Improved Detection of Haemoglobin E with Sebia Capillarys2 System

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Background:

High Performance Liquid Chromatography (HPLC) and Capillary Electrophoresis (CE) are two analytical methods that are commonly used in the identification of haemoglobin variants.

The advantage of CE includes the separation of Hb A2 from Hb E, allowing for direct quantification of Hb E in carriers. This separation is not possible in HPLC. An accurate quantitation of Hb E variant will help to identify the carrier statues of the patient. It is known that in Hb E heterozygotes, the Hb E variant is usually ≤ 30.0% of total haemoglobin; a Hb E value of > 39.0% suggests Hb E / beta thalassaemia while Hb E value of < 25% is observed in cases of Hb E with co-existing alpha thalassaemia trait.

We evaluated the performance of Sebia Capillarys2 Analyser (which uses CE method) as compared to BioRad Variant II (which uses HPLC method) in our Haematology Laboratory.

Picture 1: Sebia Capillarys2

Picture 2: BioRad Varient II

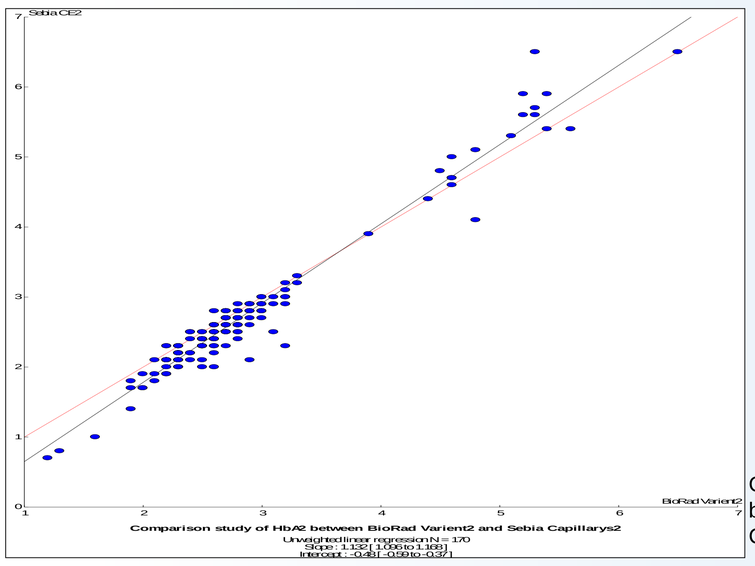
Methodology:

To determine the performance of CE and the correlation between the 2 methods:

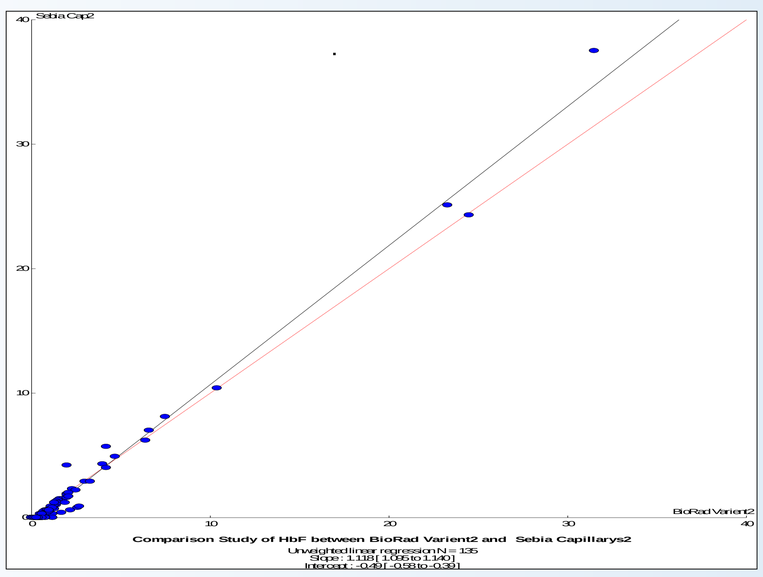
1) 200 routine patient samples without haemoglobin variants were tested. The Hb A2 (n= 170) and Hb F (n=135) results obtained from both methods were analysed using linear regression plots.
2) 10 patients with Hb E variant were analysed and compared.
3) Inter-day precision of Hb A2 and Hb F on CE were performed over 10 days using Sebia Normal Hb A2 control and over 9 days with Sebia Hb AFSC control respectively.
4) Various types of Haemoglobin variants were identified on CE over a 6-month period.

