HbA2 Prime Is a Mild Variant, but Can Be Misleading.

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INTRODUCTION

Hemoglobinopathies are defined as quantitative and qualitative changes to alpha and beta globin chains that constitute the adult normal hemoglobin (HbA). The clinical outcome of these blood disorders has been identified as significant and warrants uniform screening for early detection and medical intervention before onset of symptoms. Many analytical methods have been developed to detect the more than one thousand hemoglobin variants currently identified.

Presently, LabCorp performs High Performance Liquid Chromatography (HPLC) for screening and high resolution HPLC for confirmation, in addition to the solubility assay for sickle cell screening. The Capillary Zone Electrophoresis (CE) method (Sebia's CAPILLARYS 2 flex piercing) has emerged as a very robust separation technique that provides better separation for variants co-eluting with HbA2. This study was conducted to evaluate the CE methodology using Sebia's CAPILLARYS 2 flex piercing as a complementary method to HPLC.

METHODS

Patient samples submitted for routine hemoglobinopathy screening were analyzed using HPLC (Trinity Biotech, ultra[2])[2] as the primary methodology and Capillary Electrophoresis (Sebia, CAPILLARYS2 flex piercing)[3] as the secondary methodology. Data analysis was performed with the Trinity Biotech Resolution Version 5.3.1, and the Sebia Phoresis version 9.1.5. Over 3000 samples were assayed January - April 2019.

RESULTS

HPLC and CE methods were compared with respect to variant detection. HPLC detected more alpha and beta chain variants such as: Hb Chicago, Hb Raleigh, and Hb Hekinan. However, CE revealed Hb delta chain variants that were concealed by other hemoglobins using HPLC.

HbA2 is a normal hemoglobin formed by delta and alpha chains. HbA2 levels can be important indicators of Thalassemia and Iron Deficiency. Delta variants can falsely decrease HbA2 levels up to 50%; therefore, measurement involves combining both the HbA2 and the delta variant for a total HbA2 level.

HPLC and CE methods can easily identify HbA2', the most commonly observed delta variant. Failure to identify HbA2' can result in a miscalculation of the total HbA2 and possibly prevent the detection of Beta thalassemia. With HPLC methods, other Hb delta variants are undetectable because they co-elute with HbA. This study revealed that the CE method helped separate some delta variants into a different electrophoretic zone. This separation from HbA allowed a more accurate determination of HbA2 levels.

The study population revealed two Hb delta variants: one consistent with Hb A2-Flatbush, and another with Hb A2-Babinga. HbA2-Flatbush was observed in the D Zone and located on the x-axis at 200-201. HbA2-Babinga migrated within the E Zone on the x-axis at 225-226. Both variants are found within the African American community, a patient population that has an increased rate of alpha-thalassemia.

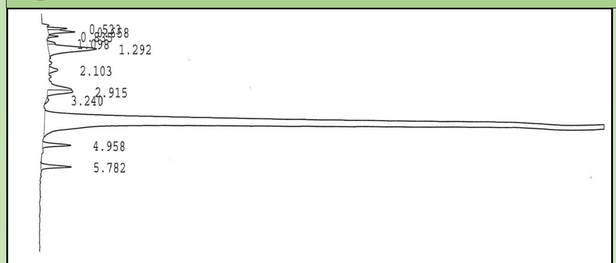
Figure 1 shows the HbA2' graphs for HPLC and CE.

Figures 2 and 3 show the HPLC and CE graphs for HbA2-Flatbush and HbA2-Babinga. HbA2 values from the HPLC method are decreased, usually indicating alpha thalassemia. However, combining HbA2 and the delta variant yields a normal HbA2 value, consistent with the RBC indices that show no hematologic abnormalities.

DATA

Figure 1 — HbA2'

