INFLUENCE OF FETAL HEMOGLOBIN ON HbA1c MEASUREMENT USING 3 DIFFERENT CAPILLARY ELECTROPHORESIS INSTRUMENTS

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INTRODUCTION

Elevated Fetal Hemoglobin (HbF) has been reported to interfere with some assay methods for HbA1c. There are many clinical conditions associated with elevated HbF (>1%), such as β-thalassemia, pregnancy, leukemia, and hereditary persistence of fetal hemoglobin. Using capillary electrophoresis (CE) method for HbA1c measurement, HbF is clearly separated from HbA1c fraction. But as HbF migrates closely to HbA0 fraction, and because HbA0 fraction is included in the calculation formula used to measure HbA1c value, an interference of HbF in the HbA1c measurement by CE method might be suspected. Here we evaluated the influence of HbF at different levels in the measurement of HbA1c by several capillary electrophoresis instruments.

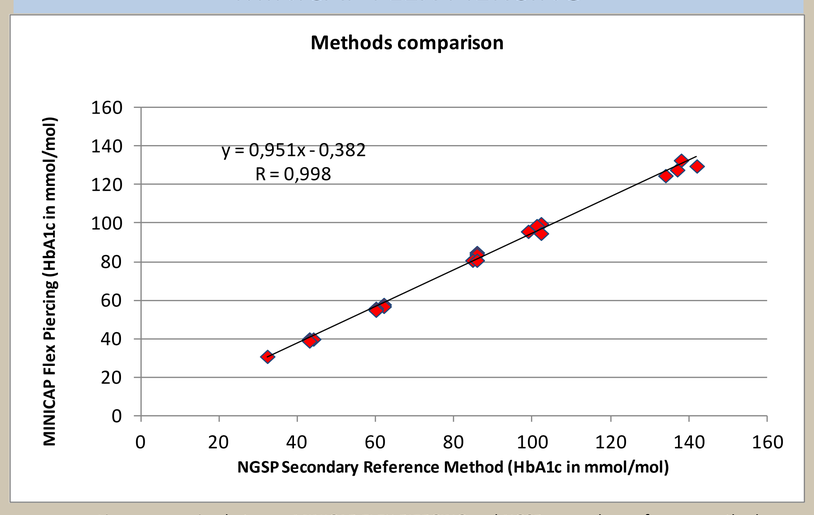
METHODS

6 adult whole blood samples showing different HbA1c levels (from 32 to 138 mmol/mol) were serially diluted with a cord blood sample with elevated HbF (>90%) to get different HbF levels (from 1.5% to 23.7 %). For all samples, the HbF level was determined on the CAPILLARYS 2 Flex Piercing Hemoglobin(e) technique (Sebia, France). Each native and diluted sample was then split in 4 aliquots. 3 aliquots were run on 3 routine CE instruments for HbA1c testing: MINICAP Flex Piercing (MCF), CAPILLARYS 2 Flex Piercing (C2FP) and CAPILLARYS 3 TERA (C3T) (Sebia, France). 1 aliquot was analyzed on a NGSP secondary reference method (TOSOH G8) that is known to be free of interference from HbF, used as the comparative method[1,2] . Limits of agreement were defined as ± 10% relative bias from the NGSP secondary reference method.

