

Development and validation of measuring hematocrit levels in dried blood spots using Near-Infrared spectroscopy

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Background: The use of dry blood spots (DBS) as a sampling method for drug monitoring has gained more popularity in recent years. For patients, DBS has several advantages over venous blood sampling. DBS is less invasive compared to standard venipuncture and can be performed by patients themselves at home. Lastly, DBS sampling is especially advantageous in infants and children due to the small volume and being less invasive. Although DBS has many advantages, a few technical issues, primarily influenced by hematocrit levels, interfere with the implementation of this method in daily clinical practice. The results for drugs that are influenced by hematocrit should be corrected for hematocrit levels. We developed a NIR method to measure hematocrit levels in DBS using a nondestructive method.

Method: A partial least squares (PLS) algorithm was used to build a quantification method with near-infrared (NIR) to measure hematocrit levels between 0.2 – 0.5 L/L. 522 venous blood samples were used to build this PLS model. The method was validated in 40 DBS samples, created by adding a small amount (50 µL) of blood on a Whatman filter paper and dried for 24 hours in a desiccator cabinet. Extra samples with high HIL-index were measured to test robustness of the method.

Results: The difference between hematocrit measurements with NIR spectroscopy and with the hematology analyzer was less than 15% for all 40 samples, during the method comparison. The accuracy and precision for all QC levels were within the acceptance criteria of <15%. By measuring samples with a high HIL-index there was no deviation visible, all samples have a relative error of <15%. The stability of hematocrit in DBS is up to 14 days for all levels.

Conclusion: During this study, we developed and analytically validated a hematocrit model using NIR spectrometry. The method is accurate, reproducible, short analysis time (51 seconds) and can analyze DBS samples up to 2 weeks, stored in a desiccator cabinet.