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Letter to the Editor

Measurement of glycated hemoglobin in a patient with homozygous hemoglobin E

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A recently diagnosed diabetic patient with homozygous HbE presented for HbA_{1c} estimation. The sample was first analyzed using ion-exchange chromatography on a Bio-Rad Variant V-II (Bio-Rad, Hercules, CA, USA) using the V2_A_{1c} _NU program and it did not produce a HbA_{1c} result but showed an A_o area peak of 84.4%. The sample was then analyzed on a second ion-exchange chromatography system, Bio-Rad D-10 analyzer using software version 3.60, and it also failed to provide a HbA_{1c} result. The D-10 variant-window peak area was 92.8%, and the A_o area peak of 1.9%. The HbF fractions by the two different analyses were 3.3% and 3.5%, respectively. The results are shown in Figure 1. The sample was then analyzed on a boronate affinity method, In2it analyzer (Bio-Rad) and the result was 9.7%. An aliquot of the sample was also analyzed on a COBAS Integra 800 (Roche Diagnostics, Germany) immunoassay method and the result was 6.9%. This was the first HbA_{1c} request received on this patient. There were three non-fasting glucose results for this patient, one with the HbA_{1c} request which was 11.8 mmol/L, and the two prior over a 2 week period were 14.4 mmol/L and 8.2 mmol/L. The 9.7% HbA_{1c} result is equivalent to an approximately average glucose concentration of 12.8 mmol/L whereas the 6.9% HbA_{1c} result equates to 8.4 mmol/L. The measured glucose results do not represent a large enough time period to provide more accurate approximation of the average glucose result. A fourth sample collected 2 months later had a glucose of 9.0 mmol/L. We measured the fructosamine (#11930010, Roche Diagnostics) on a Beckman DxC800 (Beckman Coulter, Brea, CA, USA) and the result was 349 µmol/L (RI: 190–285). This suggests the HbA_{1c} In2It result reflects the glycemic status more accurately than the Integra immunoassay result.

The patient had been confirmed 6 days prior to the HbA_{1c} request as HbE homozygous with HbA_2 of 3.6%, which is lower than usually seen in this condition (expected >4.0%) (1). The total HbA, HbA_2 and HbE area using the VII β-thalassemia short program was 93.6%. The requesting doctor was not aware how such a variant may affect HbA_{1c} estimations and the laboratory was not informed of the condition. HbE homozygote patients may present with mild hemolysis, normal or low hemoglobin and MCV and the blood film may show target cells (2). The hematology results for this patient were in line with published literature: total Hb 110 (RI: 115–160 g/L), RBC 5.5 (RI: 3.8–5.2×10^{12}/L), MCV 56 (RI: 80–100 fL) and MCH 20.0 (RI: 27.5–33.0 pg) (Sysmex XE-5000, Roche, Australia), and the blood film showed target cells.

HbE is primarily found in people originating from South-East Asia, with a prevalence of 30%–40% in some parts of Thailand, Cambodia and Laos. This varies across the remaining South Asian nations, but falls off to much lower levels across Southern Europe, the Middle East, and West Africa (1). However, with global migration HbE is likely to present more frequently in developed nations. Worldwide, HbE is the second most common hemoglobin variant, and has a single amino acid substitution, lysine for glutamic at position 26 in the β chain (3). It is estimated 30 million South-East Asians are carriers of HbE and one million are HbE homozygous (1).

The four analyzers we evaluated gave different results due to different methodologies used to detect the glycated hemoglobin. HbA accounts for approximately 60% of the glycated hemoglobins in a normal patient. HbA is glycated at the N-terminal valine residues of the β chain (4). The two ion-exchange HPLC methods correctly showed that there was no HbA_{1c}, as the patient has no HbA. We recognize that ion-exchange HPLC methods can be powerful tools in detecting and identifying the presence of some Hb variants. Incidental discovery of an index case can identify a family with a variant, prompting medical advice.

The Integra 800 immunoassay method, like many other immunoassay methods utilize an antibody that recognizes the glycated N-terminal four amino acids of the β chain (5).