Result:

1) Good precision was achieved on Sebia Capillarys2 (CE) for both Hb A2 and Hb F; Hb A2 with a CV = 3.3% and SD=0.1 and Hb F with a CV=2.8% and SD=0.69. The comparison study showed a high degree of correlation between HPLC and CE (r =0.993 for Hb F and r = 0.979 for Hb A2).

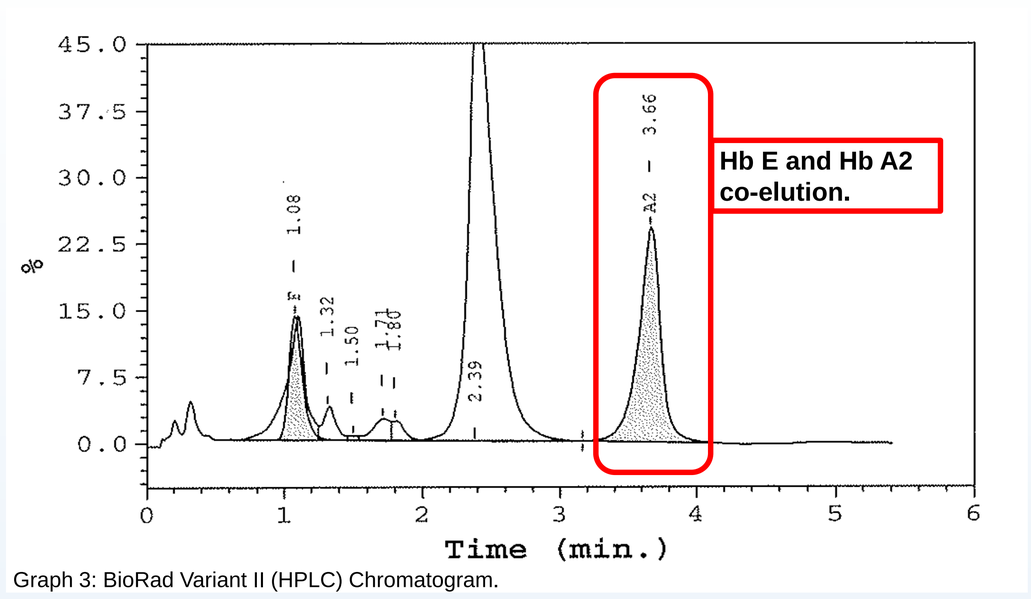


Graph 1: Comparison study of Hb A2 between BioRad Variant II and Sebia Capillarys2.

