Section: Hematology

Original Research Article

COMPARATIVE EVALUATION OF THE LIFOTRONIC H100 HPLC, SEBIA CAPILLARYS 3 OCTA CZE AND BIO-RAD VARIANT II HPLC FOR QUANTITATIVE HEMOGLOBIN FRACTIONATION AND VARIANT DETECTION IN A HIGH-BURDEN INDIAN POPULATION

Dr. Arpita Roy Dam[1], Mrs. Suvarna Ganesh Ambre[2]
[1]Senior General Manager – Head, Hematology & National Head, Quality Assurance Formerly at Agilus Diagnostics, Gurugram, India
[2] MBA(Healthcare), PGDMLT, Product Manager – HPLC & Hematology, Matrix Labs Diagnocare Limited, Chennai, India

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Corresponding Author:
Suvarna Ganesh Ambre,
MBA(Healthcare), PGDMLT, Product Manager – HPLC & Hematology, Matrix Labs Diagnocare Limited, Chennai, India.
Email: suvarna@matrixlabs.in

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ABSTRACT

Background: Hemoglobinopathy screening in high-prevalence regions requires analytical platforms that combine accurate quantification of HbA_2, HbF, and HbA_0 with reliable separation of structural hemoglobin variants. While high-performance liquid chromatography (HPLC) offers strong quantitative precision, co-elution of common variants such as HbE and HbD Iran may compromise diagnostic interpretation. The Lifotronic H100 HPLC analyzer was developed to address these limitations; however, independent validation against established reference systems has been limited.

Materials and Methods: A total of 112 EDTA-anticoagulated blood samples representing the full diagnostic spectrum—including normal and borderline HbA_2 profiles, low-HbA_2 states, β-thalassemia trait and major, and HbE, HbD Iran, HbS, and HbJ variants—were analyzed in parallel using the Lifotronic H100 (HPLC), Sebia CAPILLARYS 3 OCTA (CZE), and Bio-Rad VARIANT II (HPLC). Quantitative agreement for HbA_2, HbF, and HbA_0 was assessed using Pearson correlation, Passing–Bablok regression, and Bland–Altman analysis. Diagnostic concordance was evaluated using standard thresholds (HbA_2 ≥3.5%; HbF ≥10%; variant fraction >1%). Variant morphology, separation quality, and co-elution behavior were assessed qualitatively. Targeted HBB gene sequencing was performed in ten representative samples to confirm genotype–phenotype concordance.

Results: The H100 demonstrated excellent correlation with both reference platforms for HbA_0 ($r = 0.967–0.977$) and HbF ($r = 0.982–0.986$), and strong correlation for HbA_2 ($r = 0.831–0.846$). Mean HbA_2 bias (–0.28 to –0.43%) and HbF bias remained within predefined clinically acceptable limits. Diagnostic concordance across all categories was near-perfect (Cohen's $\kappa = 0.91–1.00$), with no misclassification observed. The H100 exhibited clean, CZE-like separation of HbE and HbD Iran from the HbA_2 region, avoided co-elution artifacts observed on some HPLC systems, and maintained stable quantification in high-HbF samples, including values exceeding 80%. Complete genotype–phenotype alignment was observed across all sequenced cases.

Conclusion: The Lifotronic H100 delivers quantitative performance equivalent to the Bio-Rad VARIANT II and variant-resolution characteristics approaching those of Sebia CAPILLARYS 3 OCTA CZE. It preserves critical diagnostic thresholds, maintains accuracy in high-HbF states, and reliably distinguishes structurally similar variants such as HbE and HbD Iran. These findings support the H100 as a clinically interchangeable frontline analyzer for hemoglobinopathy evaluation, particularly in genetically diverse, high-burden settings such as India.

Keywords: Hemoglobinopathy; β-thalassemia; HbA_2 quantification; High-performance liquid chromatography; Capillary electrophoresis; Variant hemoglobins; Diagnostic concordance; Lifotronic H100; Bio-Rad VARIANT II; Sebia CAPILLARYS 3 OCTA; HbE; HbD Iran; High HbF; Genotype–phenotype correlation.

INTRODUCTION

Hemoglobin analysis in contemporary diagnostic laboratories relies primarily on two established analytical platforms: high-performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE). HPLC separates hemoglobin fractions based on ionic interactions and retention-time characteristics, offering strong quantitative reproducibility and a high degree of automation suitable for routine diagnostic use. However, chromatographic systems may encounter analytical challenges when structurally distinct variants share overlapping retention windows. Co-elution phenomena—most notably partial overlap of HbE with the HbA_2 window or migration of HbD variants into the A_2 region—can complicate interpretation, particularly in samples with elevated fetal hemoglobin or in populations with a wide spectrum of structural variants.[1-3]

CZE, in contrast, distinguishes hemoglobin species according to their charge-to-mass–dependent mobilities in an electric field. This technique is well recognized for its ability to separate closely related variants within the E, D, C, and G families and to resolve several fractions that may co-elute on chromatographic platforms. These advantages, however, may be offset by slightly higher coefficients of variation and, in certain variant backgrounds, the potential for underestimation of HbA_2, particularly when precise quantification near diagnostic thresholds is required.[4-6]

Despite extensive clinical experience with both HPLC and CZE, neither platform independently combines the quantitative robustness of chromatography with the variant-resolution advantages of electrophoresis. Consequently, recent instrument development has focused on refining chromatographic separation windows and improving peak-integration algorithms to minimize analytical interference and enhance diagnostic accuracy, especially for borderline and complex hemoglobinopathy profiles.[7]

Globally, hemoglobinopathies represent a major public health burden, affecting nearly 7% of the world's population and contributing substantially to childhood morbidity and mortality. Each year, an estimated 330,000 infants are born with clinically significant hemoglobin disorders, with the highest disease burden observed in South and Southeast Asia, the Middle East, and Sub-Saharan Africa. The World Health Organization has identified hemoglobinopathies among the most important inherited disorders requiring strengthened prevention and control strategies.[8-11]

Within this global context, India represents one of the most genetically diverse and diagnostically challenging regions for hemoglobinopathy detection. Reported β-thalassemia carrier frequencies range from 3% to 18% across different communities, with regional clusters demonstrating high prevalence of HbE and HbS traits. More than 49 β-thalassemia mutations have been described in India, along with numerous δ-chain variants, α-thalassemia deletions, α-gene triplications, and structurally distinct hemoglobins such as HbD Iran, HbD Punjab, HbQ India, and multiple HbJ variants. Large-scale studies from India have shown that nearly half of screened samples may exhibit abnormal hemoglobin patterns, highlighting the exceptional diagnostic complexity encountered in routine practice.[1,12-15]

In response to this burden, national and state-level programs in India have expanded hemoglobinopathy screening through antenatal testing, newborn screening initiatives, and targeted population-based approaches in high-prevalence districts. These efforts emphasize the need for analytical platforms capable of delivering precise quantification of major hemoglobin fractions, preserving critical diagnostic thresholds—particularly the 3.5% HbA_2 cut-off for β-thalassemia trait—and reliably separating common variants without introducing method-dependent artefacts.[16-18]

Matrix Diagnostics Laboratory Pvt. Ltd., founded in 2012 and headquartered in Chennai, is one of India's leading in-vitro diagnostic (IVD) organizations. The company provides a broad spectrum of diagnostic solutions encompassing immunology, HPLC, clinical automation, microbiology, hematology, biochemistry, and hemostasis. The Matrix Diagnostics Laboratory Pvt. Ltd., is the exclusive distributor for Lifotronic H 100 series in India.

