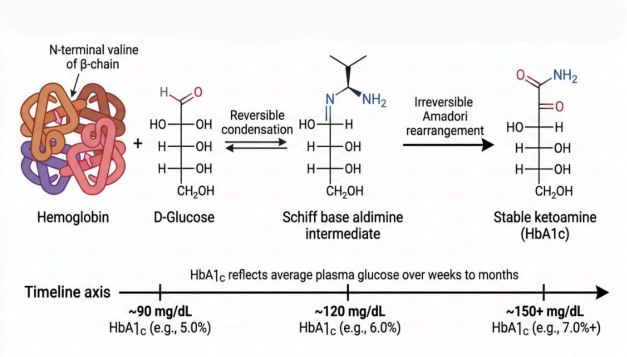


Figure-1: Mechanism of Non enzymatic glycation of Hemoglobin

2.2 Overview of Measurement Methods

2.2.1 Ion-Exchange High-Performance Liquid Chromatography (IE-HPLC)

IE-HPLC is universally regarded as the gold standard for HbA1c measurement and forms the methodological foundation of NGSP certification. The method exploits differential ionic interactions between haemoglobin fractions and a cation-exchange resin under a programmed salt gradient. HbA1c, by virtue of the glycation-mediated neutralisation of its N-terminal alpha-amino group, carries a reduced net positive charge relative to HbA0 at physiological pH, causing it to elute earlier in the chromatographic sequence. Modern platforms—including the Bio-Rad Variant II Turbo and the Tosoh G8—generate high-resolution chromatograms with discrete peaks for HbA1c, the minor HbA1a/b fractions, HbA0, and HbA2, each assigned to specific retention time windows calibrated against NGSP reference standards.[2]

The strength of IE-HPLC lies in its simultaneous generation of a complete chromatographic fingerprint of the haemoglobin profile—a feature that enables the identification of variant peaks and the triggering of automated alert flags. Its principal liability, however, is the reliance on charge-based separation: haemoglobin variants with amino acid substitutions that alter surface electrostatics may co-elute within or adjacent to the HbA1c retention time window, introducing positive or negative analytical bias. The direction and magnitude of this bias are both platform-dependent and variant-concentration-dependent, necessitating full chromatogram review rather than exclusive reliance on the numerical output.

2.2.2 Immunoassay Methods

Turbidimetric inhibition immunoassay (TINIA) and latex agglutination immunoassay platforms quantify HbA1c using antibodies directed against the glycated N-terminal hexapeptide of the beta-chain—typically the sequence Val-His-Leu-Thr-Pro-Val following glycation at valine. These methods are extensively deployed on automated general chemistry analysers and are valued for their analytical throughput, instrument accessibility, and reduced technical demands. Their central vulnerability lies in the precision and breadth of antibody epitope specificity: amino acid substitutions at or proximal to the recognition epitope—as occur with HbE (beta26 Glu→Lys) and certain other variants—may impair antibody binding affinity and generate falsely low results by reducing the apparent glycated fraction detected.[4]

2.2.3 Enzymatic Assays

Enzymatic HbA1c methods, exemplified by the Diazyme and Roche enzymatic platforms, employ a two-step biochemical procedure: proteolytic cleavage of the haemoglobin molecule by a specific protease liberates glycated amino acid residues, and the resultant fructosyl valine is then specifically oxidised by fructosyl amino acid oxidase (FAOX), generating hydrogen peroxide that is colorimetrically quantified via a peroxidase-coupled reaction system.[10] Because the method directly targets the fructosyl valine product rather than relying on the charge-based separation of intact haemoglobin chains or the structural recognition of an antibody epitope, enzymatic assays exhibit substantially reduced susceptibility to structural haemoglobin variant interference and have been certified by the NGSP for use in high-prevalence variant populations.

2.2.4 Capillary Electrophoresis

Capillary electrophoresis (CE) separates haemoglobin fractions by their differential migration velocity in a liquid-filled capillary under an applied electric field, exploiting differences in the charge-to-mass ratio of each fraction. Platforms such as the Sebia CAPILLARYS 3 TERA achieve high-resolution separation of HbA1c alongside all major variant fractions—including HbS, HbC, HbE, HbD, and HbF—with discrete, sharply defined electrophoretic zones.[11] A notable operational advantage of CE is its simultaneous haemoglobin variant identification capability during HbA1c measurement, minimising the risk of unrecognised analytical interference and enabling a single integrated workflow for both HbA1c quantification and variant screening in high-prevalence settings.

3. Haemoglobin Variants: Structural Basis and Epidemiology

3.1 Genetic and Molecular Basis

Haemoglobin variants arise from point mutations—most commonly missense substitutions—in the genes encoding the alpha-globin chains (HBA1, HBA2 on chromosome 16p13.3) or the beta-globin chain (HBB on chromosome 11p15.4). Single nucleotide substitutions result in amino acid changes that may alter the surface charge, electrophoretic mobility, chromatographic retention time, oxygen-binding affinity, solubility, or structural stability of the haemoglobin tetramer. The inheritance follows an autosomal recessive pattern; heterozygous carriers (trait states) are typically asymptomatic clinically but present with distinctive haematological and chromatographic profiles.[5]

Compound heterozygosity—wherein an individual inherits two different abnormal haemoglobin alleles—generates

clinically and analytically diverse phenotypes that are not reliably predicted from single-variant data. The haemoglobin profile of an HbSE compound heterozygote, for example, will reflect the combined interference effects of both the HbS and HbE substitutions, potentially compounding the analytical error on any single platform. Interactions between structural variants and thalassaemia mutations (gene deletions or splicing defects that reduce or abolish globin chain synthesis) further expand the spectrum of haemoglobin abnormalities relevant to HbA1c measurement.

