Development and validation of hematocrit level measurement in dried blood spots using Near-Infrared spectroscopy (NIR)

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Background

The use of dried blood spots (DBS) as a sampling method for therapeutic drug monitoring has gained more popularity in recent years. For patients, DBS sampling has several advantages over venous blood sampling. However, technical issues, primarily influenced by hematocrit levels, interfere with the implementation of this method in daily clinical practice. The results of concentration measurements of drugs that are influenced by hematocrit should be corrected for hematocrit levels. We developed a fast, nondestructive, near-infrared (NIR) method to measure hematocrit levels in DBS.

Methods

After analysis of the hematocrit concentration (range 0.2-0.5 L/L) in a venous sample, leftover material was used to pipet a blood spot (50 µL) on Whatman protein saver 903 filter paper and dried for 24h in a desiccator cabinet.

A partial least square (PLS) algorithm was used to build a quantification method from 522 DBS with near-infrared spectroscopy (NIR) using Shimadzu Fourier-transform infrared spectroscopy.

Method validation:
- Comparison to hemocytometry analyzer in 40 spotted DBS samples
- 24 extra DBS samples with high hemolysis, icterus or lipemia index (HIL-index) to test influence of these factors

Results of method comparison NIR and hemocytometry analyzer:
- Difference between hematocrit less than 15% for all 40 samples.
- Bland-Altman analysis (fig.2A): mean bias of 0.0006 (95%CI -0.0053-0.0065)
- Deming regression (fig. 2B): slope of 0.89 (95%-confidence interval 0.81-0.97) and an intercept of 0.04 (95%-confidence interval 0.01-0.07). Very small, significant (p<0.001) difference from a slope of 1 and interval of 0.
- Intra-assay CV for this method comparison was 3.0%, which is within the acceptance criterion of <10%.
- Paired t-test: no significant difference of the mean of both tests (p=0.837).
- Accuracy, precision and robustness for 5 QC levels <10% (table 1).

Validation

Measurement of 24 DBS samples with high HIL-index showed no deviations (RE<15%). The stability of hematocrit after spotting the DBS sample is up to 14 days for all QC levels.

Table 1: Validation results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hematocrit on Sysmex (L/L)</th>
<th>Inter-day</th>
<th>Intra-day</th>
<th>Robustness</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC 1</td>
<td>0.21</td>
<td>3.02</td>
<td>2.19</td>
<td>4.10</td>
</tr>
<tr>
<td>QC 2</td>
<td>0.26</td>
<td>2.24</td>
<td>1.90</td>
<td>3.04</td>
</tr>
<tr>
<td>QC 3</td>
<td>0.30</td>
<td>3.83</td>
<td>3.78</td>
<td>4.57</td>
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<tr>
<td>QC 4</td>
<td>0.40</td>
<td>3.91</td>
<td>3.72</td>
<td>4.74</td>
</tr>
<tr>
<td>QC 5</td>
<td>0.52</td>
<td>3.47</td>
<td>3.04</td>
<td>3.91</td>
</tr>
</tbody>
</table>

Accuracy = (mean accuracy) / (RSD% - RSD%)
Precision = (mean precision) / (RSD% - RSD%)
Robustness = (mean robustness) / (RSD% - RSD%)

Conclusion

We developed and validated a hematocrit model using NIR spectrometry. The method is nondestructive, accurate, reproducible, has a short analysis time (51 seconds) and can analyze DBS samples up to 2 weeks, stored in a desiccator cabinet.

The next step would be to evaluate this method in true capillary blood samples and use these results to correct for concentrations measured using DBS.

Figure 1: NIR spectrum of blank filter paper (red line) and DBS sample with hematocrit of 0.21 (green line), 0.34 (blue line) and 0.52 L/L (black line). Ranges incorporated in the NIR analysis are marked blue.

Figure 2: Method comparison of 40 patient samples. A) Bland-Altman analysis, dotted lines indicate 15% deviation and the shaded area represents the bias of 0.0006 and 95% confidence interval. B) Deming regression, dotted line represents the regression line, the continuous line and grey area indicate the line of identity and the 15% acceptance criteria.