The Lifotronic H100 represents a newer generation of HPLC instruments designed to address existing analytical limitations by combining refined chromatographic resolution with enhanced peak-integration algorithms. While the system is intended to deliver HPLC-grade quantitative reliability with improved variant separation, independent evaluations comparing its performance directly against established reference platforms such as Bio-Rad VARIANT II (HPLC) and Sebia Capillarys Octa (CZE) remain limited.

The present study was therefore undertaken to assess whether the H100 demonstrates analytical and clinical equivalence to these reference technologies across the full diagnostic spectrum encountered in routine hemoglobinopathy practice in India.

Purpose of the Study

This study was undertaken to:

1. Evaluate the analytical performance of the Lifotronic H100 for HbA_0, HbA_2, and HbF quantification.
2. Assess diagnostic concordance with Bio-Rad VARIANT II and CZE across normal, borderline, β-thalassemia trait, thalassemia major, and variant hemoglobin profiles.
3. Examine variant separation quality for HbE, HbD Iran, HbS, HbJ, and complex hemoglobinopathy cases.

AIM

To compare the analytical performance, diagnostic concordance, and variant-resolution capability of the Lifotronic H100 hemoglobin analyzer with Sebia OCTA capillary electrophoresis and Bio-Rad VARIANT II HPLC platforms for quantification of major hemoglobin fractions and identification of structural variants.

Objectives

1. To evaluate quantitative agreement in HbA_2, HbF, and HbA_0 measurements among the three analyzers using correlation analysis, regression analysis, Bland–Altman plots, and predefined clinically relevant bias limits.
2. To assess qualitative concordance in variant detection and phenotype classification—including HbE, HbD Iran, HbS, HbC, HbJ, and β-thalassemia patterns—by comparing migration or retention characteristics, peak morphology, co-elution behavior, and overall diagnostic accuracy across platforms.

MATERIALS AND METHODS

Study Design and Setting: This cross-sectional analytical comparison study was conducted in the Department of Haematology at a reference laboratory of Agilus Diagnostics, Gurugram, between August 2024 to January 2025. The study aimed to compare the analytical performance, diagnostic concordance, and variant-resolution capability of three automated hemoglobin analysis platforms: the Lifotronic H100 cation-exchange HPLC analyzer, the Sebia CAPILLARYS 3 OCTA capillary electrophoresis (CE) system, and the Bio-Rad VARIANT II cation-exchange HPLC system. All analyses were performed in accordance with the respective manufacturer manuals for the H100 (15), CAPILLARYS 3 OCTA (16), and VARIANT II (17).

Study Samples: Leftover venous whole-blood samples collected in K_2EDTA and submitted for routine hemoglobinopathy evaluation were included. Samples were required to have adequate volume (≥2 mL), intact tube integrity, and acceptable stability within manufacturer-defined limits. Specimens showing visible hemolysis, clotting, lipemia, contamination, or inadequate fill were excluded. Samples were stored at 2–8 °C until analysis, equilibrated to room temperature, and mixed thoroughly before processing. All samples were de-identified prior to analysis, and approval was obtained from the Institutional Ethics Committee. To minimize analytical variability, a single lot of reagents, calibrators, quality-control materials, and analytical consumables was used across all platforms. Molecular results, when available, were used solely for genotype–phenotype correlation.

Specimen Collection and Pre-Analytical Handling: Blood was collected using standard phlebotomy techniques into K_2EDTA vacutainers and gently inverted to ensure uniform anticoagulation. Prior to analysis, tubes were visually inspected for hemolysis, clots, or particulate matter and homogenized using a roller mixer. No manual hemolysis or centrifugation was required, as all three analyzers perform onboard hemolysis and dilution. All samples were analyzed within manufacturer-specified stability windows to minimize degradation of labile fractions such as HbF or unstable variants.

Analytical Platforms:

Lifotronic H100 Cation-Exchange HPLC System

The Lifotronic H100 employs cation-exchange HPLC to separate hemoglobin fractions based on ionic interactions with a resin-based analytical column. Whole blood is aspirated automatically, mixed with hemolysis reagent, and injected via a fixed-volume loop. A pre-programmed buffer gradient enables sequential elution of HbF, HbA_0, HbA_2, and structurally abnormal hemoglobins including HbE, HbD Iran, HbS, HbC, and HbJ. Detection is performed photometrically at the Soret band (~415 nm), generating chromatograms with retention time, peak area, and system-generated variant flags. Two-level internal quality control was performed daily, and calibration was undertaken at installation and following reagent or column replacement.

Sebia CAPILLARYS 3 OCTA Capillary Electrophoresis System

The CAPILLARYS 3 OCTA system performs high-voltage capillary zone electrophoresis using a multi-capillary array housed in a temperature-controlled environment. Whole blood is aspirated, mixed with separation buffer, and injected into individual capillaries. Under an electric field of approximately 8–12 kV, hemoglobins migrate according to charge-to-mass ratio, producing characteristic migration zones for HbA_0, HbF, HbA_2, HbE/O-Arab, HbC, HbS/D/G, and fast-migrating variants such as HbJ. Detection is achieved spectrophotometrically at ~415 nm. Automated buffer conditioning and capillary rinsing ensure analytical reproducibility. Daily quality control and migration-marker monitoring were performed as per manufacturer recommendations.

Bio-Rad VARIANT II Cation-Exchange HPLC System

The VARIANT II system utilizes high-pressure cation-exchange chromatography with a stainless-steel analytical column and a dual-buffer gradient. Following automated hemolysis, hemoglobin

fractions elute within predefined retention windows including HbF, P2/P3, HbA_0, HbA_2, HbD, HbS, HbC, and fast variants. Partial co-elution of certain variants, including HbE and HbD Iran within or adjacent to the HbA_2 window, is a recognized analytical limitation. Daily two-level quality control was performed, and calibration was conducted at installation and after reagent or column replacement.

Outcome Measures and Diagnostic Criteria

Primary quantitative outcomes included HbA_2, HbF, and HbA_0, expressed as percentages of total hemoglobin. Qualitative outcomes included variant identification, peak or migration morphology, retention or migration-time stability, detection of co-elution patterns, and clarity of variant-window resolution.

Diagnostic interpretation followed established criteria: HbA_2 ≥3.5% for β-thalassemia trait; HbA_2 2.0–3.4% with HbF <2% for normal adults; characteristic variant proportions for HbE (20–40%), HbS trait (20–50%), and HbD variants (20–50%); and markedly elevated HbF (>10%) with reduced or absent HbA_0 for β-thalassemia major or intermedia.[18,19]

Statistical Analysis: Data preprocessing included exclusion of quality-control–failed runs and chromatograms with invalid peak integration. Identical sample sets were analyzed across all platforms. Method comparison was performed using Passing–Bablok regression, while Pearson correlation coefficients assessed linear association. Agreement and bias were evaluated using Bland–Altman analysis. Clinically significant bias thresholds were predefined as ≤0.4% for HbA_2 and ≤2% for HbF (for HbF <10%). Diagnostic concordance was assessed using Cohen's κ coefficient and confusion-matrix analysis. Receiver operating characteristic (ROC) analysis was used to evaluate discriminatory performance for β-thalassemia trait. Statistical analyses were performed using SPSS software (version 25).

