



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

Yichao Huang, Peter Clements, and Robert Gibson

School of Agriculture, Food and Wine, The University of Adelaide

FOODplus

incorporating the

CENTRE OF RESEARCH EXCELLENCE

Foods for Future Australians

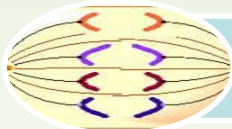
Vitamin A is important in...



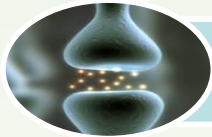
Vision



Immunity



Reproduction



Bone health



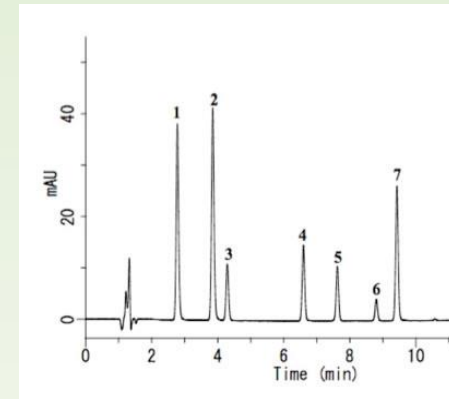
Vitamin A deficiency

Vitamin A toxicity

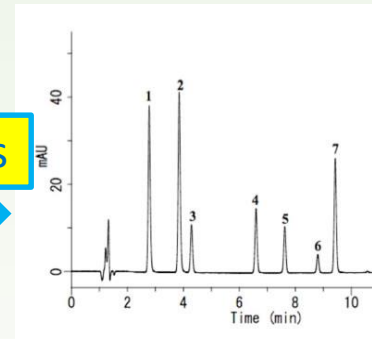
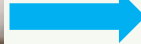
How do we measure it?