3.2 Common Variants Relevant to HbA1c Measurement

HbS results from a beta6 Glu→Val substitution that confers hydrophobic properties on the beta-chain, predisposing the deoxygenated tetramer to polymerisation and erythrocyte sickling. The homozygous state (HbSS, sickle cell disease) is characterised by severe haemolytic anaemia with markedly shortened erythrocyte survival (40–70 days), creating one of the most profoundly HbA1c-distorting conditions encountered in clinical practice. The heterozygous state (HbAS, sickle cell trait) produces a less dramatic but analytically significant reduction in HbA1c, particularly when measured by IE-HPLC platforms that fail to adequately resolve HbS from HbA0 or the HbA1c peak.

HbC (beta6 Glu→Lys) and HbE (beta26 Glu→Lys) both introduce positively charged lysine residues at positions on the beta-chain that alter surface electrostatics and, in the case of HbE, the conformation of the N-terminal region relevant to immunoassay antibody recognition. HbD-Punjab (beta121 Glu→Gln) is electrophoretically neutral, migrating similarly to HbS on alkaline electrophoresis, yet eluting at a retention time that overlaps with the HbA1c window on certain HPLC platforms—producing false elevation rather than the false depression characteristic of HbS, HbC, and HbE.[3]

Fetal haemoglobin (HbF, alpha2gamma2) contains gamma rather than beta-globin chains and therefore lacks the N-terminal valine substrate required for conventional HbA1c glycation. Elevated HbF—encountered in hereditary persistence of fetal haemoglobin (HPFH), beta-thalassaemia major, certain sickle cell syndromes, and occasionally in hydroxyurea-treated patients—dilutes the HbA-containing erythrocyte fraction and thereby reduces the glycatable haemoglobin pool, generating false-low HbA1c proportional to the HbF percentage.

3.3 Epidemiology: The Indian Perspective

India harbours one of the world's most epidemiologically complex haemoglobin variant profiles. Beta-thalassaemia trait affects approximately 3–4% of the general population, rising substantially in certain communities—up to 10–15% in Sindhis and selected Punjabi subgroups. HbE is the most prevalent structural variant in eastern India, particularly in West Bengal, Assam, Manipur, Mizoram, and Meghalaya, where carrier frequencies in tribal communities may reach 25–50%.[6] HbD-Punjab is geographically concentrated in Punjab, Haryana, and Rajasthan. Sickle cell trait and disease occur at high frequency in tribal communities across Odisha, Jharkhand, Chhattisgarh, and Maharashtra—states that simultaneously carry a substantial and growing burden of type 2 diabetes.

Compound heterozygous states, particularly HbE-beta-thalassaemia, are clinically significant in eastern and northeastern India and constitute a major cause of non-transfusion-dependent thalassaemia. The interdigitation of these haemoglobin variant phenotypes with India's estimated 77 million persons with diabetes—a population expected to expand to 134 million by 2045—creates an analytic vulnerability of national clinical significance that has not yet been comprehensively addressed in laboratory policy or clinical guidelines in most Indian states.[7,12]

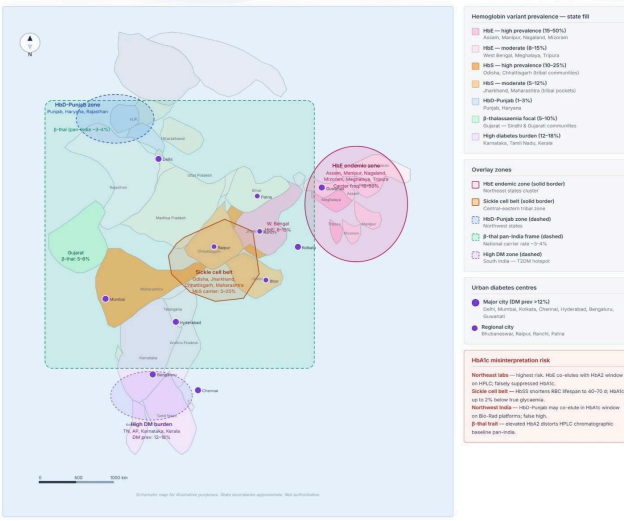


Figure:2 Geographic Distribution of Major Hemoglobin Variants and Estimated Diabetes Mellitus Prevalence Across Indian States and Regions

4. Analytical Interference: Mechanisms and Method-Specific Susceptibility

4.1 Mechanisms of Interference

4.1.1 Co-elution in Chromatographic Methods

In IE-HPLC, haemoglobin fractions are resolved on the basis of their net surface charge—a property intrinsically linked to the amino acid composition of the globin chain surface. Structural variants that introduce amino acid substitutions altering the surface charge of the beta-chain may shift the elution position of the corresponding haemoglobin fraction into proximity with the HbA1c retention time window, creating an artifactual elevation or depression in the calculated HbA1c percentage.[3] HbD-Punjab, which bears a glutamine substitution at a site that confers near-neutral surface electrostatics, elutes within the HbA1c retention window on the Bio-Rad Variant II platform, generating false elevation. The glycated forms of HbS (HbS1c) or HbC may elute adjacent to HbA0 or within the HbA1c region, complicating accurate peak area integration.

Co-elution interference is compounded by the integration algorithm employed by the instrument software, which partitions the total haemogram into predefined retention time zones. When a variant peak partially overlaps with the HbA1c window, the proportion of variant area included within the HbA1c zone depends on both the degree of peak separation

and the positioning of zone boundaries—parameters that differ between instruments and software versions. This platform-specificity underscores the importance of consulting published interference data for the specific combination of instrument model and identified variant before issuing a result.

