Robust measurement of vitamin A in plasma and blood dried on paper

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Vitamin A is important in...

- Vision
- Immunity
- Reproduction
- Bone health

Vitamin A deficiency
Vitamin A toxicity
How do we measure it?

- Finger prick
- Dried Blood Spot
- Analysis
- Clinical applications

24h
• **Background**
  – Other attempts in literature have reported losses of at least 20%
  – Clarification of how to determine level of recovery

• **Aim**
  – To develop a simple and robust determination of vitamin A in dried blood spots

• **Target**
  – System needs to be stable at room temperature for at least 2 months
Methods

- HPLC method for retinol (Biomarker for Vitamin A) – linear response 0.05-2 µg/mL
- Conventional 2-phase solvent extraction of liquid plasma/blood – 100%
- Whatman 903® filter paper
  - Worldwide used for newborn screening
  - Even distribution of blood – to relate sample (punch) size to blood volume
Experimental Procedure

Retinol recovery/stability in blood spot

HPLC Assay
Results

Retinol recovery from plasma spots with protectants

![Graph showing retinol recovery percentages with different protectants: Control, Anti-ox_BHT, Chelat_EDTA, BHT+EDTA, VitC, BHT+VitC. The graph indicates higher recovery rates with protectants compared to the control. Antioxidants (Anti-ox), chelators (Chelator), and mild acid treatments show improved recovery.]
Confirmation of the acid effect

![Bar chart showing retinol recovery% for Control, Vit C, and Anti-ox_BHT with error bars. The chart compares MeOH and HAc MeOH concentrations.](chart)
Stability of vitamin A in whole blood on paper

![Graph showing stability of vitamin A in whole blood over time. The x-axis represents days, ranging from 0 to 80. The y-axis represents retinol concentration (μg/mL), ranging from 0 to 0.7. The graph indicates >95% recovery.]
Method validation

**Linearity**

- Dried blood spot
- Direct injection

\[
y = 1.0594x + 0.8538 \\
R^2 = 0.9939
\]

\[
y = 1.0208x - 0.0143 \\
R^2 = 0.9993
\]

**Precision**

- **Intra-day and inter-day errors**

<table>
<thead>
<tr>
<th></th>
<th>Intra-day (n=6)</th>
<th>Inter-day (n=13)</th>
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<tbody>
<tr>
<td>DBS retinol (μg/mL)</td>
<td>2.02 ± 0.03</td>
<td>2.13 ± 0.13</td>
</tr>
<tr>
<td>CV%</td>
<td>1.7</td>
<td>6.2</td>
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**Limit of Quantification**

0.05 μg/mL
Validation of capillary blood (DBS) as a marker of venous retinol

N = 24 Healthy subjects
Collected blood by venepuncture and by finger prick

Venous DBS vs Capillary DBS

Venous plasma vs Capillary DBS

r=0.9724
P<0.0001

r=0.9538
P<0.0001
Conclusions

• Confirmed that losses of vitamin A on paper are due to poor extraction rather than oxidation

• Extraction problems can be overcome by mild acidic conditions

• Retinol in DBS is stable for at least 10 weeks

→ A simple and robust method for vitamin A analysis
Waite Lipid Analysis Service (WLAS)

Here are some analytical services WLAS offer:

• Formerly known as the Waite Analytical Service (WAS)
• Tissue Total Fatty acids
• Tissue Lipid Class analyses (CE, TG, FFA, DG, PL)
• DBS Total Fatty acids for blood, serum/plasma, urine and breast milk
• Plasma Phospholipid fatty acids (fast separation from plasma)
• Breast milk DHA
• DBS Free fatty acids
• Fat soluble vitamins in blood, DBS, milk and other matrices (eg. vitamin D in DBS)
• Carotenoids
• Water soluble vitamins
• Others (eg. Phthalates)