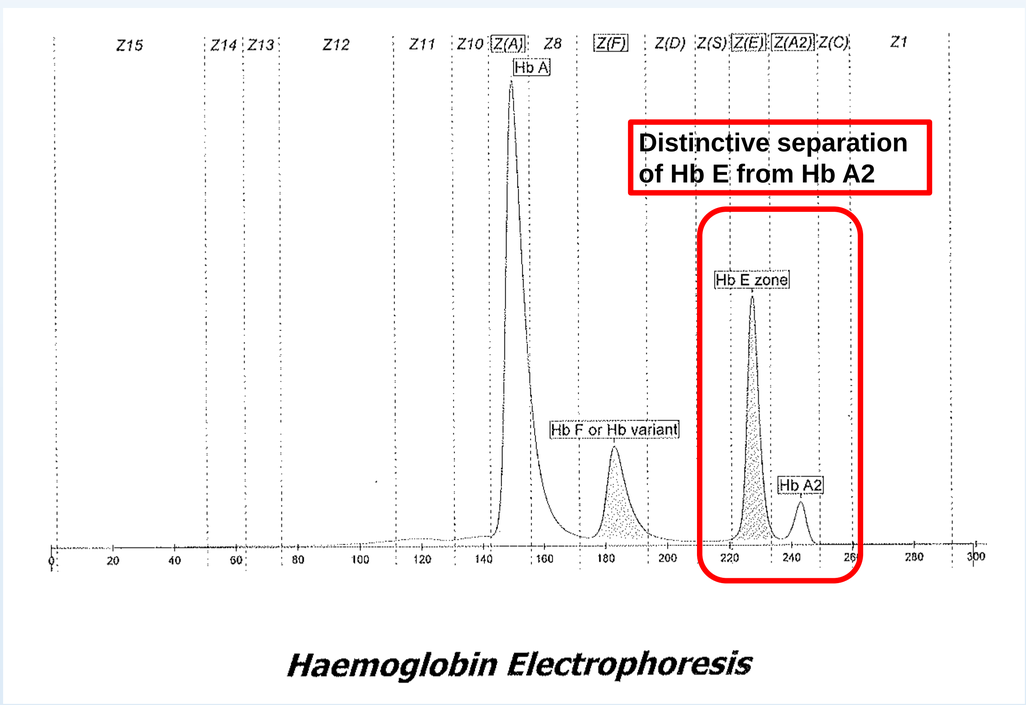


Graph 2:: Comparison study of Hb F between BioRad Variant II and Sebia Capillarys2.

2) From our evaluation, it is evident that HPLC is unable to separate Hb E from Hb A2 because both haemoglobins co-elute at the same retention time. In CE, the separation is very distinct. This observation is consistent for all 10 samples with Hb E cases tested.



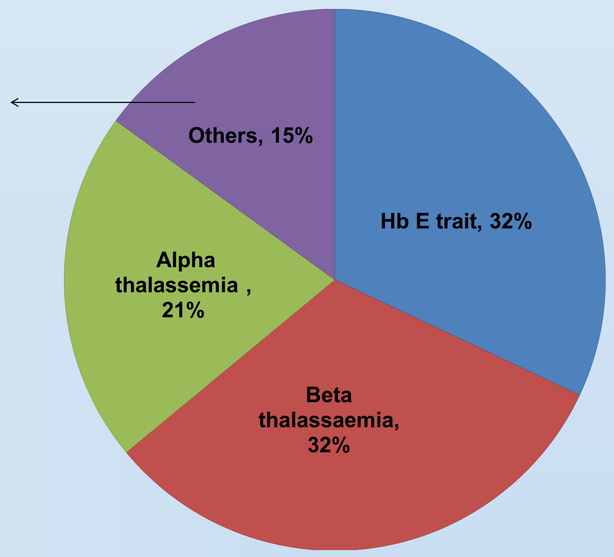
Graph 3: BioRad Variant II (HPLC) Chromatogram.



Graph 4: Sebia Capillarys2 (Capillary Zone Electrophoresis) Electrophoretogram.

3) In 6 months, about 4400 patient samples were screened. Hb E variant made up 32% of haemoglobin variants detected. Direct quantification of Hb E allowed for detection of patients with likely co-inheritance of alpha thalassaemia trait.

Others (15%);
- Compound heterozygous Hb E / beta thalassaemia
- Delta-beta thalassaemia trait
- Delta thalassaemia
- Delta haemoglobin variant
- Hb Constant-Spring trait
- Hb D trait
- HbH disease
- Hb J Kaoshiung
- Hb J trait
- Hb Lepore
- Hb New York
- Hb Q Thailand
- Hb S trait (sickle cell trait)
- Homozygous Hb D
- Homozygous Hb E



Conclusion:

There is a good correlation between the CE and HPLC methods. Sebia Capillarys2 analyser is an acceptable and accurate method of screening for thalassaemia and haemoglobinopathies. Furthermore, it is particularly suitable in our local population with a known high prevalence of Hb E variant.

Acknowledgement:

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