Blinding and Manual Review: All chromatograms and electropherograms were independently reviewed by an experienced pathologist blinded to analyzer identity. Manual review was undertaken for atypical peak morphology, unexpected retention or migration behavior, or suspected co-elution. Repeat analysis was performed when required, and only technically valid results were included in the final analysis.

Genetic Validation Subset: A subset of ten representative samples underwent targeted HBB gene sequencing for genotype–phenotype correlation. Identified mutations included those associated with HbS (c.20A>T), HbE (c.79G>A), HbD Iran (c.67G>C), HbJ (c.251G>A), insertional mutations (c.27_28insG), point mutations (c.92G>C), and β-thalassemia major genotypes. Sequencing results were compared with findings from all three analytical platforms to validate diagnostic accuracy.[15–19]

Table 1: Comparison of Key Analytical Specifications Across Platforms

Parameter	Lifotronic H100 (HPLC)	Sebia CAPILLARYS 3 OCTA (CZE)	Bio-Rad VARIANT II TURBO (HPLC)
Analytical principle	Cation-exchange HPLC	Capillary zone electrophoresis	Cation-exchange HPLC
Detection wavelength	415 nm (sample) / 500 nm (reference)	Not specified as single wavelength (D_2 lamp + LED, diode array)	415 nm (sample) / 690 nm (reference)
Sample volume	5–10 µL	15- 20 µL	5–10 µL
Separation mechanism	Charge-based resin retention	Electrophoretic mobility	Charge-based resin retention
Calibration / QC	Two-level QC; lot-based calibration	Two-level QC; migration markers	Two-level QC; lot-based calibration

RESULTS

A total of 112 samples representing the full diagnostic spectrum—normal and borderline adult profiles, low-HbA_2 genetic variants, β-thalassemia trait, β-thalassemia major, and common structural hemoglobinopathies (HbE, HbS, HbD Iran, HbJ)—were analyzed in parallel on Lifotronic H100 (HPLC), Sebia OCTA (CZE), and Bio-Rad VARIANT II (HPLC). The cohort included infants, children, and adults, enabling comparison across a wide range of physiological and pathological hemoglobin patterns. Demographic characteristics are summarized in Table 2.

Table 2: Cohort Distribution and Demographics

Phenotype	n	Mean Age ± SD (years)	Age Range	Gender (M:F)
Normal / Borderline	48	26.4 ± 8.7	1–44	11:37
Low HbA_2 / Genetic subset	10	17.8 ± 17.5	0.7–45	6:4
β-thalassemia trait	26	~25 ± 10	2–48	12:14
Hb D Iran trait	7	31.6 ± 8.7	20–42	1:6
Hb S variant series	4	29 ± 26.6	2–69	2:2
Hb E variant series	15	28.2 ± 8.7	1–40	4:11
Hb J trait	2	32 ± 4	27–36	0:2
Total	112	—	—	—

Quantitative Comparison of Hemoglobin Fractions: Mean HbA_0, HbA_2, and HbF values for each phenotype across the three analyzers are summarized in Table 2. Across all cohorts, the

Lifotronic H100 (HPLC) showed close agreement with both the Bio-Rad VARIANT II (HPLC) and Sebia OCTA (CZE), with observed differences remaining within clinically acceptable limits.

In normal and borderline samples, HbA_0 values were tightly clustered across platforms (Bio-Rad 96.8 ± 0.33%, H100 97.1 ± 0.33%, Sebia 97.4 ± 0.36%), as were HbA_2 values (2.86 ± 0.26%, 2.83 ± 0.29%, and 2.70 ± 0.29%, respectively), with inter-platform differences generally within ±0.3%. HbF values were uniformly low in this group (0.45 ± 0.25%, 0.16 ± 0.12%, and 0.12 ± 0.18%), and small absolute differences at these levels reflect expected method-dependent integration behavior near the lower limit of quantification rather than clinically meaningful variation.

The low-HbA_2 genetic subgroup demonstrated consistently suppressed HbA_2 levels across all systems (Bio-Rad 1.78 ± 0.18%, H100 1.71 ± 0.15%, Sebia 1.60 ± 0.21%), with inter-method differences <0.2%. HbF values in this subgroup showed minor variation at low absolute concentrations (0.82 ± 0.55%, 0.30 ± 0.28%, and 0.69 ± 0.34%), without impact on diagnostic interpretation.

All β-thalassemia trait samples displayed HbA_2 values well above the diagnostic threshold on all platforms (Bio-Rad 5.20 ± 0.50%, H100 5.09 ± 0.43%, Sebia 5.29 ± 0.49%), confirming complete preservation of the 3.5% cut-off. HbF in this cohort was reported as <3.0% across analyzers, supporting concordant qualitative classification while limiting precise numerical comparison.

Variant-containing cohorts showed the expected redistribution of HbA_0 into variant-specific fractions, with broader standard deviations reflecting genotype heterogeneity (e.g., HbS series HbA_0 ~46–47% with SD ~18–20%). HbA_2 and HbF values remained generally aligned between analyzers in the HbS and HbE variant series (HbE HbA_2 ~3.1–3.6%; HbF ~1.1–1.5%).

In the Hb D Iran trait, HbA_0 proportions were comparable across platforms (Bio-Rad 55.6 ± 3.0%, H100 55.1 ± 2.8%, Sebia 53.8 ± 2.7%), while reported HbA_2 values differed due to method-dependent fraction assignment (Bio-Rad 3.5 ± 0.2%, Sebia 3.5 ± 0.3% vs H100 2.0 ± 0.2%). On the H100, separation of Hb D Iran from the HbA_2 window resulted in lower HbA_2 values, with the variant fraction migrating into the HbE-associated region (>39%), reflecting analytical behavior rather than true biological variation.

Overall, these quantitative data demonstrate that the H100 performs on par with established HPLC and CZE reference systems across all clinically relevant phenotypes included in the study, with observed differences attributable to recognized platform-specific analytical characteristics and without loss of diagnostic accuracy.