Table 1. Comparison of HbA1c Measurement Methods and Their Susceptibility to Haemoglobin Variants

Method	Principle	HbS / HbC Interference	HbE / HbD Interference	Elevated HbF Effect	Other Interferences
IE-HPLC (Bio-Rad Variant II)	Cation-exchange chromatography; charge-based fractionation	Co-elution with HbA1c window; flags generated; false low or high depending on peak overlap	HbE co-elutes near HbA1c; HbD shifts retention time; false low predominates	False low; HbF lacks β-chain; separate HbF peak often visible	Carbamylated Hb (renal failure): false high; acetylated Hb: false low
IE-HPLC (Tosoh G8)	High-resolution cation-exchange chromatography; gradient elution	Better separation than Bio-Rad; HbS/HbC typically resolved but flags still raised	HbE may partially co-elute; automated flags raised; result unreliable	False low; separate HbF peak visible on chromatogram	Acetylated Hb (aspirin): false low result possible; carbamylated Hb flagged
Immunoassay (TINIA / Latex)	Antibody against glycated β-chain N-terminal hexapeptide	HbS/HbC: variable; depends on epitope preservation by antibody lot	HbE mutation at β26 near epitope may reduce antibody affinity; false low	Minimal—antibody targets β-chain only; HbF not a source of major error	Carbamylated Hb: may cause false elevation depending on kit formulation
Capillary Electrophoresis (Sebia CAPILLARYS)	Charge-to-mass migration in liquid-filled capillary under electric field	Excellent separation; HbS, HbC, and HbA1c resolved distinctly in most cases	HbE and HbD identified; HbA1c zone unaffected in the majority	HbF peak clearly delineated; low HbA1c value flagged automatically	Good resilience overall; variants flagged; recommended for high-prevalence settings
Enzymatic Assay (Diazyme / Roche)	Proteolysis of Hb → fructosyl amino acid oxidase oxidation of fructosyl valine → H_2O_2 colorimetry	Generally unaffected; variant Hb does not inhibit enzymatic substrate liberation	Minimal interference; method independent of charge or epitope topology	Minimal; glycated HbF counterpart measured if applicable	Acetylated or carbamylated Hb: minimal interference; NGSP-certified method

Abbreviations: IE-HPLC, ion-exchange high-performance liquid chromatography; TINIA, turbidimetric inhibition immunoassay; CE, capillary electrophoresis; FAOX, fructosyl amino acid oxidase; Hb, haemoglobin; H_2O_2, hydrogen peroxide.

4.1.2 Altered Erythrocyte Lifespan and Glycation Kinetics

Homozygous sickle cell disease (HbSS) and HbCC disease are characterised by chronic haemolytic anaemia with markedly abbreviated erythrocyte survival—40–70 days compared to the physiological norm of 90–120 days.[13] The consequent reduction in the cumulative exposure of circulating erythrocytes to ambient plasma glucose generates HbA1c values that systematically underestimate the prevailing glycaemia. This effect is most pronounced in HbSS, intermediate in HbSC compound heterozygotes, and demonstrably present—albeit of lesser magnitude—even in sickle cell trait (HbAS). The quantitative impact is method-independent: even an enzymatic assay that accurately measures the glycation of HbA in the sample will yield a falsely low result if the HbA-containing erythrocyte population has been exposed to plasma glucose for a substantially shorter duration than the physiological reference.

Iron deficiency anaemia represents the diametrically opposite erythrokinetic scenario: suppression of effective erythropoiesis with relative accumulation of older, more-glycated erythrocytes may generate falsely elevated HbA1c values independent of glycaemic status. Similarly, splenectomised patients retain aged erythrocytes that accumulate glycation, potentially contributing to HbA1c overestimation. These erythrokinetic influences operate as a physiological layer of interference beneath the analytical layer created by variant-specific method interactions, and both must be considered when evaluating an unexpected HbA1c result.

4.1.3 Antibody Specificity in Immunoassays

Immunoassay platforms for HbA1c rely on antibodies raised against the glycated N-terminal beta-chain hexapeptide. Amino acid substitutions at or proximal to this epitope can attenuate or abolish antibody binding affinity, introducing a negative bias into the measurement. The HbE substitution at beta26—two positions removed from the critical glycation site at beta1 valine—creates a conformational alteration in the N-terminal region that demonstrably impairs the recognition of certain anti-HbA1c antibodies, particularly those with narrower epitope specificity.[4] The magnitude of this effect varies between immunoassay kits from different manufacturers and even between reagent lot numbers from the same manufacturer—a source of variability that defies simple generalisation and demands ongoing vigilance in laboratories serving high-prevalence populations.

4.2 Method-Specific Susceptibility: Summary

IE-HPLC is the most frequently encountered method in clinical laboratories and exhibits vulnerability to all variants that alter surface charge, with co-elution being the primary mechanism. Instruments generate specific flags—including 'Variant Hb Detected,' 'Probable HbS,' 'Probable HbC,' and 'Result may be affected by Hb variant'—that serve as the critical first alert for laboratory intervention.[14] Capillary electrophoresis demonstrates superior performance in the presence of most common variants by virtue of its orthogonal separation mechanism, and is increasingly recommended as a primary or confirmatory method in regions of high haemoglobin variant prevalence.[11] Enzymatic assays are the most analytically robust against structural variant interference at the measurement level, though they remain subject to the erythrokinetic errors described above and are therefore not a complete solution in severely haemolytic states.

Table 2. Haemoglobin Variants and Characteristic Pattern of HbA1c Interference by Method

Hb Variant	Mutation	HPLC Effect	Immunoassay Effect	Enzymatic Effect	CE Effect
HbS (HbSS / HbAS)	β6 Glu→Val	False low; co-elution or short RBC lifespan dominant in HbSS	Variable; may be false low depending on antibody lot	Minimal	Separated; accurate
HbC (HbCC / HbAC)	β6 Glu→Lys	False low; altered peak morphology	Variable	Minimal	Resolved; accurate
HbD-Punjab (HbAD)	β121 Glu→Gln	Co-elution with HbA1c window possible on Bio-Rad platforms	Usually unaffected	Minimal	Usually resolved
HbE (HbAE / HbEE)	β26 Glu→Lys	Co-elutes near HbA1c; significant false low	Reduced antibody affinity; false low	Minimal	Mostly resolved; HbE peak identified
HbF (elevated)	Fetal γ-chain; $\alpha_2\gamma_2$ tetramer	Separate peak; dilutes HbA1c fraction	Not targeted (γ-chain); minimal direct effect	Minimal	HbF peak visible and quantified

Abbreviations: HPLC, high-performance liquid chromatography; CE, capillary electrophoresis; RBC, red blood cell.