RESULTS

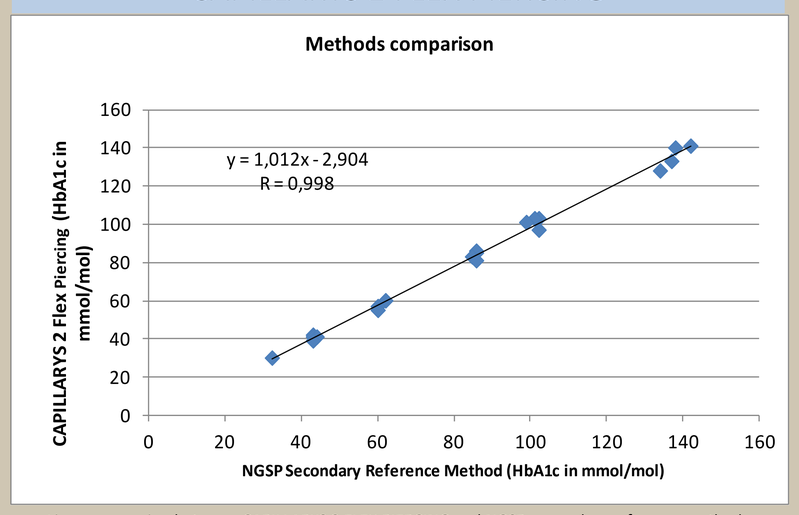
Methods comparison showed a good correlation between each CE method and the NGSP method when all native and diluted samples were analyzed (linear regression y= 0.951x -0.382 and a coefficient of correlation R=0.998 for MCF; y=1.012x -2.904 and R=0.998 for C2FP; y= 0.980x -1.175 and R=0.997 for C3T). The mean deviations at 30, 60 and 90mmol/mol were successively 1.9, 3.3 and 4.8 mmol/mol on MCF; 2.6, 2.2 and 1.9mmol/mol on C2FP; 1.8, 2.4 and 2.9mmol/mol on C3T, showing no major deviation from the comparative method. No result exceeded 10% bias from the NGSP secondary reference method.

MINICAP FLEX PIERCING



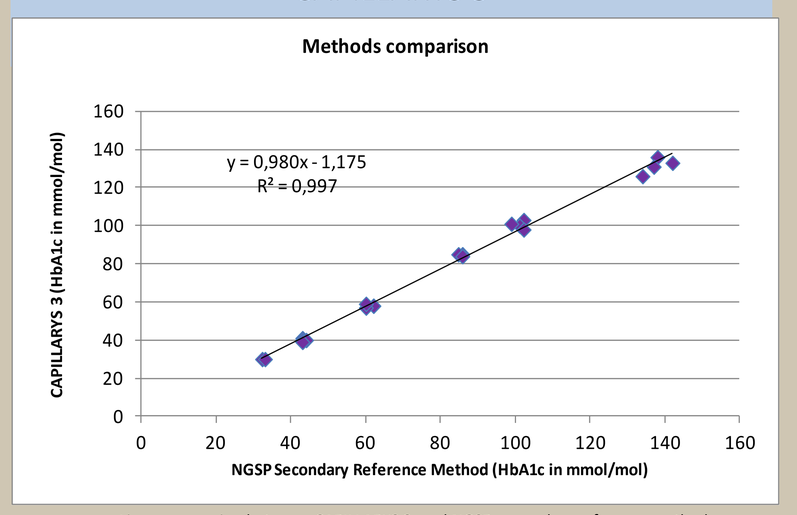
Linear regression between MINICAP FLEX PIERCING and NGSP secondary reference method

CAPILLARYS 2 FLEX PIERCING

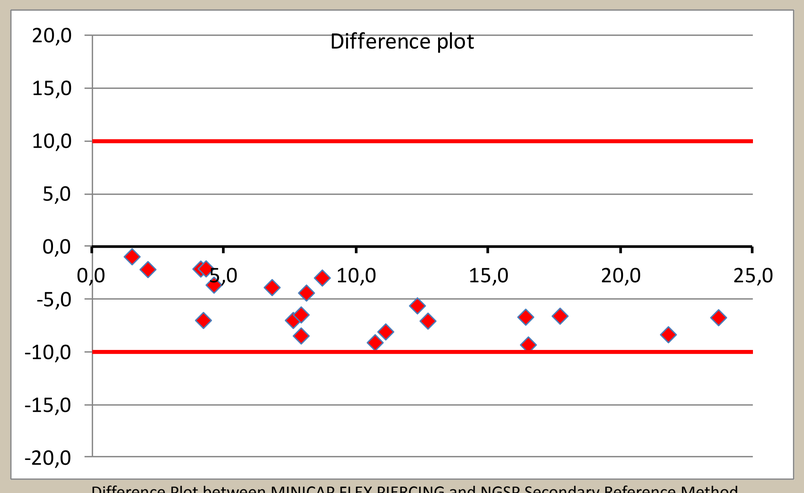


Linear regression between CAPILLARYS 2 FLEX PIERCING and NGSP secondary reference method