24h



Weeks



Finger prick

Dried Blood Spot

Analysis

Clinical applications

- **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

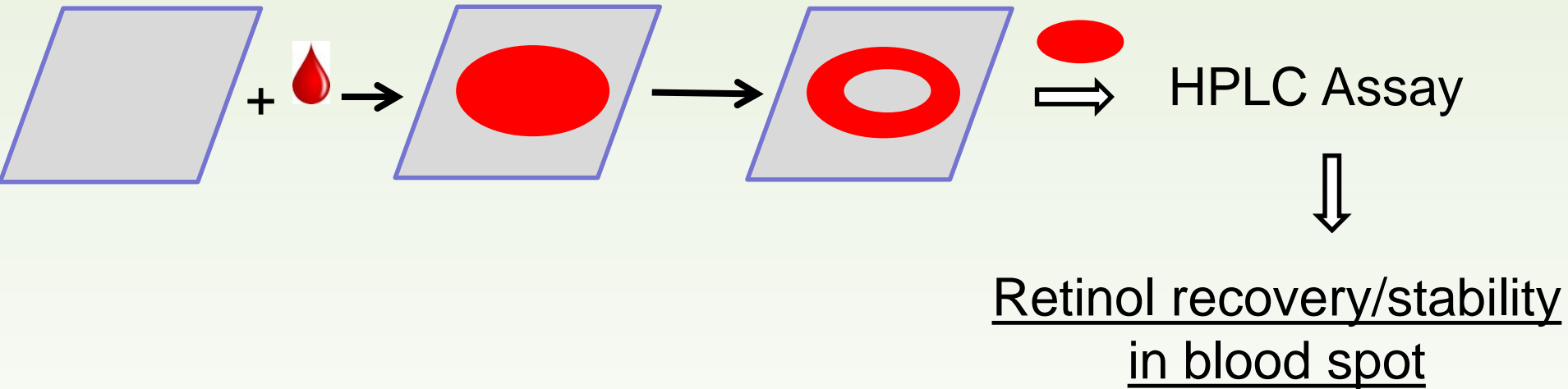
- **Target**

- System needs to be stable at room temperature for at least 2 months

Methods

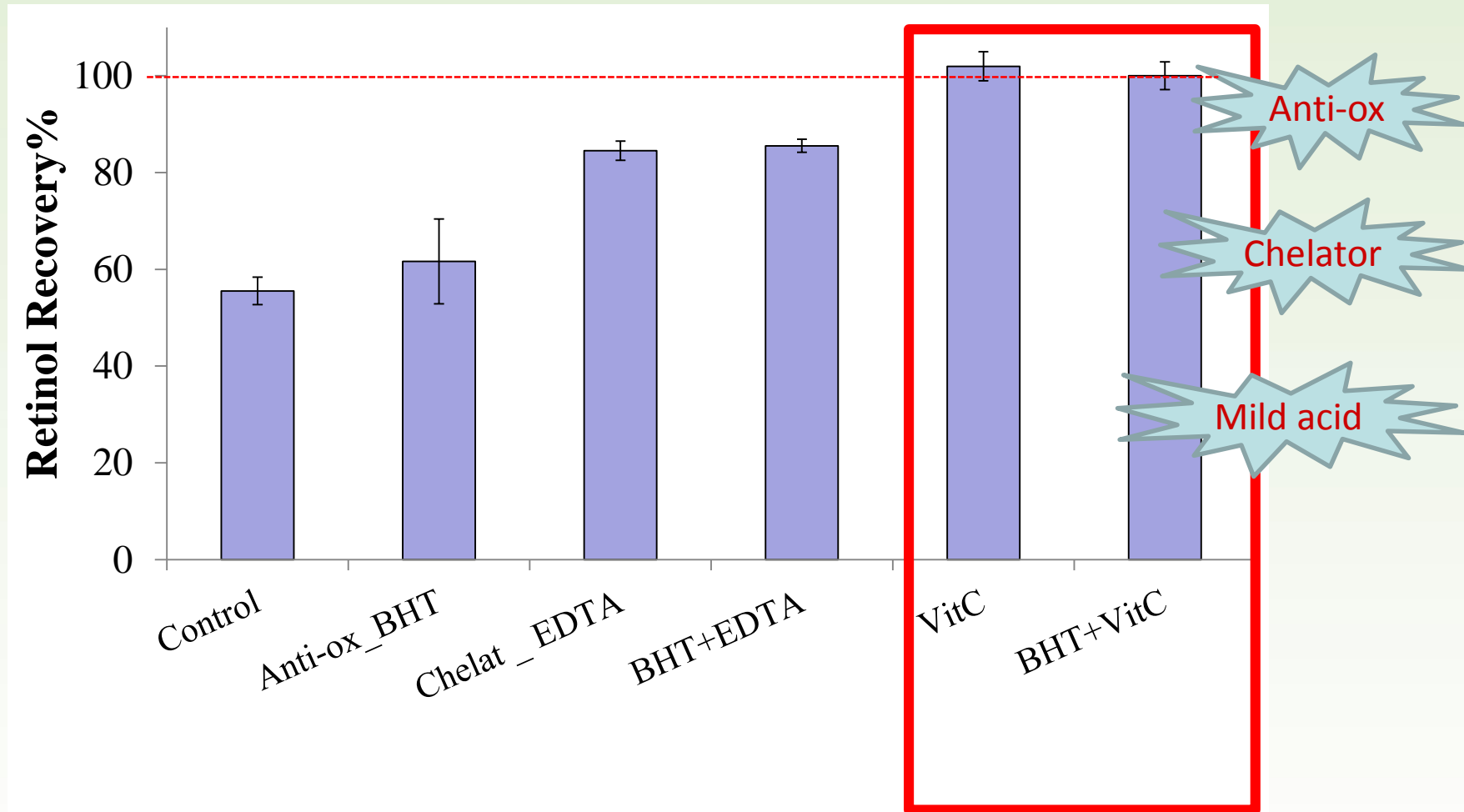
- HPLC method for retinol (Biomarker for Vitamin A) – linear response 0.05-2 $\mu\text{g}/\text{mL}$
- Conventional 2-phase solvent extraction of liquid plasma/blood – 100%
- Whatman 903[®] filter paper
 - Worldwide used for newborn screening
 - Even distribution of blood – to relate sample (punch) size to blood volume