4.3 False Elevation and False Depression: A Mechanistic Summary

False elevations of HbA1c arise through three primary mechanisms: (1) co-elution of an abnormal haemoglobin fraction—most notably HbD-Punjab—within the HbA1c retention time window on certain HPLC platforms; (2) prolonged erythrocyte lifespan in iron deficiency anaemia, splenectomy, or aplastic states that enrich the circulating pool with older, more-glycated cells; and (3) cross-reaction of carbamylated haemoglobin (prevalent in uraemic patients) with immunoassay antibodies or HPLC peak overlap in the HbA1c window.[9]

False decrements are more prevalent in clinical practice and arise from: (1) haemolysis-mediated shortening of erythrocyte lifespan in HbSS, HbCC, and HbE-beta-thalassaemia; (2) quantitative dilution of the HbA pool by elevated HbF, which lacks the glycatable beta-chain; (3) impaired antibody recognition of structural variants in immunoassay platforms; (4) suppression of the HbA1c chromatographic peak area by a large adjacent variant peak in HPLC; and (5) the presence of unstable haemoglobins, which are partially removed during sample preparation and may reduce the apparent HbA1c.[15]

5. Chromatographic Interpretation: A Practical Guide

5.1 Normal Chromatogram Architecture

A normal IE-HPLC chromatogram on the Bio-Rad Variant II Turbo platform displays a characteristic elution sequence: the minor HbA1a/b fractions elute first (approximately 0.7–0.9 min), followed by HbA1c (retention time 1.10–1.30 min), HbA0 (approximately 3.5–4.0 min), and HbA2 (approximately 4.5–5.5 min), the last-named constituting 2.2–3.3% of total haemoglobin in normal adults. Total haemoglobin is calculated from the integrated area beneath all peaks, and the HbA1c percentage is expressed relative to this total. The chromatogram should be free of unassigned peaks, with each fraction falling within calibrated reference windows and the peak morphology exhibiting smooth Gaussian profiles without shoulder artefacts.[14]

5.2 Recognition of Abnormal Chromatographic Patterns

The presence of a haemoglobin variant manifests on the chromatogram as: (1) an unassigned peak appearing at a retention time outside established reference windows; (2) a shift in the elution position of a nominally normal fraction; (3) an anomalously large or broad HbA2 fraction that may reflect co-elution of HbE or another variant; (4) an irregular or non-Gaussian peak morphology suggestive of peak overlap; or (5) a markedly reduced HbA0 area coupled with an increase in unidentified fractions, as seen in high-percentage variant states. An unassigned peak eluting between the HbA1c and HbA0 retention windows should immediately raise suspicion for a structural variant, and its area relative to HbA0 provides a preliminary estimate of the carrier state: 35–45% variant area is characteristic of heterozygosity, while >80% indicates homozygosity or compound heterozygosity.

HbE warrants particular attention, as it elutes at a retention time very close to that of HbA2 on several HPLC platforms, generating an apparently elevated HbA2 area—a pattern that is frequently misidentified as beta-thalassaemia trait by laboratories unfamiliar with this interference. The distinction carries significant clinical consequences: the co-elution of HbE with HbA2 will inflate the measured HbA2, and may simultaneously displace area from the HbA1c peak if the HbE peak margin encroaches on the HbA1c window, generating a combined false HbA2 elevation and false-low HbA1c.

5.3 Peak Area Integration and Analytic Significance

In HPLC chromatography, peak area—rather than peak height—is the quantitatively definitive parameter for fraction calculation, integrating the totality of analyte eluting within the chromatographic band. When a variant peak partially merges with the HbA1c retention window, area-integration algorithms may incorporate the variant contribution within the HbA1c fraction, inflating the reported percentage. Conversely, a narrow, sharply separated variant peak that falls entirely outside the HbA1c window exerts minimal direct influence on peak area calculation. Laboratory scientists must therefore review the full graphical chromatogram—not merely the instrument-generated numeric report—in any case where variant interference is suspected or flagged.[10]

5.4 Instrument Flags: Recognition and Response

Contemporary IE-HPLC instruments incorporate automated flagging algorithms that alert the operator to aberrant chromatographic patterns. Common flag classes include: 'Variant Hb Detected,' 'Probable HbS/HbC/HbE,' 'Elevated HbF,' 'Abnormal Chromatogram,' and 'Result may be

unreliable.' It is imperative that laboratory staff are trained to interpret these alerts as actionable clinical signals rather than system artefacts to be overridden. Each flagged result should trigger a structured laboratory response: full chromatogram review, correlation with available clinical and haematological data, and—where indicated—referral for confirmatory haemoglobin variant analysis. Failure to act on HPLC flags represents a preventable and potentially medicolegally significant source of patient harm.

