



Comparison of the “Hemoglobin(e)” and “HbA1c” kits for the Hb A2 and Hb S quantification on the CAPILLARYS 2 Flex Piercing (Sebia)

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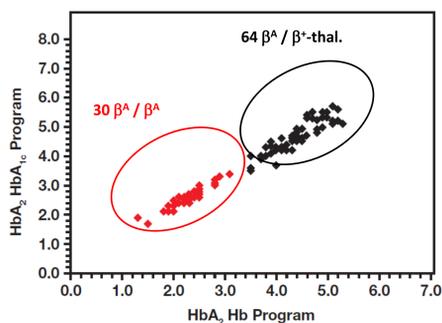
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Introduction

Quantification of Hb A2 and Hb S levels is classically done by cation-exchange HPLC or capillary electrophoresis (CE), with a dedicated kit often different from the one used for Hb A1c quantification. However, a reliable and accurate estimation of these two parameters during Hb A1c measurement could be very interesting in several situation: (i) For Hb A2; fortuitous diagnosis of a Beta-thalassemic trait, a status that is especially important to diagnose for a diabetic patient as it might lead to a decrease of the red blood cells lifespan (80 to 90 days) (1) and thus to an under-estimation of the Hb A1c level compared to the real glycemic equilibrium; (ii) For Hb S: sporadic demands of Hb S measurement for laboratories that did not realize hemoglobin status assessment in routine. Even for these lasts, in case of urgent request of Hb S quantification (Sickle Cell Disease patient that need to be transfused urgently), the possible use of the “HbA1c” kit would avoid the change of the kit if it is not the right one installed.

Hb A2 level measurement with the “HbA1c” kit

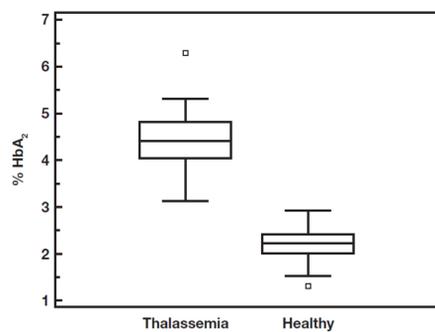
Princeps study: Dr. Urrechaga, 2012 (2)



Linear regression versus the “Hemoglobin(e)” kit (n=94)

$$\text{Hb A2 ("HbA1c" kit)} = 0.98 \text{ Hb A2 ("Hemoglobin(e)" kit)} + 0.35, \text{ with } r^2=0.98$$

Correlation is excellent but probable negative bias

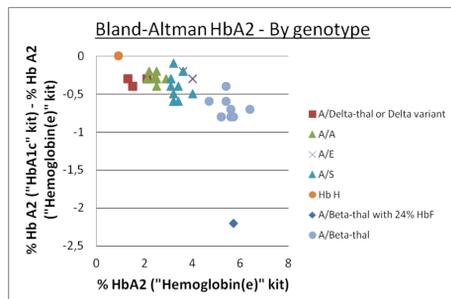


Beta-thalassemic trait diagnosis (n=279)

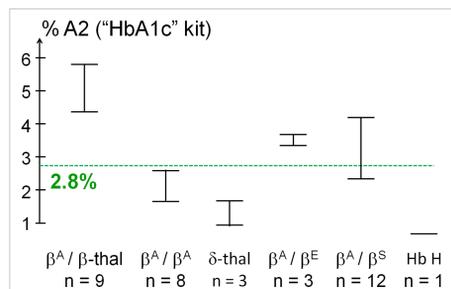
With a cut-off of 2.8%, sensitivity and specificity are equal to 100%

Validation study: Dr. Joly, 2013

Smaller number of patients but wider panel of genotypes



Non informative Hb A2 level if associated Beta variant



Invalid Hb A2 measurement with the “HbA1c” kit when Hb F > 15%

Conclusion

Despite a negative bias of approximately 0.6% compared to the reference “Hemoglobin(e)” kit, the “HbA1c” kit proves to be perfectly able to diagnose a Beta-thalassemic trait, by using a cut-off of 2.8%. The bias observed between the two kits can be partially explained by the separation of the Hb A2 glycosylated fraction.

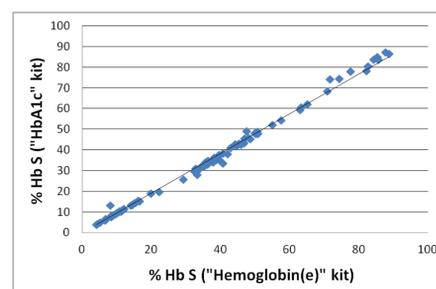
References

- Gallo E, Pich P, Ricco G, et al. The relationship between anemia, fecal stercobilinogen, erythrocyte survival, and globin synthesis in heterozygotes for beta-thalassemia. Blood. 1975 Nov;46(5):693-8.
- Urrechaga E. High-resolution HbA(1c) separation and hemoglobinopathy detection with capillary electrophoresis. Am J Clin Pathol. 2012 Sep;138(3):448-56.

Hb S level measurement with the “HbA1c” kit

Correlation study

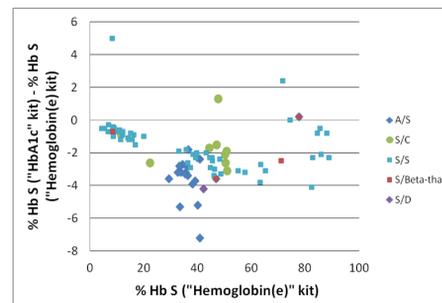
n=91 patients: 17 A/S, 4 S/beta-thal., 9 S/C, 1 S/D-Punjab and 60 S/S before and after transfusion. Hb S level covers all the analytical range.



Linear regression versus the “Hemoglobin(e)” kit

$$\text{Hb S ("HbA1c" kit)} = 0.96 \text{ Hb S ("Hemoglobin(e)" kit)}, \text{ with } r^2=0.99$$

Correlation is excellent but probable negative bias for the “HbA1c” kit

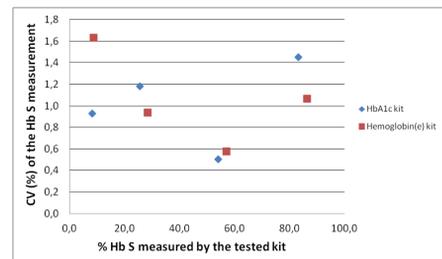


Bland-Altman (by genotype) versus the “Hemoglobin(e)” kit

~2% negative bias compared to the “Hemoglobin(e)” kit, independently of the beta-globin genotype

Intra-assay precision study

Assessed on 3 series of 10 measurements, at 4 concentration levels



CV from 0.5 to 1.5% for the “HbA1c” kit

CV from 0.6 to 1.6% for the “Hemoglobin(e)” kit

Inter-assay precision study

Assessed on 10 series of 3 measurements, at 4 concentration levels

CV from 0.3 to 1.1% for both “HbA1c” and “Hemoglobin(e)” kits

Conclusion

The “HbA1c” kit seems to be perfectly suitable for the Hb S level follow-up in Sickle Cell Disease patients, as the systematic observed bias compared to the reference “Hemoglobin(e)” kit is not clinically significant. The use of the “HbA1c” kit could also allow the pathologist to fortuitously screen heterozygous Hb A/S, keeping in mind that this diagnosis will need to be confirmed by two other independent biochemical techniques.