Letter to the Editor
Diagnostic Hematology

Ann Lab Med 2025;45:101-104
https://doi.org/10.3343/alm.2024.0182
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Ability of the Capillary Electrophoresis-based HbA1c Assay to Detect Rare Hemoglobin Variants

Melania Olivieri, Ph.D.[1], Marco Rosetti, M.S.[1], Giovanni Poletti, M.D.[1], Massimo Maffei, M.S.[2], Domenico Coviello, M.D.[2], Massimo Mogni, M.S.[2], Francesca Capalbo, B.S.[1], Morandini Maria Caterina, B.S.[1], Valentina Polli, M.S.[1], Alice Clementoni, M.S.[1], Evita Massari, M.S.[1], Marta Monti, M.D.[1], Sauro Maoggi, M.S.[3], and Tommaso Fasano, M.D., Ph.D.[1]

[1]Clinical Pathology Unit, Hub Laboratory, AUSL della Romagna, Cesena, Italy; [2]Laboratory of Human Genetics-IRCCS Istituto Giannina Gaslini, Genoa, Italy; [3]Sebia Italia srl, Bagno a Ripoli, Firenze, Italy

Dear Editor,

Capillary electrophoresis (CE) is an accurate method for quantifying glycated hemoglobin (Hb)A1c and detecting HbF, A2, and Hb variants [1]. The CE technology used in our laboratory (Capillarys 3 TERA; Sebia, Lisses, France) facilitates the quantification of HbA1c with the HbA1c kit and of Hb fractions (A, A2, F, and variants) with the HEMOGLOBIN(E) kit. In most cases, the presence of Hb variants can be assessed using both methods. One report suggests that Hb Melusine [α114(GH2)Pro→Ser] can only be detected using the HbA1c method [2]. To the best of our knowledge, there are no reports of other Hb variants with such anomalous behavior.

We report the cases of three Hb variants (Hb Bleuland, La Desirade, and Novara) encountered in 2023 that could only be detected using the CE HbA1c method. Once Hb variants were suspected, molecular analysis was performed using next-generation sequencing with the Devyser Thalassemia kit or Sanger sequencing with primers targeting the α- and β-globin genes. Written informed consent for genetic analysis and research use of data was obtained from patients involved in the study. Local Ethics Committee waived the need for a formal study authorization in view of the recognized clinical path of the laboratory testing reported in the study. Fig. 1 shows electropherograms of the three Hb variants incidentally detected during routine HbA1c analysis (Fig. 1C, 1E, 1G) and electrophoretic profiles for the same samples obtained using the Hb variant kit (Fig. 1D, 1F, 1H). In all cases, HbA1c quantification was invalidated by the presence of a double peak in the HbA0 fraction. However, the electropherograms obtained with the HEMOGLOBIN(E) kit showed no abnormality, and HbA2 measurements (2.6%, 3.2%, and 2.6%, respectively) were within the reference range (2.2–3.5%) used in our laboratory. Therefore, without the HbA1c method, these electrophoretic profiles would have been reported as normal, without a comment (Fig. 1).

The first case involved a 55-yr-old man of Asian origin with diabetes. His electropherogram showed split peaks for the HbA0 and HbA2 fractions, suggesting the presence of a heterozygous interfering α-variant, identified as Hb Bleuland [α108(G15) Thr→Asn; HBA2: c.326C>A]. By analyzing historical data, we found a previous normal chromatogram for HbA1c obtained using HPLC for this patient (data not shown). This Hb variant, which is reportedly unstable, is associated with a mild, clinically silent α-thalassemia phenotype [3]. In combination with an α0-thal allele or mutations in the polyadenylation sites of the α2

Received: April 9, 2024
Revision received: May 21, 2024
Accepted: September 20, 2024
Published online: November 12, 2024

Corresponding author: Melania Olivieri, Ph.D.
Clinical Pathology Unit, Hub Laboratory, AUSL della Romagna, Piazza della Liberazione, 60, 47522 Cesena (FC), Italy
E-mail: melania.olivieri@auslromagna.it