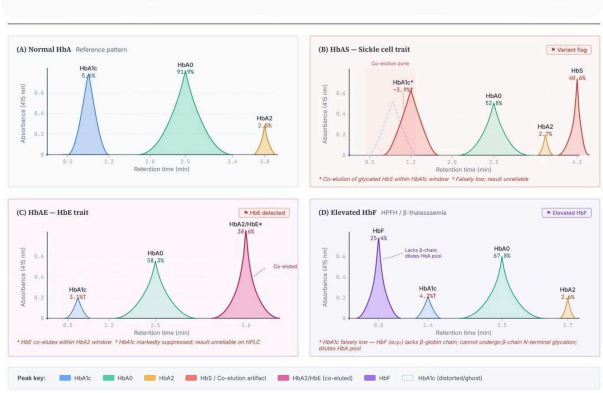


Figure:3 Representative Ion-Exchange High-Performance Liquid Chromatography Chromatograms Illustrating Normal and Hemoglobin Variant Patterns

6. Clinical Implications of Erroneous HbA1c Values

6.1 Discordance Between HbA1c and Plasma Glucose

The most practically impactful manifestation of haemoglobin variant interference in clinical practice is a clinically meaningful discordance between the HbA1c value and the patient's ambient plasma glucose measurements or self-monitored blood glucose log. A patient with well-documented hyperglycaemia—fasting glucose consistently above 126 mg/dL, postprandial values exceeding 200 mg/dL—and an HbA1c that appears unexpectedly low should prompt systematic consideration of variant interference. The converse pattern—an elevated HbA1c in a patient with apparently controlled blood glucose—should equally prompt interrogation of the haemoglobin profile.[15]

The clinical threshold for concern is a discordance between measured HbA1c and estimated average glucose (eAG)—derived from the formula eAG (mg/dL) = 28.7 × HbA1c (%) − 46.7—that exceeds the expected analytical and biological variation. When this discrepancy exceeds approximately 15–20 mg/dL, variant interference should be a leading diagnostic consideration, particularly in patients from ethnically high-prevalence populations.

6.2 Diagnostic Misclassification

The diagnostic consequences of unrecognised haemoglobin variant interference range from incorrect diabetes diagnosis or exclusion to potentially life-altering therapeutic errors. A falsely low HbA1c in a patient with HbSS disease and poorly controlled type 2 diabetes may lead the clinician to accept a spurious appearance of adequate glycaemic control, withhold intensification of therapy, and thereby expose the patient to chronic hyperglycaemia with acceleration of microvascular complications—retinopathy, nephropathy, and peripheral neuropathy—on a background already compromised by sickle cell end-organ damage. [16]

In the diagnostic context, reliance on a falsely low HbA1c to exclude diabetes in a carrier of a haemolytic variant—without complementary oral glucose tolerance testing or fasting plasma glucose measurement—may critically delay diagnosis and initiation of protective lifestyle and pharmacological interventions. Conversely, false elevation—as may occur with HbD-Punjab on Bio-Rad HPLC platforms—could precipitate inappropriate escalation of therapy, with attendant risks of hypoglycaemia, particularly in older patients or those with impaired hypoglycaemic awareness.

6.3 Illustrative Case Scenarios

Case A: A 38-year-old tribal woman from Odisha with known HbSS disease presents with polyuria and polydipsia of two months' duration. Fasting plasma glucose is 178 mg/dL and the two-hour postprandial glucose is 310 mg/dL. HbA1c measured by IE-HPLC on the Bio-Rad Variant II platform is reported as 5.8%. The HPLC chromatogram shows an unassigned peak at a retention time consistent with HbS, with associated instrument flags. The discordance between plasma glucose values and the HbA1c result is attributable to both shortened erythrocyte lifespan and chromatographic co-elution artefacts in HbSS disease. Glycated albumin measurement yields 22.4%, consistent with significant chronic hyperglycaemia, leading to appropriate initiation of antihyperglycaemic therapy.[17]

Case B: A 55-year-old Punjabi man recently diagnosed with type 2 diabetes has an HbA1c of 9.2% measured on the Bio-Rad Variant II platform, despite self-monitored blood glucose readings averaging 115–130 mg/dL. A review of the HPLC chromatogram reveals an unassigned peak at a retention time consistent with HbD-Punjab, with co-elution into the HbA1c window. Haemoglobin electrophoresis confirms HbAD heterozygosity. Repeat HbA1c measurement by enzymatic assay yields 7.1%, concordant with the patient's continuous glucose monitoring data. The false elevation of 2.1% percentage points, if unrecognised, would have prompted unnecessary intensification of therapy.[18]

7. Alternative Glycaemic Markers in the Presence of Haemoglobin Variants

7.1 Fructosamine

Fructosamine quantifies the total pool of glycated serum proteins, of which albumin—with its high circulating concentration and relatively uniform glycation kinetics—constitutes the dominant fraction. Measurement employs a colorimetric nitroblue tetrazolium (NBT) reduction assay, in which fructosamine at alkaline pH reduces NBT in proportion to the total glycated protein concentration.[19] Because fructosamine is determined by the glycation of circulating plasma proteins rather than haemoglobin, it is unaffected by erythrocyte lifespan or haemoglobin structure. Reflecting the half-life of serum albumin (approximately 20 days), fructosamine provides a glycaemic retrospective window of approximately two to three weeks. The established reference range in non-diabetic adults is approximately 200–285 µmol/L.

The principal limitations of fructosamine as an alternative glycaemic marker are its dependence on albumin concentration and turnover, and its susceptibility to interference from conditions that alter plasma protein metabolism. Nephrotic syndrome, hepatic cirrhosis, protein-losing enteropathy, acute illness with a negative acute-phase protein response, and thyroid dysfunction—all of which alter albumin levels—will confound fructosamine interpretation. Fructosamine is not approved by the ADA as a diagnostic criterion for diabetes mellitus and serves exclusively as a monitoring surrogate in contexts where HbA1c is analytically unreliable.

7.2 Glycated Albumin

Glycated albumin (GA) is a more specific measure of albumin glycation, expressed as a percentage of total albumin concentration rather than as an absolute glycated protein quantity. This proportional expression corrects for inter-individual variation in total albumin concentration, improving comparability across patients. GA is quantified by enzymatic assay (Lucica GA-L method) or affinity chromatography, and reflects glycaemia over the preceding two to four weeks.[20] GA has been extensively validated in Japan, where it has attained widespread clinical adoption as a glycaemic monitoring tool, and is increasingly recognised in international endocrinology literature as a reliable alternative to HbA1c in patients with haemolytic anaemia, end-stage renal disease on dialysis—where accelerated albumin glycation occurs—and liver disease.