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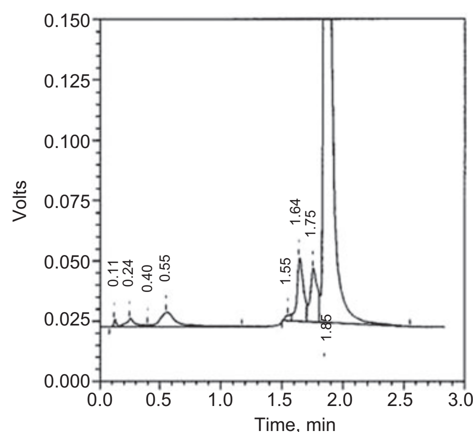
A

Peak name	Calibrated area%	Area%	Retention time, min	Peak area
A_{1a}	---	0.3	0.11	4818
A_{1b}	---	1.1	0.24	19,477
Unknown	---	0.2	0.40	3605
F	---	3.1	0.55	55,383
P3	---	0.5	1.55	9179
P4	---	5.3	1.64	95,367
A_o	---	6.5	1.75	116,355
E,D	---	83.1	1.85	1,498,590
			Total area:	1,802,773

A_{1c} Concentration=%



B

Peak name	Calibrated area%	Area%	Retention time, min	Peak area
A_{1a}	---	0.8	0.19	27,641
A_{1b}	---	1.9	0.30	64,932
F	---	0.4	0.54	12,360
LA1C	---	2.3	0.70	75,856
A_{1c}	8.5	---	0.92	204,696
P3	---	3.6	1.51	120,055
P4	---	1.9	1.61	63,403
A_o	---	83.0	1.73	2,783,172
			Total area:	3,352,115

A_{1c} Concentration=8.5%

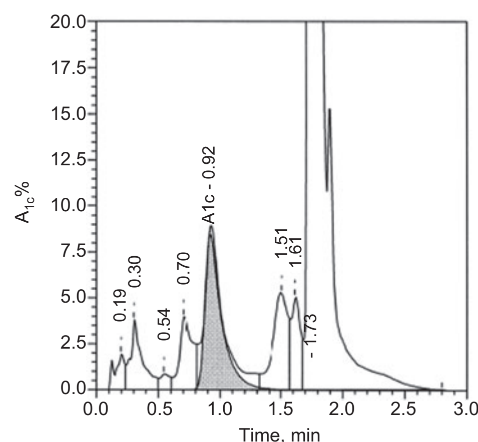


Figure 1

Since these amino acids are the same for HbA and for HbE, one would expect that the Integra result would be accurate. The Integra method has been shown not to be affected by heterozygous HbE variants (3). However, our other patient data suggests that the immunoassay underestimated the patient's glycemic status, perhaps due to a decreased affinity of the antibody to HbE. Therefore, we recommend that each immunoassay be examined for accuracy with homozygous HbE samples. Boronate affinity measures all glycated hemoglobins, and it has been reported to be unaffected by variant