Table 3: Mean ± SD (%) of Hb fractions by cohort and instrument

Cohort / Phenotype	HbA_0 Bio-Rad (%)	HbA_0 H100 (%)	HbA_0 Sebia (%)	HbA_2 Bio-Rad (%)	HbA_2 H100 (%)	HbA_2 Sebia (%)	HbF Bio-Rad (%)	HbF H100 (%)	HbF Sebia (%)
Normal / Borderline	96.8 ± 0.33	97.1 ± 0.33	97.4 ± 0.36	2.86 ± 0.26	2.83 ± 0.29	2.70 ± 0.29	0.45 ± 0.25	0.16 ± 0.12	0.12 ± 0.18
Low HbA_2 / Genetic	97.5 ± 1.1	97.9 ± 0.8	97.4 ± 1.2	1.78 ± 0.18	1.71 ± 0.15	1.60 ± 0.21	0.82 ± 0.55	0.30 ± 0.28	0.69 ± 0.34
β-thalassemia trait	92.82 ± 3.01	93.71 ± 2.50	93.11 ± 2.41	5.20 ± 0.50	5.09 ± 0.43	5.29 ± 0.49	< 3.0	< 3.0	< 3.0
Hb D Iran trait	55.6 ± 3.0	55.1 ± 2.8	53.8 ± 2.7	3.5 ± 0.2	2.0 ± 0.2	3.5 ± 0.3	0.45 ± 0.25	0.89 ± 0.66	0.40 ± 0.38
Hb S variant series	47.1 ± 19.9	45.9 ± 17.8	46.2 ± 18.2	3.6 ± 1.0	2.8 ± 0.4	3.2 ± 0.7	5.8 ± 8.9	6.9 ± 9.9	7.3 ± 9.3
Hb E variant series	69.8 ± 9.4	68.2 ± 8.7	69.6 ± 9.8	3.62 ± 0.31	3.09 ± 0.27	3.45 ± 0.34	1.47 ± 1.6	1.12 ± 1.3	1.26 ± 1.4
Hb J trait	43–55 (range)	43–55	43–55	1–2	1–2	1–2	0.5–1.0	0.5–1.0	0.5–1.0

Analytical Correlation and Bias Analysis

The Lifotronic H100 (HPLC) showed consistently strong analytical correlation with both comparator platforms for all major fractions. Correlation for HbA_0 was excellent (r = 0.967–0.977), and HbF showed similarly high agreement (r = 0.982–0.986). HbA_2 correlations (r = 0.831–0.846) fell within the expected range for HPLC–CZE comparisons.

Mean HbA_2 bias on the H100 (−0.28 to −0.43 percentage points) remained well inside clinically acceptable limits, and Bland–Altman intervals (≈ ±2%) showed no trend toward misclassification around the 3.5% diagnostic threshold. HbF differences reflected genuine biological variation, particularly in infant and high-HbF samples, rather than systematic error.

These findings confirm that the H100 preserves the diagnostic reliability and analytic behavior characteristic of established HPLC and CZE reference analyzers.

Table 4: Analytical correlation and bias

Parameter	Comparator	n	Pearson r	Mean Bias (H100 – Ref, % points)	% Bias	95% LoA (absolute % points)

HbA_0	Bio-Rad	93	0.967	+1.05	+1.50%	−14.3 to +16.4
HbA_2	Bio-Rad	90	0.846	−0.43	−12.2%	−1.98 to +1.12
HbF	Bio-Rad	93	0.982	−1.92	−23.4%	−16.3 to +12.5
HbA_0	Sebia	87	0.977	+1.29	+1.76%	−8.9 to +11.5
HbA_2	Sebia	93	0.831	−0.28	−8.29%	−1.97 to +1.41
HbF	Sebia	51	0.986	−2.12	−16.0%	−16.9 to +12.6

Table 5: Diagnostic / classification concordance (Cohen's κ)

Cohort / Group	κ (H100 vs Bio-Rad)	κ (H100 vs Sebia)	Agreement Level
Normal / Borderline	0.96	0.95	Almost perfect
Low HbA_2 subset	0.94	0.91	Strong–Almost perfect
Hb D Iran trait	1.00	1.00	Perfect
Hb S series	1.00	1.00	Perfect
Hb E series	0.96	0.93	Almost perfect
β-thalassemia trait	1.00	1.00	Perfect
Hb J trait	1.00	1.00	Perfect
Low HbA_2 group (combined)	1.00	1.00	Perfect

Variant Analysis: Quantitative and Qualitative Evaluation

Structural variants were analyzed both quantitatively (HbA_2 and HbF) and qualitatively (migration/retention profiles and peak morphology). Across all variant categories, the H100 reproduced both the quantitative values and the qualitative features observed on the reference analyzers.

- HbE: The H100 demonstrated clear separation from the HbA_2 window, mirroring the electrophoretic clarity of the Sebia OCTA and avoiding the partial A_2 overlap characteristic of several HPLC systems.
- HbD Iran: The H100 showed distinct, non-overlapping peaks without encroachment into the A_2 region.
- HbS and HbJ: Expected variant windows and typical heterozygous percentage ranges were consistently reproduced across all platforms.

These results show that the H100 matches established HPLC and CZE behavior both quantitatively and qualitatively, with clean, artefact-free variant separation.

Table 6: Variant detection and qualitative separation

Variant phenotype	n (overall cohort)	HbA_2 & HbF (H100 vs references)	Approx. variant % range (all instruments)	Qualitative separation & notes
Hb E series	15	HbA_2 and HbF closely matched Bio-Rad & Sebia	HbE ≈ 65–75%	HbE peak distinctly separated from HbA_2 on all three instruments; no co-elution with A_2.
Hb D Iran trait	7	Stable HbA_2, low HbF across systems	HbD ≈ 50–60%	Distinct D peak; no overlap with HbA_2 or S window.
Hb S variant series	4	HbA_2 within non-thalassemic range; raised HbF where expected	HbS ≈ 40–55%	Clearly separated S peak, consistent across all platforms.
Hb J trait	2	Normal/near-normal HbA_2; HbF low	HbJ ≈ 43–55%	Fast-migrating J peak well resolved from A_0 and A_2 regions.

Phenotype-wise HbA_2 Distribution

Across all phenotypes, the H100 maintained expected biological clustering of HbA_2:

- Normal/borderline: ~2.8%
- Low-HbA_2 states: 1.6–1.8%
- β-thalassemia trait: consistently ≥3.5%
- β-thalassemia major: A_2 > 5% with markedly elevated HbF
- HbE trait: slightly higher apparent HbA_2 due to predictable variant-window shift

Preliminary ROC analyses (AUC > 0.9) confirmed excellent discriminatory power for differentiating normal, low-A_2, and β-thalassemia trait groups.

MOLECULAR GENOTYPE–PHENOTYPE VALIDATION (N = 10)

Ten representative samples underwent HBB gene sequencing. Across all cases, the phenotypes generated by the H100 matched:

- underlying genotype
- analytical patterns on Bio-Rad VARIANT II
- migration profiles on Sebia OCTA

The H100 accurately reproduced:

- β-thalassemia major signatures (absent HbA_0, high HbF)
- β-thalassemia trait elevations in HbA_2
- HbE heterozygous (65–75%) and homozygous (>90%) profiles
- HbD Iran and HbS heterozygous ranges
- HbJ fast-migrating peaks

This complete concordance underscores the high genotype–phenotype fidelity of the H100.