HPLC

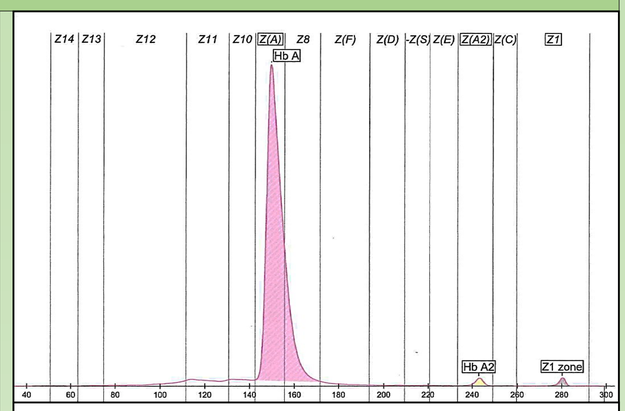


PEAK	RT	REL RT	% CONC	AREA	COMMENT
1	0.533	F 0.35	0.5%	9925	
2	0.658	F 0.43	0.9%	18765	
3	0.835	F 0.55	0.2%	4360	
4	1.098	F 0.72	0.3%	5127	
5	1.292	F 0.85	3.8%	75524	A1c or possibly HbH
6	2.103	F 1.38	0.5%	9668	
7	2.915	A 0.74	1.4%	27102	
8	3.240	A 0.82	0.2%	3194	
9	4.010	A 1.01	89.9%	1791870	A0 peak
10	4.958	S 0.88	1.2%	23125	8 A2 peak
11	5.782	S 1.02	1.2%	24013	4

Total Area: 1992673

HbA2: 4.958/RT 1.2% HbA2': 5.782/RT 1.2%
Total HbA2 (RI 1.8-3.2): (1.2 + 1.2) = 2.4

Capillary Electrophoresis

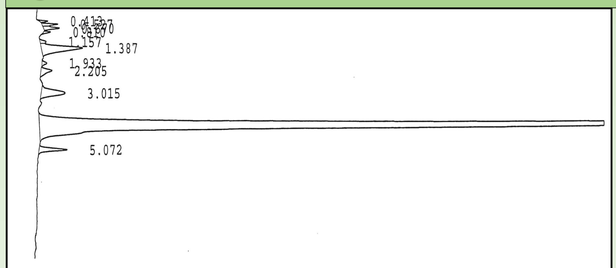


Name	%
Hb A	97.8
Hb A2	1.2
Z1 zone HbA2'	1.0

HbA2: Z(A2) x=243 1.2% HbA2': Z1 x=288 1.0%
Total A2(RI 1.8-3.2): (1.2 + 1.0) = 2.2

Hematology: RBC(RI 3.77-5.28): 4.13 HGB(RI 11.1-15.9): 12.6 MCV(RI 79-97): 92

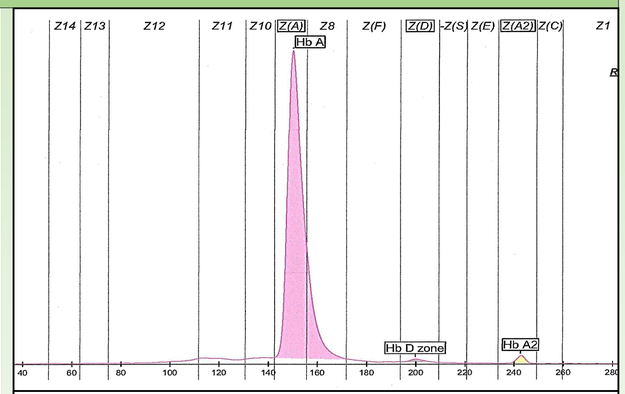
Figure 2 — HbA2-Flatbush



PEAK	RT	REL RT	% CONC	AREA	COMMENT
1	0.413	F 0.25	0.2%	3351	
2	0.527	F 0.32	0.4%	7784	
3	0.670	F 0.40	0.5%	9939	
4	0.810	F 0.49	0.2%	3245	
5	1.157	F 0.69	0.3%	4714	
6	1.387	F 0.83	3.3%	61573	A1c or possibly HbH
7	1.933	F 1.16	0.2%	3888	
8	2.205	F 1.32	0.7%	13591	
9	3.015	A 0.72	2.1%	39012	
10	4.232	A 1.01	90.9%	1710458	A0 peak
11	5.072	S 0.88	1.3%	23518	8 A2 peak

Total Area: 1881073

HbA2: 5.072/RT 1.3% HbA2-Flatbush: With HbA 0.0%
Total HbA2 (RI 1.8-3.2): (1.3 + 0.0) = 1.3

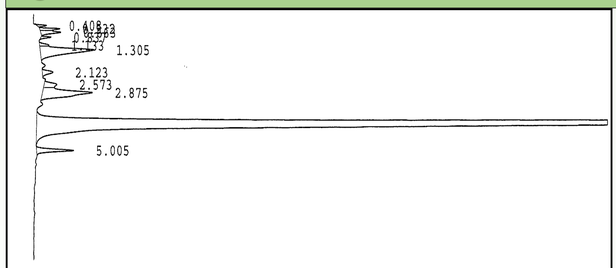


Name	%
Hb A	98.1
Hb D zone HbA2-Flatbush	0.6
Hb A2	1.3

HbA2: Z(A2) x=243 1.3% HbA2-Flatbush: Z(D) x=200 0.6%
Total HbA2(RI 1.8-3.2): (1.3 + 0.6) = 1.9