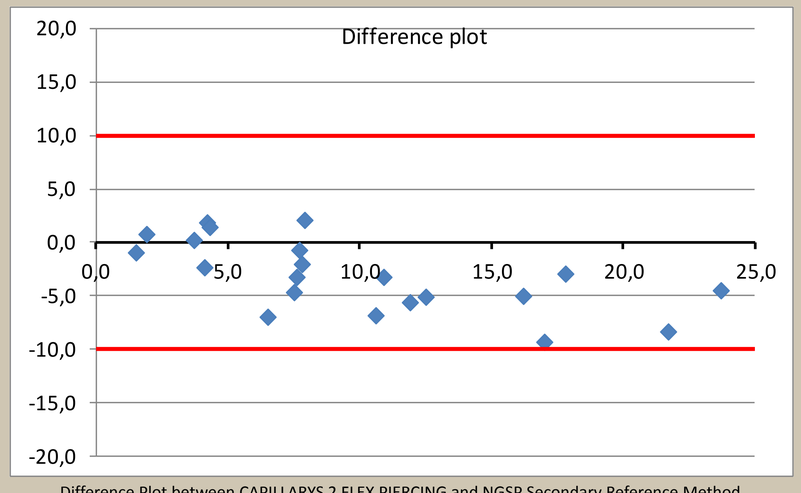
CAPILLARYS 3



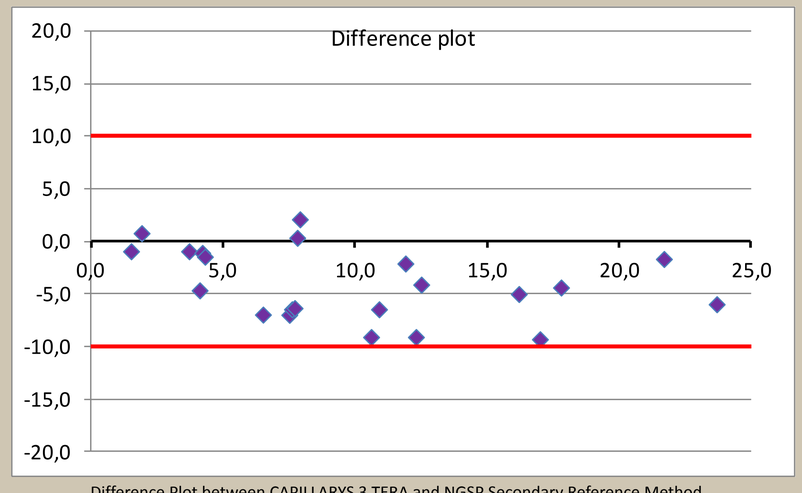
Linear regression between CAPILLARYS 3 and NGSP secondary reference method



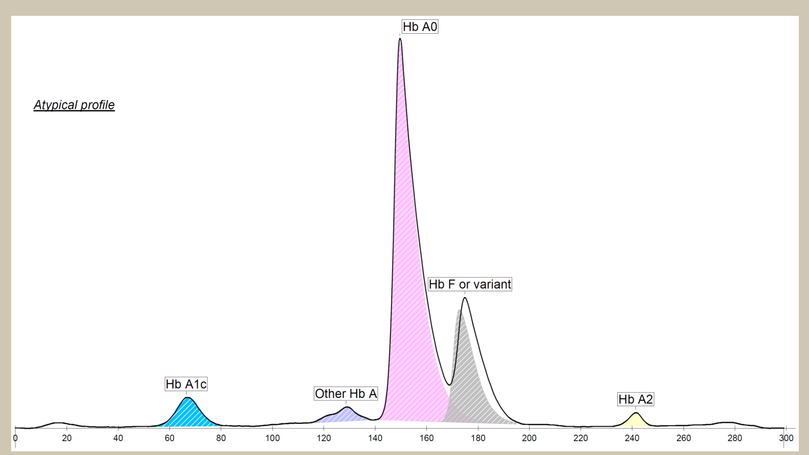
Difference Plot between MINICAP FLEX PIERCING and NGSP Secondary Reference Method
X-axis: HbF values obtained on CAPILLARYS 2 Flex Piercing Hemoglobin(e) technique, (%);
Y-axis: relative difference between MINICAP FLEX PIERCING and NGSP Seconday Reference Method on HbA1c value, (%); red lines represent the limits of agreement