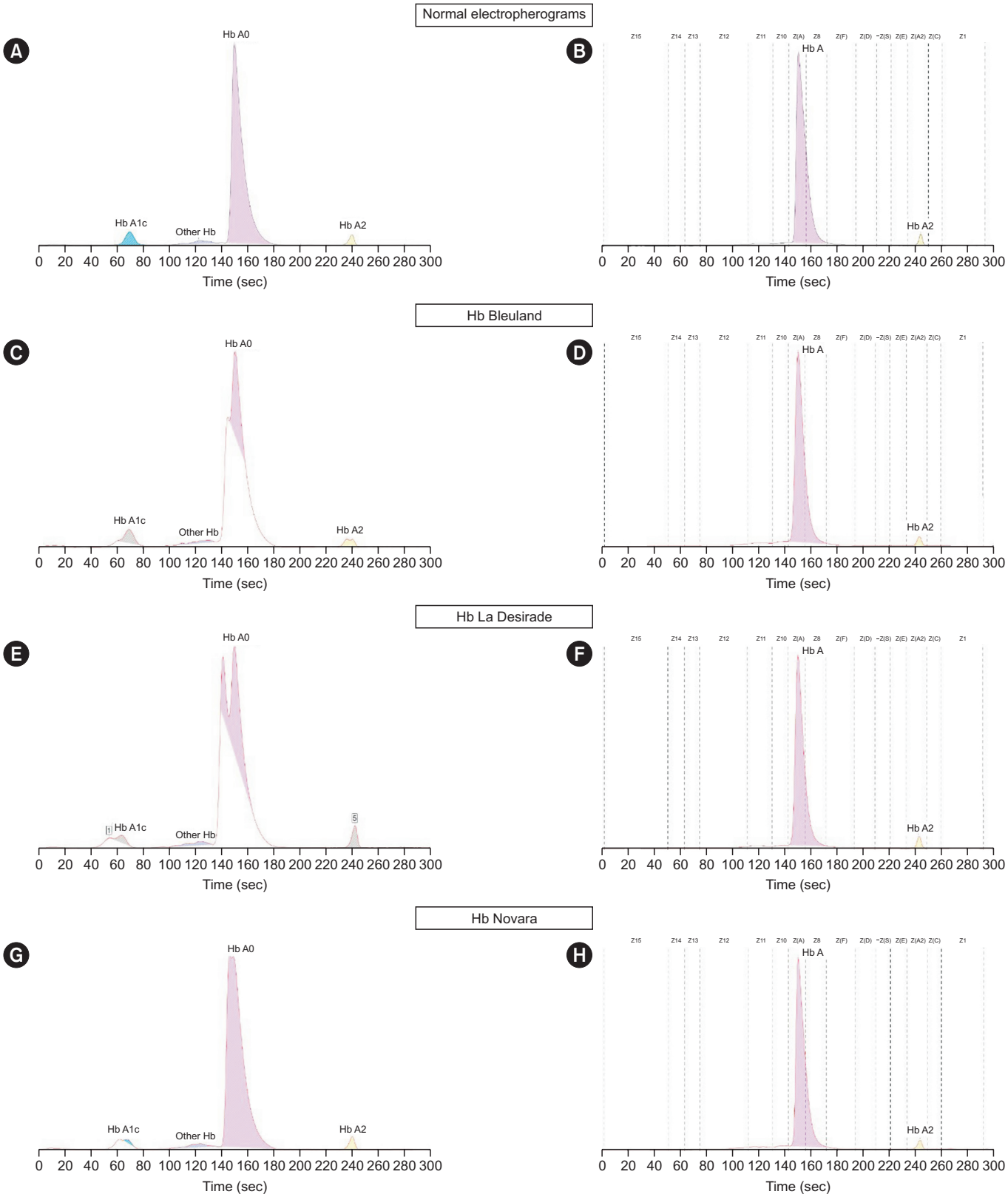

Fig. 1. Hb analysis using capillary electrophoresis. Examples of normal electropherograms obtains the HbA1c kit (A) and HEMOGLOBIN(E) kit (B). Atypical electropherograms obtained using the HbA1c kit (C, E, G) and normal electropherograms obtained using the HEMOGLOBIN(E) kit (D, F, H) for samples from three patients with Hb Bleuland, Hb La Desirade, and Hb Novara.

https://doi.org/10.3343/alm.2024.0182

gene or in the α-hemoglobin-stabilizing protein, Hb Bleuland can have a major clinical impact in the form of Hb H disease [4].

In the second case, the electropherogram of a 30-yr-old pregnant woman of African origin was atypical because of the presence of two different peaks in the HbA0 and HbA1c fractions; no duplication of the HbA2 fraction was observed, suggesting anomalies in the β-chain. In this case, the unstable Hb La Desirade [β129(H7) Ala→Val; HBB: c.389C>T] variant was found in heterozygosity. This Hb variant reportedly cannot be detected by HPLC [5]. When combined with other variants, such as Hb Louisville [β42(CD1)Phe→Leu; HBB: c.127T>C], several significant clinical presentations have been observed [6]. Because of its unstable nature, any association with beta-thalassemia can cause variably severe clinical conditions [7].

In the third case, the CE electropherogram of an 86-yr-old Italian woman with diabetes showed a small double peak in both the HbA0 and HbA1c fractions, suggesting the presence of a disruptive β-variant identified as Hb Novara [β125(H3) Pro→Thr; HBB: c.376C>A] in heterozygosity. This variant was discovered by Crespi, *et al*. [8], who reported a case of microcytic anemia due to this Hb variant combined with a deletion in the α-chain.