Hematology: RBC(RI 3.77-5.28): 4.46 HGB(RI 11.1-15.9): 11.4 MCV(RI 79-97): 81

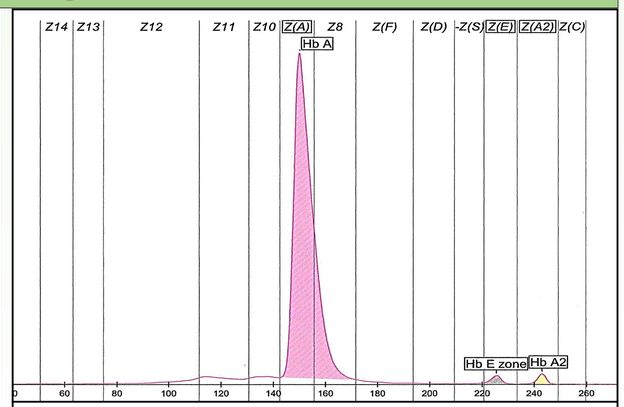
Figure 3 — HbA2-Babinga



PEAK	RT	REL RT	% CONC	AREA	COMMENT
1	0.408	F 0.26	0.2%	4218	
2	0.532	F 0.34	0.5%	10646	
3	0.663	F 0.42	0.7%	14605	
4	0.837	F 0.53	0.2%	4479	
5	1.133	F 0.72	0.3%	5897	
6	1.305	F 0.82	3.8%	86005	A1c or possibly HbH
7	2.123	F 1.34	0.5%	10366	
8	2.573	F 1.63	0.7%	14927	
9	2.875	A 0.72	3.6%	81589	
10	4.045	A 1.01	88.3%	1984320	A0 peak
11	5.005	S 0.88	1.3%	29586	8 A2 peak

Total Area: 2246638

HbA2: 5.005/RT 1.3% HbA2-Babinga: With HbA 0.0%
Total HbA2 (RI 1.8-3.2): (1.3 + 0.0) = 1.3



Hb A		97.2
Hb E zone	HbA2-Babinga	1.4
Hb A2		1.4

HbA2: Z(A2) x=243 1.4% HbA2-Babinga: Z(E) x=266 1.4%
Total HbA2(RI 1.8-3.2): (1.4 + 1.4) = 2.8

Hematology: RBC(RI 3.77-5.28): 4.47 HGB(RI 11.1-15.9): 13.9 MCV(RI 79-97): 91

DISCUSSION

HbA2' is a frequently observed delta variant, and although the variant itself has no known clinical significance, identification is important for accurate HbA2 levels. This has been well documented in cases of beta thalassemia[1]. This study has shown that by using an alternative method, other delta variants can be identified, and their detection is equally important to avoid misleading information.

In reference laboratories, hemoglobinopathy interpretations are sometimes performed in the absence of supporting data. Routine testing such as CBC and Iron studies, while integral to interpretation, is usually performed at the originating hospital or physician's office. More often than not, this data is not submitted to the reference laboratory. Low HbA2 levels, without corroborating data, can be interpreted as alpha thalassemia or iron deficiency anemia, and come with recommendations of additional testing. This additional testing would be expensive and unnecessary, while still leaving questions as to the root cause of the HbA2 decrease.

CONCLUSIONS

Quantifying HbA2 plays an important role in interpretation and detection of Thalassemia. Difficulties arise when qualitative abnormalities interfere with HgA2 measurement. More than 80 delta variants have been identified so far and detection can be hindered by using only one methodology. No one method is proficient at detecting all variants and this study has shown that CE and HPLC can assist and complement each other mutually. Employing both HPLC and CE increases detection of all variants, especially delta variants, and can reduce misinterpretation and misleading results.

REFERENCES

1. D. Abdel-Gadir, L. Phelan, B.J. Bain: *Haemoglobin A2' and its significance in B thalassemia diagnosis*, International Journal of Laboratory Hematology. 2009, 31(3), 315-319.
2. Trinity Biotech USA, *ultra*2 with Resolution™ Software
3. Sebia, Inc., Norcross, Georgia, USA, Capillarys™2 flex piercing

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