Reference intervals for GA in non-diabetic adults are typically 11–16%, varying marginally with the specific assay platform. GA is not yet endorsed by major international guidelines for the diagnostic classification of diabetes mellitus, precluding its use as a standalone diagnostic criterion. Its role remains that of a validated monitoring surrogate in patients where HbA1c accuracy is compromised.

Table 3. Alternative Glycaemic Markers: Characteristics and Clinical Decision Framework

Alternative Marker	Biological Basis	Reporting Window	Indications & Limitations
Fructosamine	Total glycated serum proteins (predominantly albumin); NBT colorimetric reduction assay	2–3 weeks (albumin t½ ≈ 20 days)	Useful in haemolytic states; affected by hypoalbuminaemia, nephrotic syndrome, liver disease, thyroid dysfunction; not approved for diabetes diagnosis by ADA
Glycated Albumin (GA)	Specific albumin glycation expressed as % of total albumin; enzymatic or affinity chromatography method	2–4 weeks	Widely validated in Japan; reliable in dialysis, hepatic disease, haemolytic anaemia; reference range 11–16%; increasingly recognised internationally
1,5-Anhydroglucitol (1,5-AG)	Competitive renal tubular reabsorption of 1,5-AG with glucose; reflects short-term postprandial glucose excursions	1–2 weeks	Highly sensitive to acute hyperglycaemia; affected by renal impairment; not widely available in India; primarily a monitoring tool

Abbreviations: ADA, American Diabetes Association; NBT, nitroblue tetrazolium; 1,5-AG, 1,5-anhydroglucitol.

7.3 Clinical Decision Framework for Alternative Marker Selection

Alternative glycaemic markers should be preferentially deployed when: (1) a clinically significant discordance between HbA1c and blood glucose monitoring data is identified; (2) IE-HPLC flags or an abnormal chromatogram indicate the presence of a haemoglobin variant; (3) the patient is known to carry a haemolytic haemoglobin variant or thalassaemia syndrome; (4) conditions independently altering erythrocyte lifespan are present (haemolytic anaemia of any aetiology, iron deficiency, renal failure, or post-splenectomy state); or (5) pregnancy is confirmed, wherein physiological changes in haemoglobin dynamics and the possibility of elevated HbF may compromise HbA1c accuracy.[21]

8. Laboratory Approach and Practical Recommendations

8.1 When to Suspect a Haemoglobin Variant

A structured index of suspicion for haemoglobin variant interference should be maintained in clinical laboratory practice. Suspicion is warranted when: (1) the HPLC chromatogram displays unassigned peaks, unusual peak morphology, retention time shifts, or automated variant flags; (2) the HbA1c result is discordant with serial plasma glucose measurements or continuous glucose monitoring data; (3) the reported HbA1c is implausibly low (<3.5%) or implausibly high (>15%) without an evident clinical explanation; (4) the patient belongs to an ethnic group with a known high prevalence of haemoglobin variants; or (5) the HbA1c has changed dramatically between serial measurements performed on different instrument platforms without an intervening clinical event.[22]

Triggers for Haemoglobin Variant Suspicion in HbA1c Testing

- IE-HPLC variant flag generated by the instrument software
- Clinically significant discordance between HbA1c and plasma glucose / CGM data
- Implausibly low HbA1c (<3.5%) or high (>15%) without clinical explanation
- Patient from an ethnic group with high haemoglobin variant prevalence (e.g., northeastern India, tribal belt, Punjabi communities)
- Known haematological diagnosis of haemolytic anaemia, thalassaemia, or haemoglobinopathy
- Established renal failure (risk of carbamylated haemoglobin interference)

8.2 Confirmatory Variant Analysis

When haemoglobin variant interference is suspected, confirmatory characterisation should include: (1) dedicated haemoglobin variant analysis using a purpose-built HPLC platform (e.g., Bio-Rad D-10 or the Variant II haemoglobin variant programme), which reports the full haemoglobin fraction profile with variant identification; (2) high-resolution capillary electrophoresis (Sebia CAPILLARYS 3 TERA) for independent orthogonal separation and quantification; or (3) conventional alkaline and acid haemoglobin electrophoresis as a complementary confirmatory method in resource-limited settings.[11] These studies collectively quantify HbA, HbA2, HbF, and variant fractions with appropriate identification. Molecular confirmation by PCR-RFLP or next-generation sequencing of globin genes is reserved for diagnostic uncertainty or when genetic counselling is being pursued.

8.3 Reporting Strategies and Clinician Communication

When a haemoglobin variant is identified that compromises HbA1c accuracy, the laboratory report must explicitly and unambiguously state: (1) the variant identity and genotype; (2) the nature and likely direction of the interference; (3) whether the HbA1c result issued is reliable, flagged as unreliable, or accompanied by an alternative assay result; (4) the alternative marker employed and its result; and (5) a recommendation for future glycaemic monitoring strategy.[23] A direct telephone call or electronic alert to the requesting clinician is advisable when interference has already influenced clinical decisions or when the erroneous result may prompt immediate therapeutic action. This communication obligation is both a professional responsibility and a medicolegal safeguard.

9. Relevance in Resource-Limited Settings: The Indian Perspective

The challenge of haemoglobin variant interference with HbA1c measurement is particularly acute in the Indian healthcare landscape, where the high prevalence of both haemoglobin variants and diabetes mellitus intersects with significant disparities in laboratory infrastructure, clinical biochemistry expertise, and access to confirmatory testing. In many peripheral and district-level laboratories, HbA1c is measured using a single platform—often a basic turbidimetric immunoassay or an entry-level HPLC system—without locally available access to confirmatory haemoglobin variant analysis. Point-of-care HbA1c devices, increasingly employed in community diabetes screening programmes and primary care outreach, vary widely in their susceptibility to variant interference and are generally not equipped to flag abnormal haemoglobin profiles.[24]

The laboratory workforce in peripheral settings may lack specific training in IE-HPLC chromatogram interpretation or familiarity with the clinical significance of instrument flags—a gap that is not addressed by current undergraduate or postgraduate medical laboratory technology curricula in most Indian states. The affordability and availability of alternative glycaemic markers such as glycated albumin remain restricted outside urban tertiary care institutions, further limiting the operational toolkit available to clinicians managing diabetes in patients with known haemoglobin variants.

