VALIDATION OF A NOVEL NON-CONTACT METHOD TO ESTIMATE THE HEMATOCRIT OF DRIED BLOOD SPOTS

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The hematocrit (Hct) effect is considered to be one of the most crucial issues in dried blood spot (DBS) analysis. Since the Hct of a blood sample affects blood viscosity and hence, the volume of blood contained in a fixed-size DBS punch, deviating Hct values can significantly impact DBS-based quantitation. To evaluate the extent of the Hct effect for a given DBS we previously developed a method that allows estimating the Hct of a DBS based on its potassium (K^+) content [1]. Additionally, using caffeine and paraxanthine as model compounds, it was shown that the K^+ content could also be employed to introduce a Hct specific correction factor (utilizing a K^+-based correction algorithm) which alleviates the Hct effect [2].
Although this K^+-based method yielded good results when applied to patient samples, it also suffered from some practical drawbacks, as it consumed part of the DBS and required additional sample preparation. Therefore, we now developed a non-destructive method, which allows to predict a DBS' Hct using non-contact diffuse reflectance spectroscopy. This way, mere scanning of a DBS suffices to derive its Hct.

This non-contact method was successfully validated based on FDA guidelines. A linear calibration model after log/log transformation best described the data. The bias, intra- and interday imprecision at low, mid and high Hct, as well as at the lower (0.20) and upper (0.67) limit of quantitation were always within 15%. Stability at ambient conditions after storage for up to five months and stability at 60°C for up to three days (the latter mimicking extreme transport conditions) were evaluated at three Hct levels. Additionally, DBS-specific aspects (i.e. the volcano effect and the volume effect) were evaluated during validation, again at three Hct levels. Although a slight volcano and volume effect were discerned, this was of no practical influence (i.e. within 15%).

In a next step, the non-contact Hct estimation method was applied to 233 patient DBS with varying Hct values (Hct = 0.20 - 0.50). The Hct of these patient DBS was estimated using the non-contact method, whilst the true Hct of the patient samples was determined on the corresponding venous whole blood samples using a Sysmex XE-5000 hematology analyzer. The non-contact method was found fit for purpose, since i) a good correlation was observed between the estimated and true Hct ($r = 0.95$), ii) the limits of agreement obtained with Bland and Altman analysis were very similar to those obtained with the K^+-based method and since iii) over 95% of the estimated Hct values were within 15% of the corresponding true Hct values. In addition, incurred sample reanalysis demonstrated the excellent reproducibility of the method, since, with a single exception of 5.4%, all reanalysis results were within 3.5% of the corresponding average of the original and reanalysis result.

[1] Capiau *et al*. Anal Chem, 2013.
[2] De Kesel *et al*. Anal Bioanal Chem, 2014.