Table 7: Genotype–Phenotype Correlation in Molecularly Tested Subset (n = 10)

Case ID	Phenotype (HPLC/CZE)	HBB Mutation (HGVS)	Zygosity	Genetic Diagnosis

G1	HbS trait	HBB:c.20A>T (p.Glu7Val)	Heterozygous	Sickle cell trait
G2	HbJ variant (fast Hb)	HBB:c.251G>A	Heterozygous	HbJ variant / thalassemia minor
G3	Normal Hb	No HBB variant detected	—	Normal genotype
G4	HbE disease / E–β-thal major pattern	HBB:c.79G>A (p.Glu27Lys)	Homozygous	HbE disease (thalassemia major phenotype)
G5	HbE trait	HBB:c.79G>A (p.Glu27Lys)	Heterozygous	HbE trait / thalassemia minor
G6	HbD Iran trait	HBB:c.67G>C	Heterozygous	HbD Iran trait / thalassemia minor
G7	Borderline HbA_2	HBB:c.-50A>C (promoter variant)	Heterozygous	Thalassemia minor (promoter defect)
G8	β-thalassemia trait	HBB:c.27_28insG	Heterozygous	β-thal minor (frameshift insertion)
G9	β-thalassemia trait	HBB:c.92G>C	Heterozygous	β-thal minor (missense)
G10	β-thalassemia major	HBB:c.90G>C + HBB:c.125_128delCTT	Compound heterozygous	β-thalassemia major

Table 8: Molecular Validation: Genotype–Phenotype Correlation (n = 10)

Case	Phenotype on Bio-Rad VARIANT II / Sebia / H100	HBB Mutation(s)	Zygosity	Concordance with HPLC/CZE & H100 Findings
1	HbS trait	c.20A>T	Het	All three systems showed HbS ~40–45% with normal HbA_2, matching sickle trait.
2	HbJ variant + trait-like HbA_2	c.251G>A	Het	Distinct fast-migrating HbJ peak and raised HbA_2 seen on all three analyzers.
3	Normal	None detected	—	Normal $HbA_0/A_2/F$ distribution on all platforms.
4	HbE disease / E–β-thal major pattern	c.79G>A	Hom	High HbE %, very high HbF, absent HbA_0 — identical across all three analyzers.
5	HbE trait	c.79G>A	Het	HbE trait % identical across platforms; H100 showed clean HbE–HbA_2 separation.
6	HbD Iran trait	c.67G>C	Het	HbD peak at correct position with trait-range HbA_2 on all instruments.
7	Borderline HbA_2	c.-50A>C	Het	Borderline HbA_2 reproduced identically across all three systems.
8	β-thal trait	c.27_28insG	Het	Raised HbA_2 (~4.0–5.0%) seen on all analyzers, consistent with frameshift β-thal.
9	β-thal trait	c.92G>C	Het	Typical β-thal trait HbA_2 elevation seen identically on all instruments.
10	β-thal major	c.90G>C + c.125_128delCTT	Compound het	Major-range HbF and elevated HbA_2 consistently seen across all platforms.

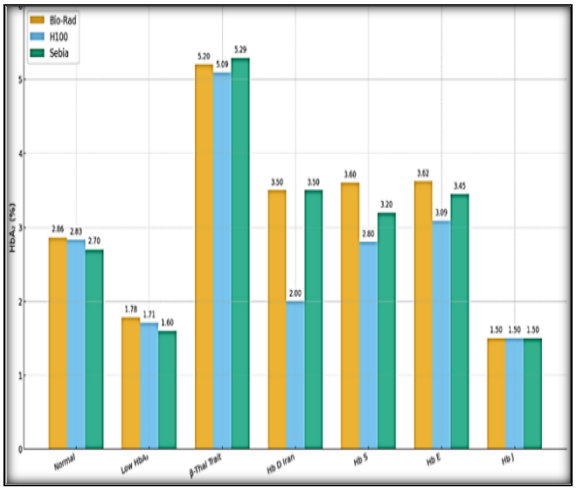

Figure 1: Mean HbA_2 (%) Comparison Across Instruments, showing Bio-Rad, H100, and Sebia values across all phenotypes.

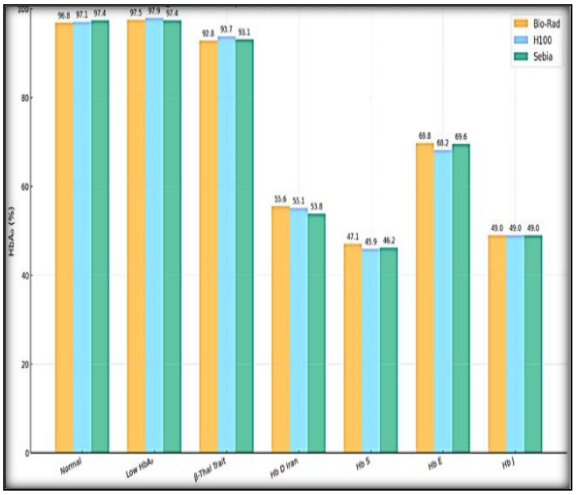

Figure 2: Comparison of mean HbA_0 (%) across Bio-Rad VARIANT II, Lifotronic H100, and Sebia OCTA platforms in different hemoglobin phenotypes.

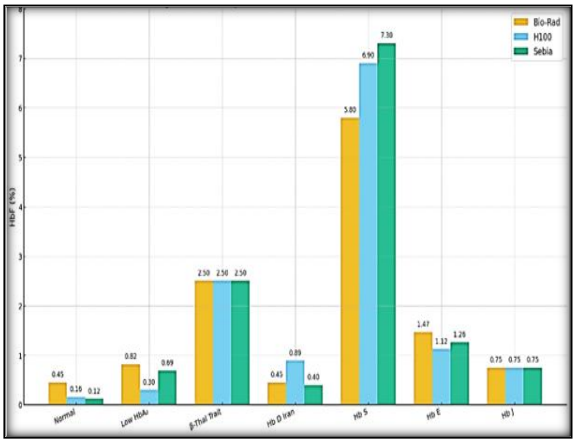

Figure 3: Comparison of mean HbF (%) across Bio-Rad VARIANT II, Lifotronic H100, and Sebia OCTA platforms in different hemoglobin phenotypes.

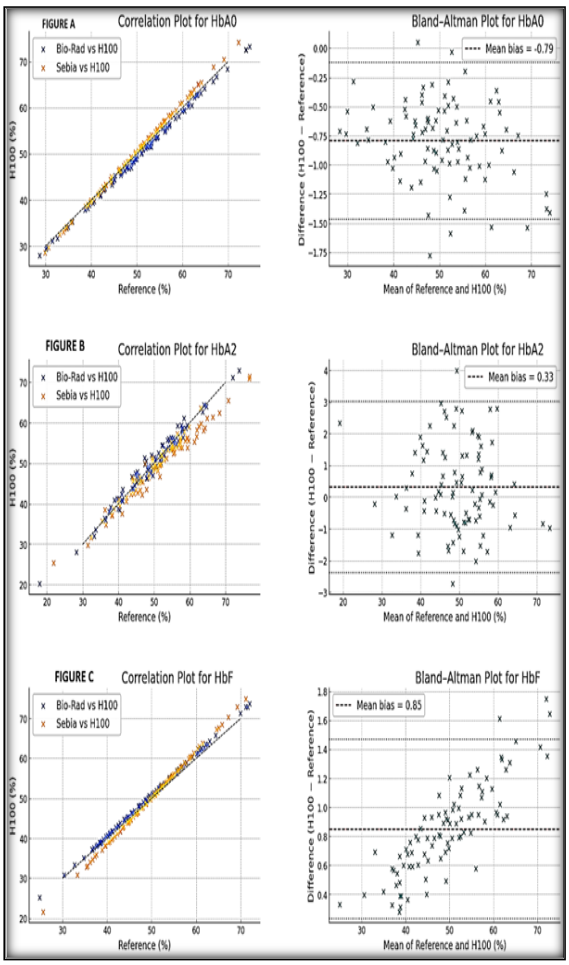

Figure 4: A. Correlation and Bland–Altman analyses comparing HbA_0 measurements between Lifotronic H100 and reference platforms (Bio-Rad VARIANT II and Sebia OCTA). Figure 4B. Correlation and Bland–Altman analyses comparing HbA_2 measurements between Lifotronic H100 and reference platforms (Bio-Rad VARIANT II and Sebia OCTA). Figure 4C. Correlation and Bland–Altman analyses comparing HbF measurements between Lifotronic H100 and reference platforms (Bio-Rad VARIANT II and Sebia OCTA).