A pragmatic and proportionate response to these challenges demands multi-level interventions: mandatory integration of haemoglobin variant screening into diabetes diagnostic pathways in high-prevalence districts; targeted training of laboratory personnel in chromatogram interpretation and flag-response protocols; adoption of enzymatic HbA1c assays or capillary electrophoresis as primary platforms in regions of high haemoglobin variant prevalence; and the development of national consensus guidelines—by the Indian Council of Medical Research, the Association of Clinical Biochemists of India, and the Research Society for the Study of Diabetes in India—specifically addressing HbA1c interpretation in the context of Indian haemoglobin variant epidemiology.

10. Future Perspectives

The field of HbA1c measurement is undergoing a progressive engineering evolution directed at minimising susceptibility to haemoglobin variant interference. Next-generation enzymatic assay platforms incorporating highly specific recombinant fructosyl valine oxidases with broadened substrate specificity offer the prospect of accurate HbA1c quantification across virtually the entire spectrum of structural haemoglobin variants, provided erythrocyte lifespan is not significantly compromised—the one interference mechanism that no analytical improvement can rectify without addressing the underlying biology.[10]

In the immunoassay domain, manufacturers are progressively reformulating antibody epitopes—deploying bispecific or multivalent anti-HbA1c antibodies that maintain recognition across a wider range of beta-chain variants near the glycated hexapeptide region—and reformulating assay buffers to mitigate the effects of carbamylated haemoglobin cross-reactivity. These engineering advances, while iterative rather than transformative, are progressively narrowing the range of conditions in which immunoassay HbA1c values are analytically unreliable.

Mass spectrometry—specifically electrospray ionisation mass spectrometry (ESI-MS) of intact haemoglobin—enables direct measurement of the mass difference between the glycated and non-glycated forms of the beta-globin chain, circumventing all of the analytical challenges inherent in charge-based separation, antibody recognition, and enzymatic substrate accessibility.[25] The IFCC has endorsed a mass spectrometry-based reference method for HbA1c as the highest-order metrological anchor of the entire measurement hierarchy, underscoring the long-term trajectory of the field towards analyte-specific, variant-independent measurement. The clinical translation of mass spectrometry from reference laboratory to routine deployment remains constrained by instrument cost and technical complexity, but these barriers are progressively diminishing.Integration of molecular genomic data into laboratory information systems offers another forward-looking pathway: targeted NGS panels for globin gene variants are now clinically available and declining in cost, and their integration with electronic health records could enable the laboratory information system to automatically select the analytically

appropriate HbA1c method—or flag the result and recommend alternative markers—when a known interfering variant is identified in a patient's genomic record.

11. Conclusion

Haemoglobin variants constitute a clinically important and systemically underappreciated source of HbA1c measurement error, with direct and potentially serious consequences for the diagnosis and management of diabetes mellitus. The mechanisms of interference—co-elution in IE-HPLC, antibody epitope disruption in immunoassays, and erythrocyte lifespan truncation in haemolytic states—are individually well characterised, yet their combined clinical impact remains inadequately recognised at the point of care in most healthcare settings, including in India. The laboratory scientist occupies a central and irreplaceable role in detecting, flagging, and communicating these interferences, particularly in populations where the co-prevalence of haemoglobin variants and diabetes is high.

A systematic laboratory approach—encompassing trained chromatogram review, instrument flag-triggered investigation, selection of appropriate alternative glycaemic biomarkers, confirmatory variant analysis, and transparent and timely clinician communication—constitutes the operational foundation of accurate glycaemic assessment in affected patients. As measurement technology evolves toward variant-independent enzymatic and mass spectrometric methods, the field is positioned to progressively eliminate the analytical dimension of this problem. In the interim, awareness, education, and methodological vigilance remain indispensable tools.

Key Takeaways for Laboratory Practice

- Always review the full HPLC chromatogram, not merely the numerical HbA1c output; unassigned peaks, retention time shifts, and automated flags must be investigated.
- HbE, HbS, HbC, HbD, and elevated HbF each interfere through distinct mechanisms; no single method is immune to all variants under all conditions.
- Enzymatic HbA1c assays and capillary electrophoresis generally outperform IE-HPLC and immunoassays in the presence of common haemoglobin variants.
- Significant discordance between HbA1c and plasma glucose data should prompt systematic investigation for variant interference before a clinical conclusion is drawn.
- Fructosamine and glycated albumin are validated alternative monitoring markers; neither is approved for the diagnostic classification of diabetes mellitus.
- In India's haemoglobin variant-prevalent regions, laboratorians should integrate variant awareness into HbA1c reporting workflows as a routine practice.
- Proactive direct communication with the requesting clinician when HbA1c is unreliable is a professional and medicolegal obligation of the laboratory.
- All HbA1c measurement methods used for clinical reporting must be NGSP-certified and IFCC-traceable.

