Introduction
Vitamin D₂ and D₃ are secosteroids found in supplements and dietary sources and, in the case of vitamin D₂, biosynthesised in skin on exposure to sunlight. Although there is disagreement about the effectiveness of vitamin D₂ supplementation for raising and maintaining plasma concentrations [1,2], the 25-hydroxy metabolites of both forms (25-OHD and 25-OHD₃) are usually quantified in serum and summed to assess an individual's vitamin D status.

Dried whole blood collected from finger-pricks, using volumetric absorptive microsampling (VAMS) devices, has been explored as an alternative matrix to serum / plasma for vitamin D status assessment with promising results [3]. This sampling technique could potentially allow individuals to self-collect samples at home and post them to laboratories for analysis.

There are additional considerations when designing and validating an assay suitable for quantification of an endogenous analyte from home-collected dried whole blood VAMS samples. These include:

- Calibration line construction
- Shipping and storage stability
- Sampling collection error
- The effect of haematocrit
- Comparison of analyte concentrations in VAMS finger-prick samples vs. serum

Methods
Whole blood samples were extracted and analysed from 10μL Neoteryx Mitra® devices as follows:

1. Internal Standard Addition
2. Protein-Clump
3. Liquid & Liquid Separation
4. Derivationation
5. Measuring and Sensing

Whole blood Mitra® samples were dried for a minimum of 16 hours at room temperature before extraction. Serum samples were extracted in an analogous manner to the VAMS samples, using a validated assay.

Calibration Lines
Calibration lines were prepared and tested using a range of different matrices including whole blood (i.e. standard addition), washed red blood cells (WRBC) combined with characterised serum, and WRBC combined with analyte-free matrix (e.g. phosphate buffered saline: PBS). A range of different haematocrits (or simulated haematocrits) were examined.

Assay Assessment
Assay validation included the following tests:

- Linearity
- Sensitivity and Selectivity
- Inter- / Intra- Precision and Accuracy
- Matrix Effects
- Carryover
- Recovery at Different Concentrations
- Recovery at Different Haematocrits
- Re-inject Stability
- Stability on Device at Different Storage Conditions
- Device Batch Comparison
- Effect of Anticoagulant
- Haematocrit Effect
- Device Loading
- Finger-Prick vs. Venepuncture Whole Blood Comparison
- Finger-Prick vs. Serum Comparison

Stability on Device
Stability of the analytes was assessed at different concentrations and using blood from different individual haematocrits under a range of conditions simulating those likely to be experienced during shipment and storage of samples (both with and without anticoagulant mimicking finger-prick samples and calibration standards / QC's).

Table 1: Example stability data for 25-OHD

<table>
<thead>
<tr>
<th>Sex / Hct</th>
<th>Anticoagulant?</th>
<th>Storage conditions</th>
<th>Serum concentration (nmol/L)</th>
<th>Average stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Yes</td>
<td>-20°C / 4 months</td>
<td>32.3</td>
<td>112% (CV 7.4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35.5</td>
<td>105% (CV 3.9%)</td>
</tr>
<tr>
<td>Male</td>
<td>Yes</td>
<td>-20°C / 10 months</td>
<td>13.5</td>
<td>105% (CV 4.9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18.9</td>
<td>105% (CV 5.8%)</td>
</tr>
<tr>
<td>Female</td>
<td>No</td>
<td>-20°C / 1 month</td>
<td>11.5</td>
<td>94% (CV 7.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.5</td>
<td>100% (CV 6.8%)</td>
</tr>
<tr>
<td>Male</td>
<td>No</td>
<td>-20°C / 1 month</td>
<td>13.2</td>
<td>95% (CV 7.4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31.4</td>
<td>97% (CV 5.7%)</td>
</tr>
</tbody>
</table>

Stability was found to be acceptable under the majority of conditions tested.

Device Loading
Different loading approaches replicating common sampling errors (e.g. double loading) were simulated and found to have a negligible impact on the measured concentrations of the analytes.

Haematocrit
The effect on sampler absorption was explored by spiking 25-OHD and labelled 25-OHD₂ into blood from different individual haematocrits, then loading onto VAMS devices. The differences in haematocrit did not introduce a significant bias into the measured analyte concentrations.

Table 2: Precision (coefficient of variance; CV) and accuracy (relative error; RE) of labelled 25-OHD₂ from whole blood VAMS samples of different haematocrits

<table>
<thead>
<tr>
<th>Hematocrit</th>
<th>Precision (CV)</th>
<th>Accuracy (RE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30%</td>
<td>F30%</td>
<td>F30%</td>
</tr>
<tr>
<td>40%</td>
<td>F40%</td>
<td>F40%</td>
</tr>
<tr>
<td>50%</td>
<td>F50%</td>
<td>F50%</td>
</tr>
</tbody>
</table>

These results were further supported by P and A tests where calibration lines were prepared using bloods with different haematocrits to the quality control samples.

Finger-prick vs. Serum Concentrations
Wet serum vs. dried finger-prick whole blood VAMS samples (taken independently by the donor) show good correlation between measured 25-OHD and 25-OHD₂ levels. Further sample collection is currently underway.

Conclusions
- An assay has been developed and tested that is suitable for 25-OHD₂ and D₃ quantification from VAMS samples.
- Measured analyte concentrations were not significantly affected by the sampling and storage conditions investigated (simulating home-collection).
- The relationship between dried whole blood versus serum has been tested, but further assessment is required as an area of continuing and future work.

References

Figure 1: 25-OHD (left) and 25-OHD₂ (right)

Figure 2: Schematic of the extraction procedure

Figure 3: Calibration lines prepared using different matrices

Figure 4: 25-OHD₂ concentrations from devices loaded using different approaches (red = male sample, blue = female sample). Shaded areas show concentrations within 15% of the prepared level

Figure 5: Comparison of serum vs. finger-prick whole blood VAMS concentrations for 25-OHD₂ (A and B) and 25-OHD₂ (C and D). VAMS concentrations in B and D have been corrected for haematocrit