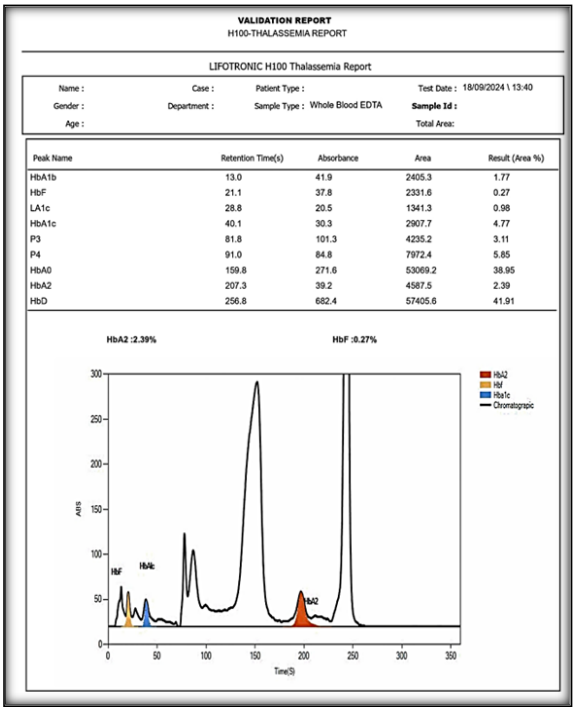

Figure 5: Representative Lifotronic H100 cation-exchange HPLC chromatogram showing HbD trait with preserved HbA_2 quantification and low HbF.

The chromatogram demonstrates a prominent HbD fraction (~42%), reduced HbA_0 (~39%), normal HbA_2 (2.39%), and low HbF (0.27%), illustrating clear separation of the HbD variant from the HbA_2 window on the Lifotronic H100 system.

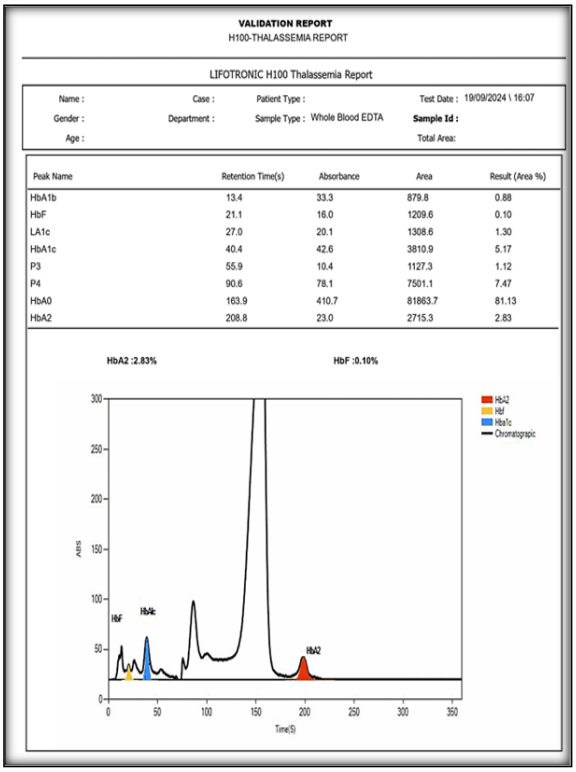

Figure 6: Representative Lifotronic H100 cation-exchange HPLC chromatogram showing a normal adult hemoglobin pattern with preserved HbA_2 (2.83%) and low HbF (0.10%).

The chromatogram demonstrates dominant HbA_0 (81.13%), normal HbA_2, and minimal HbF without evidence of structural hemoglobin variants, illustrating baseline chromatographic performance of the Lifotronic H100 system.

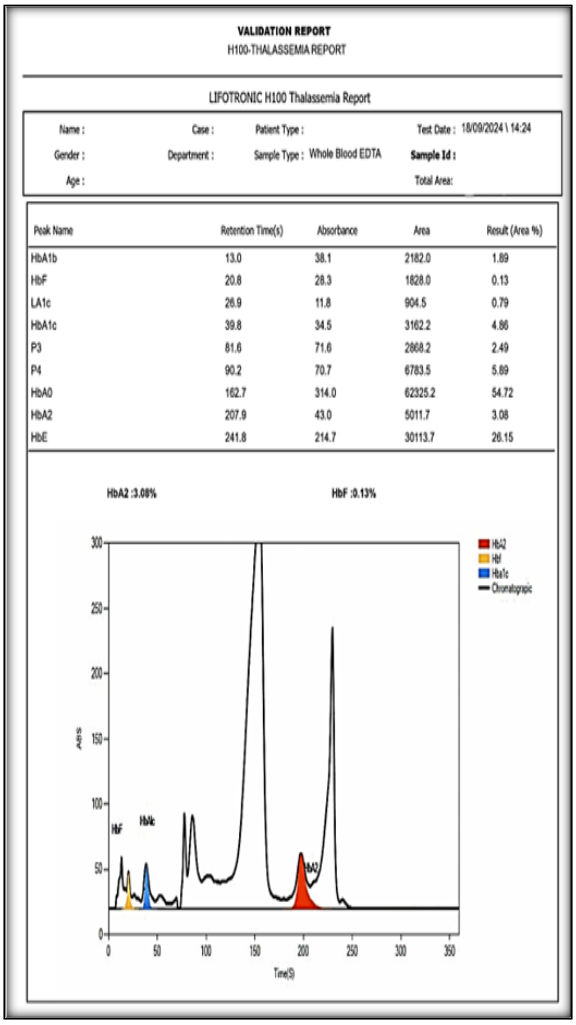

Figure 7: Lifotronic H100 cation-exchange HPLC chromatogram demonstrating HbE trait with clear separation from the HbA_2 window (HbE 26.15%, HbA_2 3.08%, HbF 0.13%).

Representative HbE trait pattern on the Lifotronic H100 showing preserved HbA_2 quantification and minimal HbF.