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REFERENCES

1. American Diabetes Association Professional Practice Committee. Standards of Care in Diabetes—2024. Diabetes Care. 2024;47(Suppl 1):S1–S321. doi:10.2337/dc24-SINT

2. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). IFCC Reference Method for Measurement of HbA1c in Human Blood. Clin Chem Lab Med. 2021;59(1):2–13. doi:10.1515/cclm-2020-0328
3. Little RR, Roberts WL. A review of variant hemoglobins interfering with hemoglobin A1c measurement. J Diabetes Sci Technol. 2009;3(3):446–51. doi:10.1177/193229680900300307
4. Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. Clin Chem. 2001;47(2):153–63. doi:10.1093/clinchem/47.2.153
5. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008;86(6):480–7. doi:10.2471/BLT.06.036673
6. Narang A, Das S, Sharma M. Hemoglobin E disease and its implications in HbA1c measurement: experience from a tertiary care laboratory. J Lab Physicians. 2021;13(1):37–42. doi:10.1055/s-0041-1723083
7. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of type 2 diabetes: Indian scenario. Indian J Med Res. 2007;125(3):217–30.
8. Weykamp C. HbA1c: a review of analytical and clinical aspects. Ann Lab Med. 2013;33(6):393–400. doi:10.3343/alm.2013.33.6.393
9. Radin MS. Pitfalls in hemoglobin A1c measurement: when results may be misleading. J Gen Intern Med. 2014;29(2):388–94. doi:10.1007/s11606-013-2595-x
10. Higgins T. HbA1c—an analyte of increasing importance. Clin Biochem. 2012;45(13–14):1038–45. doi: 10.1016/j.clinbiochem.2012.03.027
11. Keren DF, Hedstrom D, Gulbranson R, Ou CN, Bak R. Comparison of Sebia Capillarys capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies. Am J Clin Pathol. 2008;130(5):824–31. doi:10.1309/AJCPQXD2MJJMVZYL
12. Aggarwal P, Jain S. Impact of hemoglobin E (HbE) on HbA1c estimation: analytical challenges. Indian J Clin Biochem. 2020;35(2):202–8. doi:10.1007/s12291-019-0844-6
13. Panzer S, Kronik G, Lechner K. Glycosylated hemoglobins (GHb): an index of red cell survival. Blood. 1982;59(5):1111–4.
14. National Glycohemoglobin Standardization Program (NGSP). Factors that interfere with HbA1c test results [Internet]. 2023 [cited 2024 Jan 10]. Available from: http://www.ngsp.org/factors.asp
15. Rohlfing CL, Hanson S, Funnell MM, Little RR. Hemoglobin A1c and estimated average glucose in patients with sickle cell trait. Clin Chim Acta. 2012;413(17–18):1394–5. doi:10.1016/j.cca.2012.04.019
16. Quigley A, Mason J, Bhatt H. Haemoglobin variants and HbA1c: a major source of error in diabetes management. J Diabetes Metab. 2019;10:845. doi:10.35248/2155-6156.19.10.845
17. Shrivastava M, Navaid S, Chabbra A, Garg S. Analytical interference by hemoglobin variants in HbA1c estimation by immunoturbidimetric method: a prospective study. Ann Pathol Lab Med. 2017;4(3):A265–70.
18. Bhutani G, Gupta G, Bhatt D, Singh G. Study of glycated hemoglobin in patients with haemoglobin D disease. J Assoc Physicians India. 2019;67(4):48–50.
19. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care. 2023;46(10):e151–99. doi:10.2337/dc23-0179
20. Shrivastava S, Navaid S, Chhabra A. Correlation of HbA1c with fructosamine in diabetes patients with hemoglobin variants: a prospective study. Int J Diabetes Dev Ctries. 2018;38(4):452–7. doi:10.1007/s13410-018-0637-y
21. Mosca A, Paleari R, Dalfra MG, Di Cianni G, Cuccuru I, Pellegrini G, et al. Reference intervals for hemoglobin A1c in pregnant women: data from an Italian multicenter study. Clin Chem. 2006;52(6):1138–43. doi:10.1373/clinchem.2005.064899
22. John WG; on behalf of UK HbA1c Standardisation Group. Glycated haemoglobin analysis. Ann Clin Biochem. 2009;46(Pt 1):31–5. doi:10.1258/acb.2008.008103
23. Pani LN, Korenda L, Meigs JB, Driver C, Chamany S, Fox CS, et al. Effect of aging on A1C levels in individuals without diabetes: evidence from the Framingham Offspring Study and NHANES 2001–2004. Diabetes Care. 2008;31(10):1991–6. doi:10.2337/dc08-0577
24. Jain S, Bhatt S, Kishore S, Singh R. Prevalence of hemoglobin variants and their interference with HbA1c estimation in a tertiary care hospital of India. Natl J Lab Med. 2022;11(2):BO04–08. doi:10.7860/NJLM/2022/53012.2600
25. Weykamp C, John WG, Mosca A, Hoshino T, Little R, Jeppsson JO, et al. The IFCC Reference Measurement System for HbA1c: a 6-year progress report. Clin Chem. 2008;54(2):240–8. doi:10.1373/clinchem.2007.097402
26. Selvin E, Rawlings AM, Bergenstal RM, Coresh J, Brancati FL. No racial differences in the association of glycated hemoglobin with kidney disease and cardiovascular outcomes. Diabetes Care. 2013;36(10):2995–3001. doi:10.2337/dc12-2715
27. Carson AP, Reynolds K, Fonseca VA, Muntner P. Comparison of A1C and fasting glucose criteria to diagnose diabetes among U.S. adults. Diabetes Care. 2010;33(1):95–7. doi:10.2337/dc09-1227
28. Khanna P, Torigoe T, Thangam R. Enzymatic HbA1c assay in the presence of common hemoglobin variants: clinical validation. Clin Chem Lab Med. 2020;58(6):943–52. doi:10.1515/cclm-2019-1023
29. Schnedl WJ, Trinker M, Lipp RW, Rabold-Schertler E, Krause R. Falsely low glycated haemoglobin in a patient with haemolytic anaemia due to hereditary spherocytosis. Diabetes Nutr Metab. 2004;17(1):49–51.
30. Sumita NM, Andriolo A, Neto AM, et al. Revisao das recomendacoes brasileiras para HbA1c. Rev Bras Med. 2009;66(1):12–18.