The variants described are extremely rare and, as in the case of other known variants, remain undetected when using several analytical systems that analyze the separation and quantification of Hb components, as they co-migrate with normal HbA0. This behavior contrasts with that of more common variants (HbS, HbC, HbD, and HbE), which are identifiable by a distinct peak in a specific migration or elution zone [2, 9]. These three cases highlight the different capacities of two CE-dedicated kits (HbA1c and Hb variant) to detect rare Hb variants and demonstrate the clinical importance of accurately identifying these Hb variants. The inclusion of dicarboxyphenylboronic acid in the HbA1c kit likely enhances its discriminatory power compared with that of the HEMOGLOBIN(E) kit [10]. Although most rare Hb variants detected incidentally are clinically asymptomatic, their potential risk, when combined with other variants or thalassemic conditions, should not be underestimated. A critical evaluation of the methodology employed can help recognize its potential and limitations and, when appropriate, facilitate its comparison with other methods. Regardless of the screening method employed for hemoglobinopathies, laboratory personnel must recognize that no single method can identify all Hb variants; thus, in certain clinical scenarios, employing two distinct methods is essential to ensure an accurate diagnosis.

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

Olivieri M, Rosetti M, and Fasano T wrote the manuscript; Maffei M, Coviello D, and Mogni M carried out molecular analyses; Capalbo F and Morandini MC carried out capillary electrophoresis; and Poletti G, Polli V, Clementoni A, Massari E, Monti M, and Maoggi S contributed to the interpretation of the results and manuscript revision.

CONFLICTS OF INTEREST

None declared.

RESEARCH FUNDING

None declared.

REFERENCES

1. Dessi M, Pieri M, Pignalosa S, Martino FG, Zenobi R. Performances of capillary electrophoresis and HPLC methods in HbA1c determination: diagnostic accuracy in HbS and HbD-Iran variants' presence. J Clin Lab Anal 2015;29:57-60.
2. Peeters B, Brandt I, Desmet K, Harteveld CL, Kieffer D. Hb Melusine and Hb Athens-Georgia: potentially underreported in the Belgian population? Four cases demonstrating the lack of detection using common CE-HPLC methods either for glycated hemoglobin (HbA1c) analysis or Hb variant screening. Acta Clin Belg 2016;71:458-461.
3. Harteveld CL, Versteegh FGA, Kok PJMJ, van Rooijen-Nijdam IH, van Delft P, Giordano PC. Hb Bleuland [α108(G15) Thr→Asn, A C C→A A C (α2)]: a new abnormal hemoglobin associated with a mild α-thalassemia phenotype. Hemoglobin 2006;30:349-54.
4. Wajcman H, Traeger-Synodinos J, Papassotiriou I, Giordano PC, Harteveld CL, Baudin-Creuza V, et al. Unstable and thalassemic alpha chain hemoglobin variants: a cause of Hb H disease and thalassemia intermedia. Hemoglobin 2008;32:327-49.
5. Strickland SW, Campbell ST, Little RR, Bruns DE, Bazydlo LAL. Recognition of rare hemoglobin variants by hemoglobin A1c measurement procedures. Clin Chim Acta. 2018;476:67-74.
6. Kamseng P, Trakulsrichai S, Trachoo O, Yimniam W, Panthan B, Jittorntam P, et al. Low oxygen saturation and severe anemia in compound heterozygous Hb Louisville [β42(CD1) Phe→Leu] and Hb La Desirade [β129(H7)Ala→Val]. Hematology 2017;22:114-8.
7. Alkindi S, Al Zadjali S, Al Rawahi M, Al Haddabi H, Daar S, ElSadek R A, et al. Clinical and laboratory features of hemoglobin La Desirade variant in association with sickle cell and alpha thalassemia genes. Mediterr J Hematol Infect Dis 2021;13:e2021010.
8. Crespi I, Campisi, M.P, Duranti D, Serino R, Saliva E, Marchiando M, et al. Incidental detection of a new hemoglobin beta variant performing

HbA1c measurement. Biochim Clin 2018;42:E56-8.
9. Yuan Y, Zhou X, Gao L, Ren Q, Ji L. Silent hemoglobin variant during capillary electrophoresis: a case report. J Diabetes Investig 2020;11:1014-7.
10. Deschamps G, Robert F, Simonin D, inventors; SEBIA, assignee. Analysis and assay of glycated haemoglobins by capillary electrophoresis, buffer compositions and kits for capillary electrophores. United States patent US 9.222,913B2. 2015 Dec 29.

https://doi.org/10.3343/alm.2024.0182