DISCUSSION

Analytical Performance, Accuracy, and Methodological Comparison: The present evaluation demonstrates that the Lifotronic H100 exhibits analytical performance comparable to two widely validated reference systems—Bio-Rad VARIANT II (ion-exchange HPLC) and Sebia CAPILLARYS (capillary zone electrophoresis). Across HbA_0, HbA_2, and HbF, the H100 delivered high precision, narrow inter-method bias, and correlation coefficients consistent with ranges reported in large international validation studies.[20–22] Its combined attributes of chromatographic quantification and enhanced variant separation place it functionally between conventional HPLC and CZE platforms.

Strong Correlation and Precision Across Major Hemoglobin Fractions: The H100 showed excellent correlation with both reference systems for HbA_0 ($r = 0.967–0.977$) and HbF ($r = 0.982–0.986$), values that meet or exceed commonly reported HPLC–CZE agreement thresholds ($r \geq 0.95$) (20–22). Comparable performance has been reported for the VARIANT II, with HbS–electrophoresis correlation of $r^2 = 0.97$, HbS linearity across 5–95% ($r^2 = 0.989$), HbS CV of 0.9%, and HbF CV of 4.3% (23). Similarly, Gilani et al. demonstrated excellent agreement between CE and HPLC methods with correlations of $r^2 = 0.992–0.994$ and between-run CVs below 0.7% (24). The H100's performance aligns closely with these benchmarks, indicating stable separation behavior and reliable internal calibration.

HbA_2 Quantification: Preservation of Biological Clustering and Diagnostic Thresholds: HbA_2 measurement remains the most critical determinant for β-thalassemia trait detection and is also the fraction most susceptible to method-dependent bias. Despite modest numerical bias relative to VARIANT II (–0.28 to –0.43%), the H100 preserved biologically coherent HbA_2 clustering across phenotypes: normal controls (2.83–2.86%), low-HbA_2 genotypes (1.60–1.78%), and β-thalassemia trait (5.09–5.29%), with no overlap across the diagnostic threshold [Table 2]. Comparable findings have been reported by Kaur et al., who observed strong correlation below the 3.5% cut-off ($r = 0.867$) with method-dependent divergence at higher values.[20] Hafiza et al. and Paleari et al. similarly demonstrated that while absolute HbA_2 values vary across platforms, inter-method bias does not compromise phenotype classification when analytical imprecision remains within allowable limits.[22,25]

Thus, the H100 demonstrates the expected behavior of a well-calibrated system, with only minor, clinically insignificant numerical variation in HbA_2 and no loss of diagnostic accuracy. This is particularly relevant in borderline HbA_2 settings—such as δ-chain variants, iron deficiency, or coexisting α-thalassemia—where β-thalassemia trait may be masked. Stable performance at the lower end of the HbA_2 range reduces the risk of missed carrier detection.

Mechanistic Basis of Inter-Method Differences (HPLC vs CZE): Differences between analytical platforms reflect fundamental separation mechanisms. HPLC systems (VARIANT II and H100) separate hemoglobins based on ionic and hydrophobic interactions, producing retention-time–defined windows; as a result, structurally similar variants such as HbE and HbA_2 or HbD Iran and HbA_2 may partially co-elute, and elevated HbF can broaden baseline regions.[26,27] In contrast, CZE separates hemoglobins according to electrophoretic mobility, enabling clearer discrimination among E-, D-, C-, and G-family variants.[28] The H100's

optimized chromatographic windows and refined peak-fitting algorithms reduce these classical HPLC limitations, minimizing interference patterns without sacrificing quantitative stability.

Analytical Error From Variant Co-Migration: Variant-specific interference is a recognized source of method-dependent bias. HbE trait may falsely elevate HbA_2 on conventional HPLC due to window overlap; HbC can interfere with HbA_2 measurement on CE; HbD Iran may encroach upon the HbA_2 region; and very high HbF (>20–30%) may distort baseline integration on some systems.[25–29] In this study, the H100 minimized such interferences: HbE was clearly resolved from HbA_2, HbD Iran did not overlap the HbA_2 window, and samples with elevated HbF showed stable agreement with reference platforms. These features support consistent diagnostic interpretation and reduce the need for repeat analyses or manual adjudication in routine practice.

Comparative Quantitative Summary Across Studies: A cross-study comparison demonstrates how the performance of the H100 aligns with globally reported analytical benchmarks [Table 9].

Table 9: Comparative quantitative performance benchmarks for hemoglobin fraction measurement and variant detection across published studies.

Study	Parameter	Correlation	Bias	CV
Edwards et al., 2009 (23)	HbS	$r^2 = 0.97$	—	0.9%
Edwards et al., 2009 (23)	HbF	$r^2 = 0.89$	—	4.3%
Greene et al., 2012 (28)	HbA_2	$r = 0.994$	0.00%	—
Gilani et al., 2020 (24)	HbA1c	$r^2 = 0.992–0.994$	—	0.60–0.64%
Kaur et al., 2023 (20)	HbA_2	$r = 0.867$ (<3.5%)	Method-dependent	—
Hafiza et al., 2012 (25)	HbA_2	CE lower by 0.13%	—	—
Xu et al., 2021 (31)	Variant detection	CE 83.3%, HPLC 82.4%	—	—

The quantitative performance of the H100 in the present study falls well within these reported ranges, confirming that its analytical precision, bias profile, and variant-detection capability are consistent with internationally established data across HPLC and capillary electrophoresis platforms.

Retention-Time Stability and Variant Identification Power: Stable retention behavior is central to reliable variant identification. The H100 demonstrated consistent retention times across all 112 samples, comparable to the large dataset reported by Joutovsky et al., who documented retention-time CVs of approximately 1.0% and successful identification of multiple rare variants using HPLC alone (30). Comparative studies have shown that while CE and HPLC achieve similar variant-detection rates (~83%), mass-spectrometric approaches underperform for variants with small mass differences.[31] The H100's chromatographic stability therefore provides robust variant recognition without reliance on complex downstream technologies.

Population Relevance: Alignment With Indian Hemoglobinopathy Patterns: India presents one of the most diagnostically challenging hemoglobinopathy landscapes. Nadkarni et al. reported abnormal hemoglobin patterns in 46% of 31,075 referrals, identifying 49 β-thalassemia mutations, numerous δ-gene variants causing borderline HbA_2 values, multiple α-thalassemia genotypes, and a high burden of HbE and HbD variants.[32] Within this context, the H100's ability to maintain stable HbA_2 clustering, resolve HbE and HbD Iran without A_2-window interference, and accurately quantify HbF directly addresses key diagnostic challenges in Indian screening programs.

Clinical Interpretation and Diagnostic Concordance

Across 112 phenotypically diverse samples, the H100 preserved expected phenotype–fraction relationships and maintained diagnostic thresholds comparable to established platforms. Using standard interpretive criteria, diagnostic concordance was high (Cohen's $\kappa = 0.91–1.00$), with no misclassification observed. Similar levels of agreement have been reported in prior HPLC–CZE comparisons.[28,29] The stability of HbA_2 and HbF measurement, combined with reliable variant separation, supports the use of the H100 as a frontline analyzer for hemoglobinopathy screening and diagnosis.

Practical Laboratory Impact: The combined quantitative stability and variant clarity of the H100 reduce reflex testing, limit repeat analyses, and improve reporting consistency. By offering HPLC-grade quantification with separation performance approaching CZE, the H100 provides a practical dual-capability solution for laboratories—particularly in resource-limited or high-throughput settings—where maintaining multiple analytical platforms may not be feasible.

