Inconsistent Hb A_{1c} Results Observed after Implementing a New Method

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CASE DESCRIPTION

A 66-year-old White male with a history of type 2 diabetes mellitus (T2DM), congestive heart failure, and chronic kidney disease visited an outpatient clinic for regular follow-up with T2DM and congestive heart failure. A hemoglobin A_{1c} (Hb A_{1c}) test was performed using Sebia CAPI3 TERA capillary electrophoresis (CE) and the apparent Hb A_{1c} was 9.1% (normal range 4.0–6.0%) with an unknown peak cathodal to Hb A_0. This peak partially overlapped with Hb A_0 and had a similar size (Fig. 1A). The patient had no history of anemia or related symptoms at the time of the visit. Because of this atypical profile, the Hb A_{1c} result was not reported, and the case was further investigated. The concurrent fasting glucose concentration was 156 mg/dL (8.7 mmol/L) [normal range: 70–100 mg/dL (3.9–5.6 mmol/L)], and the fasting glucose concentrations ranged from 110 mg/dL (6.1 mmol/L) to 156 mg/dL (8.7 mmol/L) with an average of 135 mg/dL (7.5 mmol/L) in the past 3 months. The patient's previous Hb A_{1c} and fasting glucose concentration were 6.4% and 124 mg/dL (6.9 mmol/L), tested 6 months prior (Table 1). HbA_{1c} was measured by a cation exchange HPLC method (Tosoh G8, v.5.24F) at that time, and no atypical pattern or hemoglobin variant was detected.

Since the unknown hemoglobin variant was in the zone of Hb F, there was a concern for potential interference with Hb A_{1c} quantification (i.e., if Hb F > 23% as per the manufacturer's instructions). To determine whether the variant hemoglobin was Hb F, the specimen was tested for hemoglobin variants using CE (Sebia Capillarys 2, hemoglobin program). A similar peak overlapping with Hb A was identified, but it was not in the Hb F zone (Fig. 1B). According to Sebia's user manual, the variant peak was in a zone that did not contain any common pathological variants. For a comparison with the previous HPLC method, we also measured the Hb A_{1c} of this specimen by Tosoh G8, and the result was 5.8% without any hemoglobin variants (Fig. 1C).

QUESTIONS TO CONSIDER	
1.	What are the principles of common methods for Hb A_{1c} testing?
2.	What are the limitations of cation exchange HPLC and CE in Hb A_{1c} measurement?
3.	When should an alternative Hb A_{1c} testing method be considered?

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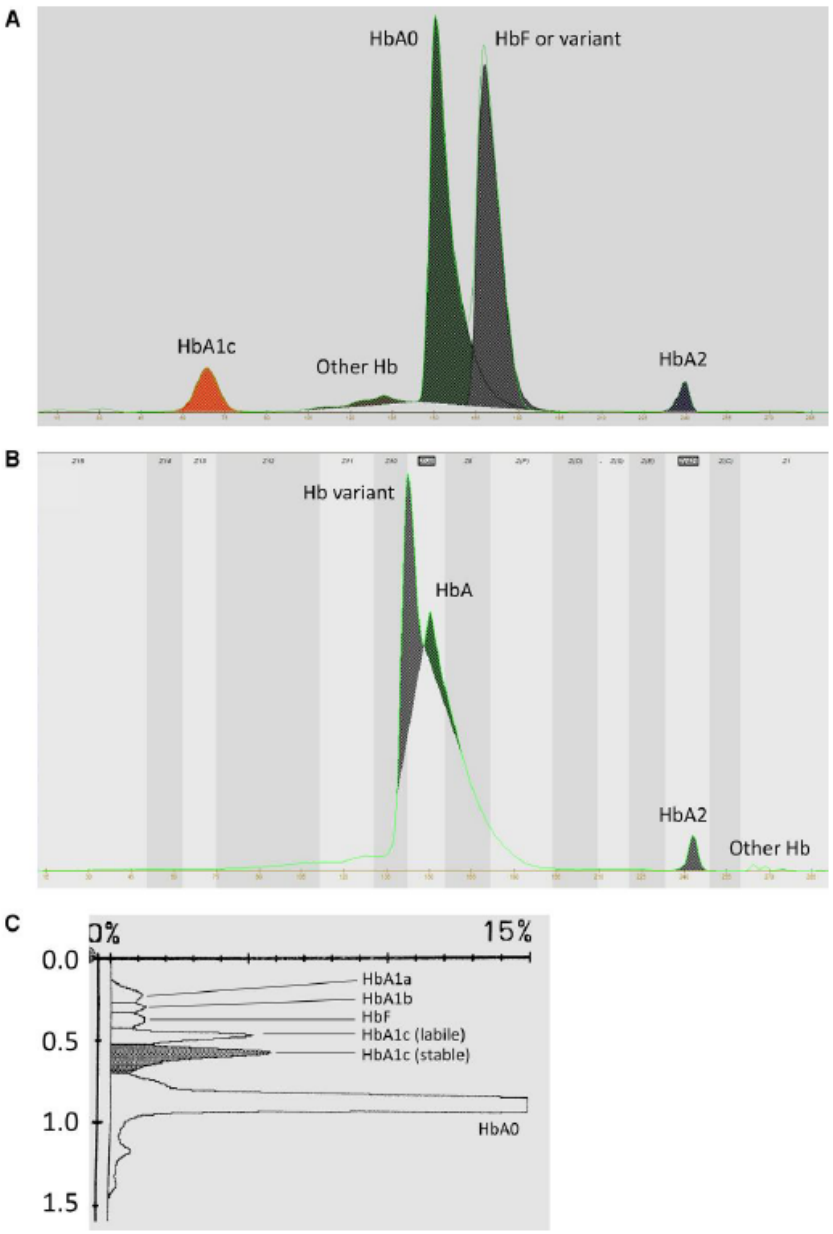

Fig. 1. An unknown hemoglobin variant co-eluted with Hb A_0 in a cation exchange HPLC method but was partially separated from Hb A_0 in a CE method. (A), Hb A_{1c} test by CE (Sebia, CAPI3 TERA). Hb A_0 = 48.4%, F or variant = 41.9%, A_2 = 2.2%, other Hb = 2.1%. The software calculated Hb A_{1c}% = 9.1%; (B), Hb variants identified by CE (Sebia, Capillarys 2); (C), Hb A_{1c} test by HPLC (Tosoh G8, v.5.24F). Stable Hb A_{1c} = 5.8% and labile Hb A_{1c} = 2.7%. For visibility, all peak names were manually relabeled according to the software's assignment. Color figure available at https://academic.oup.com/clinchem.

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Table 1. Comparison of Hb A_{1c} results by different methods.

Date of collection	Method	Instrument	Hb A_{1c} (%)	eAG[a] (mg/dL)	Fasting glucose (mg/dL)
December 29, 2022	Cation exchange HPLC	Tosoh G8, v.5.24F	6.4	137	124
June 14, 2023	CE	Sebia, CAPI3 TERA	9.1	214	156
	Cation exchange HPLC	Tosoh G8, v.5.24F	5.8	120	156
	Enzymatic assay		5.6	114	156

[a]eAG = 28.7 × A_{1c}—46.7. mg/dL = 0.056 mmol/L.

Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the August 2024 issue of *Clinical Chemistry*. To view the case and comments online, go to https://academic.oup.com/clinchem/issue/70/8 and follow the link to the Clinical Case Study and Commentaries.

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