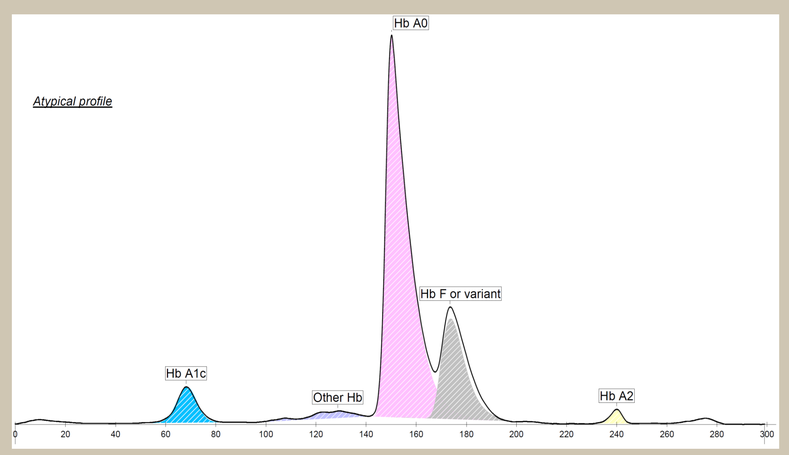
Difference Plot between CAPILLARYS 2 FLEX PIERCING and NGSP Secondary Reference Method
X-axis: HbF values obtained on CAPILLARYS 2 Flex Piercing Hemoglobin(e) technique, (%);
Y-axis: relative difference between CAPILLARYS 2 FLEX PIERCING and NGSP Secondary Reference Method on HbA1c value, (%); red lines represent the limits of agreement



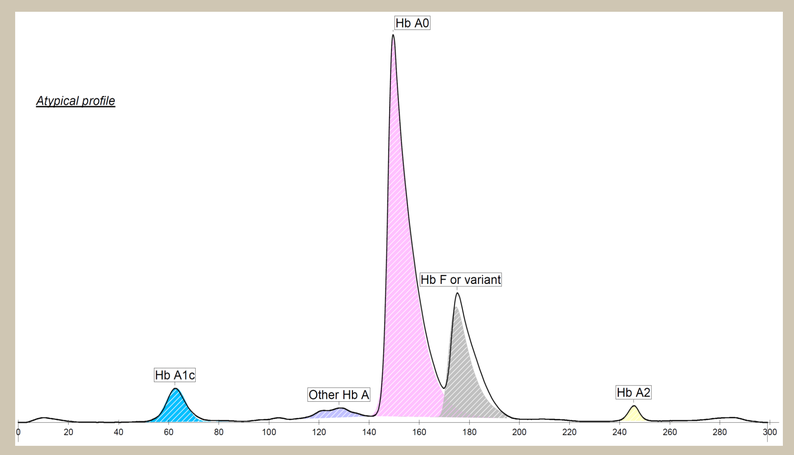
Difference Plot between CAPILLARYS 3 TERA and NGSP Secondary Reference Method
X-axis: HbF values obtained on CAPILLARYS 2 Flex Piercing Hemoglobin(e) technique, (%);
Y-axis: relative difference between CAPILLARYS 3 TERA and NGSP Seconday Reference Method on HbA1c value, in (%); red lines represent the limits of agreement



Profile MINICAP FLEX PIERCING with 21.7% HbF



Profile CAPILLARYS 2 FLEX PIERCING with 21.7% HbF



Profile CAPILLARYS 3 with 21.7% HbF

CONCLUSION

This evaluation showed that none of the CE methods tested is subject to interference with HbF up to 23% on the measurement of HbA1c. MINICAP Flex Piercing, CAPILLARYS 2 Flex Piercing and CAPILLARYS 3 TERA can reliably report accurate HbA1c results in case of elevated HbF.

REFERENCES

[1]Little RR , Rohlfing CL, Hanson SE, Schmidt RL, Lin C-N, Madsen RW, and Roberts WL. The Effect of Increased Fetal Hemoglobin on 7 Common Hb A1c Assay Methods. Clin Chem 2012 58: 945-6

[2]Shu I, Devaraj S, Hanson SE, Little RR, Wang P. Comparison of hemoglobin A1c measurements of samples with elevated fetal hemoglobin by three commercial assays. (Letter) Clin Chim Acta 2012;413:1712-1713.

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