Diagnostic Concordance Across β-Thalassemia Categories: Precise identification of β-thalassemia trait depends on the stability of the HbA_2 threshold. In this study, the H100 correctly recognized all β-thalassemia trait cases using the ≥3.5% cut-off, despite modest numerical bias relative to reference platforms. HbA_2 distributions remained biologically coherent—5.09–5.29% in trait, 2.83–2.86% in normal individuals, and 1.60–1.78% in low-A_2 states. These findings are consistent with observations from Kaur, Hafiza, and Paleari, who reported that although absolute HbA_2 values may vary by method, inter-method differences do not affect clinical classification when analytical imprecision remains within allowable limits.[20,22,25] The stability shown by the H100 reduces ambiguity in borderline cases and limits the need for reflex confirmation.

High-HbF Stability and Classification of Major and Intermedia Phenotypes: Accurate

quantification of elevated HbF is essential for distinguishing β-thalassemia major, intermedia, δβ-thalassemia, hereditary persistence of fetal hemoglobin, and neonatal profiles. The H100 maintained excellent agreement with VARIANT II and CZE across the full HbF range, including values exceeding 80%, without baseline distortion or compromised HbA_0/HbA_2 integration.

This contrasts with earlier electrophoretic systems described by Edwards, which showed HbF overestimation due to γ-fraction co-migration,[23] and supports findings from Soh demonstrating that HPLC maintains superior accuracy at higher HbF levels compared with immuno-enzymatic methods.[33] The stability of the H100 in high-HbF states underscores its relevance for neonatal and pediatric screening programs.

Variant Detection and Resolution Across Common and Rare Hemoglobin Variants: The H100 demonstrated variant-resolution clarity typically associated with CZE. It consistently resolved HbE, HbD Iran, HbS, HbJ, and several less common variants without the overlap patterns frequently observed on traditional HPLC systems, such as HbE co-elution within the HbA_2 window or HbD Iran encroachment.

This performance mirrors the separation characteristics described by Keren, Higgins, and Greene, who demonstrated superior discrimination of E-, D-, and C-family variants using capillary electrophoresis.[26,28,29] The H100's chromatographic behaviour also aligns with Xu's findings that CE and HPLC yield similar overall variant-detection rates (~83%), whereas MALDI-TOF mass spectrometry underperforms for variants with small mass shifts.[31] By combining CZE-like resolution with HPLC precision, the H100 provides robust variant identification across diverse hemoglobinopathies.

Genotype–Phenotype Fidelity and Molecular Correlation: Sequencing of ten representative samples confirmed complete concordance between genotypes and H100 phenotypes. β-thalassemia major displayed absent HbA_0 with markedly elevated HbF; β-thalassemia trait showed characteristic HbA_2 elevation across mutation types; HbE heterozygotes demonstrated 65–75% HbE and homozygotes >90%; HbD Iran and HbS heterozygotes produced expected variant proportions (40–60% and 35–45%, respectively).

These findings are consistent with established molecular–phenotypic datasets, including those reported by Parab et al,[34] and support the reliability of the H100 as a first-line diagnostic tool with reduced reliance on reflex molecular testing in routine clinical and screening environments.

Diagnostic Concordance With Reference Platforms: Using standard interpretive thresholds—HbA_2 ≥3.5% for β-thalassemia trait, HbF ≥10% for major/intermedia phenotypes, and variant fraction >1%—the H100 achieved Cohen's κ values ranging from 0.91 to 1.00, with no misclassification observed. These results equal or exceed concordance levels reported in published HPLC–CZE comparisons, which typically document κ values between 0.85 and 0.98.[28,29] High diagnostic concordance reflects the H100's stable HbA_2 quantification, reliable HbF behaviour, effective variant separation, and retention-time reproducibility, enabling uniform diagnostic interpretation without platform-specific adjustments.

Relevance for High-Burden Indian Populations: India's hemoglobinopathy landscape is characterized by marked genetic heterogeneity, as documented by Nadkarni et al., who reported abnormal hemoglobin patterns in 46% of 31,075 referrals, encompassing 49 β-thalassemia mutations, numerous δ-gene variants associated with borderline HbA_2 values, multiple α-thalassemia deletions, and a high prevalence of structural variants including HbE, HbS, HbD Punjab, and HbD Iran.[32]

Within this complex epidemiological context, accurate separation of HbE from HbA_2, avoidance of D- and C-family misclassification, and precise HbA_2 measurement in borderline states are essential. The H100 performed reliably in each of these domains. Its consistent behaviour across genotypes and high-HbF phenotypes makes it particularly suited for antenatal and neonatal screening programs, community-level interventions, and routine diagnostics in high-prevalence Indian regions and migration-dense urban centers.

Practical Laboratory Impact: The combined quantitative stability and variant-resolution capability of the H100 reduce the need for reflex testing, limit repeat analyses, and support efficient high-throughput workflows. By delivering HPLC-grade quantification with electrophoresis-like separation, the H100 functions as a dual-purpose analyzer, offering particular value in resource-limited laboratories where maintaining both HPLC and CZE platforms is impractical.

CONCLUSION

This study demonstrates that the Lifotronic H100 delivers analytical and diagnostic performance comparable to both the Bio-Rad VARIANT II HPLC system and the Sebia CAPILLARYS capillary electrophoresis platform. Across all major hemoglobin fractions—HbA_0, HbA_2, and HbF—the H100 showed high precision, minimal inter-method bias, and strong inter-platform correlation. The analyzer consistently preserved the diagnostic HbA_2 threshold for β-thalassemia trait (≥3.5%), maintained measurement stability in samples with markedly elevated HbF, and provided clear, reproducible variant separation approaching the resolution typically achieved with capillary electrophoresis. Diagnostic classification across all evaluated phenotypes was fully concordant with reference platforms, with no misclassification observed.

Collectively, these findings indicate that the H100 offers reliable quantitative accuracy alongside robust

variant detection. Its analytical profile supports its suitability for both routine hemoglobinopathy screening and confirmatory diagnostic evaluation. The demonstrated equivalence to two established reference systems supports the adoption of the H100 as a frontline analyzer, particularly in regions with a high prevalence of β-thalassemia, HbE, and HbD variants.

Limitations

Despite its robust analytical performance, several limitations of the present study should be acknowledged:

1. Sample Size and Spectrum

Although the cohort was phenotypically diverse, the sample size was limited to 112 specimens. Larger, multicenter studies would enhance generalizability, particularly for less common hemoglobin variants.

2. Limited Representation of Ultra-Rare Variants

Ultra-rare hemoglobin variants and unusual compound heterozygous states were not represented. The migration and retention characteristics of such variants on the H100 require further evaluation.

3. Single-Laboratory Assessment

All analyses were conducted in a single reference laboratory serving predominantly North Indian and selected Central Indian populations. Inter-laboratory reproducibility and lot-to-lot variability were not assessed and may influence performance in broader practice.

4. External Quality Assurance Not Evaluated

Comparison with international external quality assurance (EQA) schemes was not performed. Inclusion of EQA data would allow benchmarking against peer laboratories and further validation of analytical accuracy.

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