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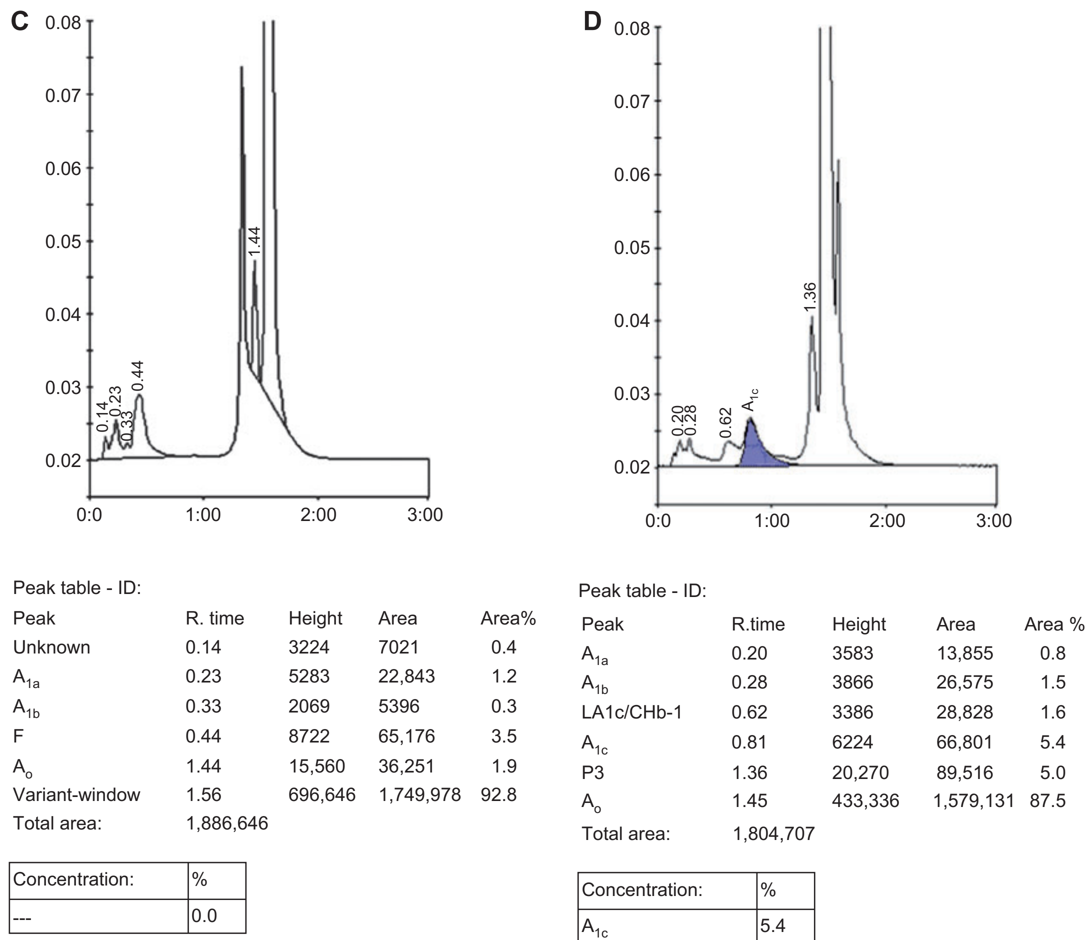
Figure 1 (continued)



Peak table - ID:

Peak	R. time	Height	Area	Area%
Unknown	0.14	3224	7021	0.4
A_{1a}	0.23	5283	22,843	1.2
A_{1b}	0.33	2069	5396	0.3
F	0.44	8722	65,176	3.5
A_o	1.44	15,560	36,251	1.9
Variant-window	1.56	696,646	1,749,978	92.8
Total area:	1,886,646			

Concentration:	%
---	0.0

Peak table - ID:

Peak	R.time	Height	Area	Area %
A_{1a}	0.20	3583	13,855	0.8
A_{1b}	0.28	3866	26,575	1.5
LA1c/CHb-1	0.62	3386	28,828	1.6
A_{1c}	0.81	6224	66,801	5.4
P3	1.36	20,270	89,516	5.0
A_o	1.45	433,336	1,579,131	87.5
Total area:	1,804,707			

Concentration:	%
A_{1c}	5.4

Figure 1 Chromatograms from Bio-Rad HPLC systems.
(A) Variant II V2_A_{1c} _NU program; (B–D) 10 software version 3.60 of a sample with homozygous HbE variant and normal patient samples.

hemoglobin forms, although it cannot detect the presence of variants (6). Based on published data and the lack of interference from Hb variants on affinity chromatography systems, it seems likely that the In2it result provides the best reflection of the patient's glycemic status. Availability of more glucose and fructosamine data for this patient would have provided a better guide as to whether this is actually true. To our knowledge there is no data comparing the In2it method to a more sophisticated affinity chromatography method with homozygous HbE samples.

In South-East Asian countries particularly where the homozygous HbE variant is prevalent the affinity chromatography methods are the methods of choice if diabetic patients are to be managed appropriately. Additionally, in cases where hemolytic anemia is present, appropriate management to achieve good glycemic control will need to be supplemented by fructosamine estimates.

In summary, to provide reliable and useful glycated hemoglobin results when variants or chromatograms with potential variants are identified on HPLC systems, results should be checked with an affinity chromatography method that has been shown not to have interference from the particular variant. Affinity chromatography systems as point of care systems are now readily available (and relatively inexpensive). Therefore, laboratories running HPLC systems should invest in such analyzer combinations, specifically in a point of care method that has been thoroughly evaluated for interferences. Another possibility would be to send out the sample for analysis by an affinity chromatography method to enable accurate results to be obtained to best guide patient care. Fructosamine also must be considered as an alternative method for monitoring glycemic control in such patients, especially if the erythrocyte lifespan may be altered.

Conflict of interest statement

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