Experimental Procedure

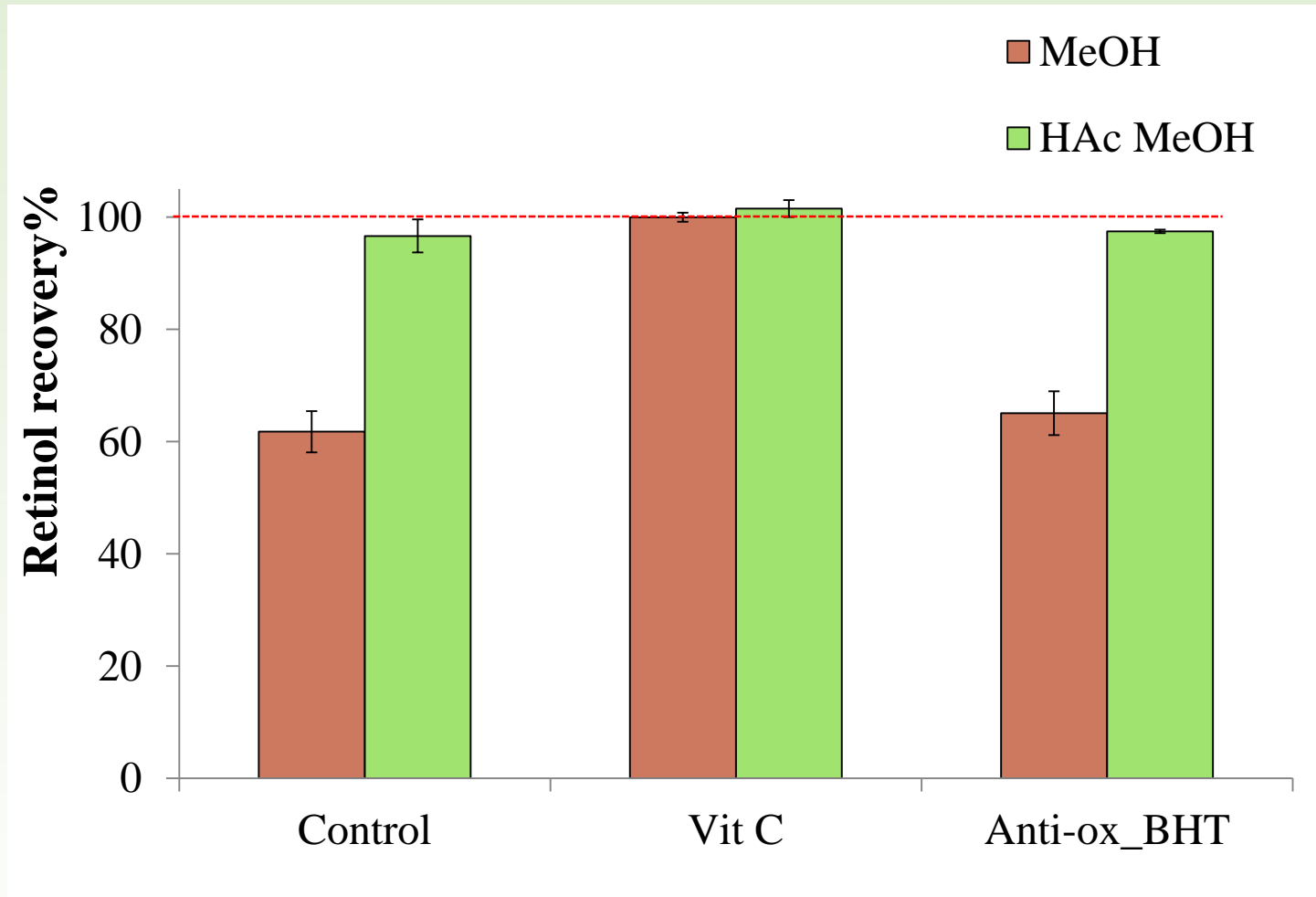


Results

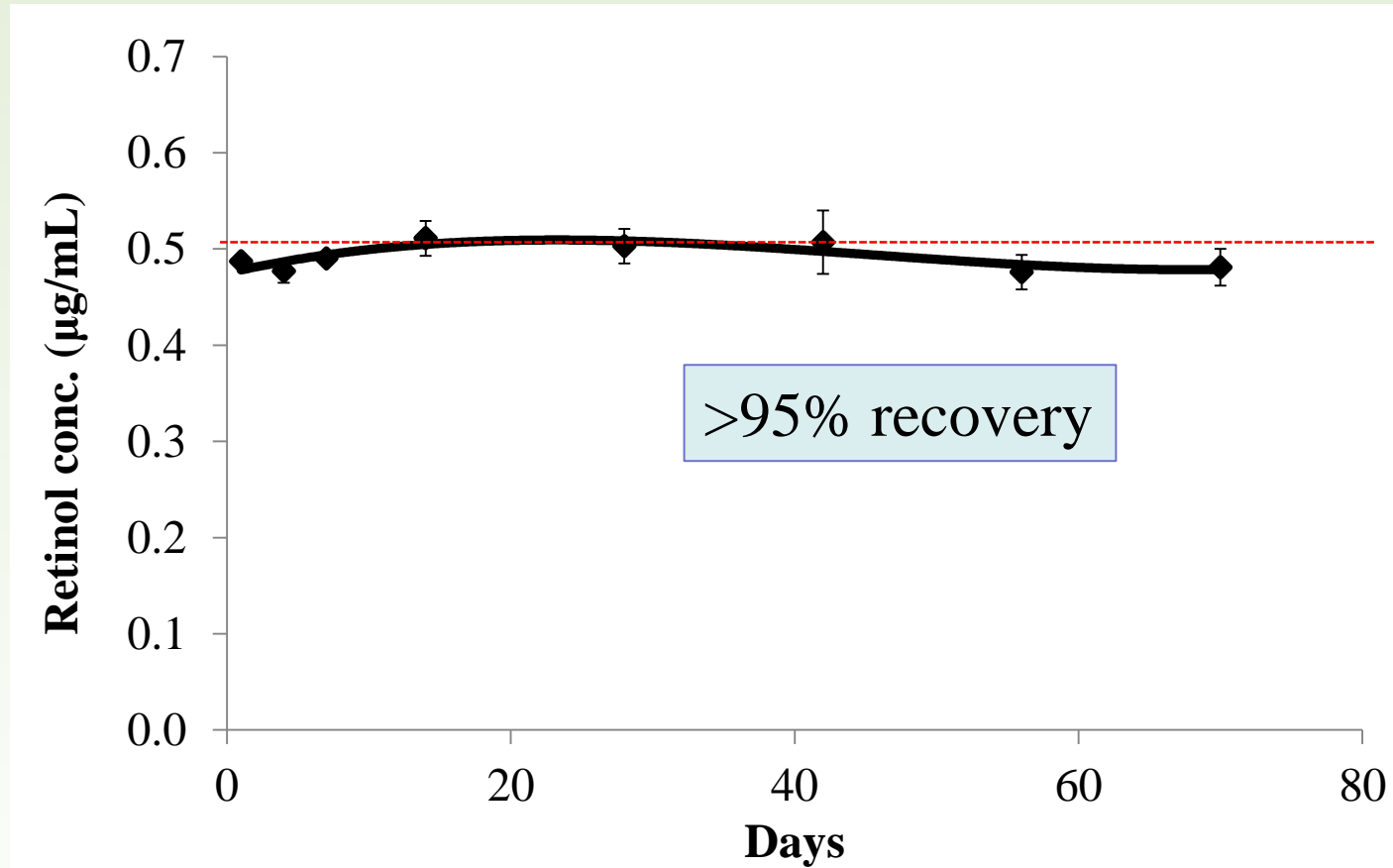
Retinol recovery from plasma spots with protectants



Confirmation of the acid effect

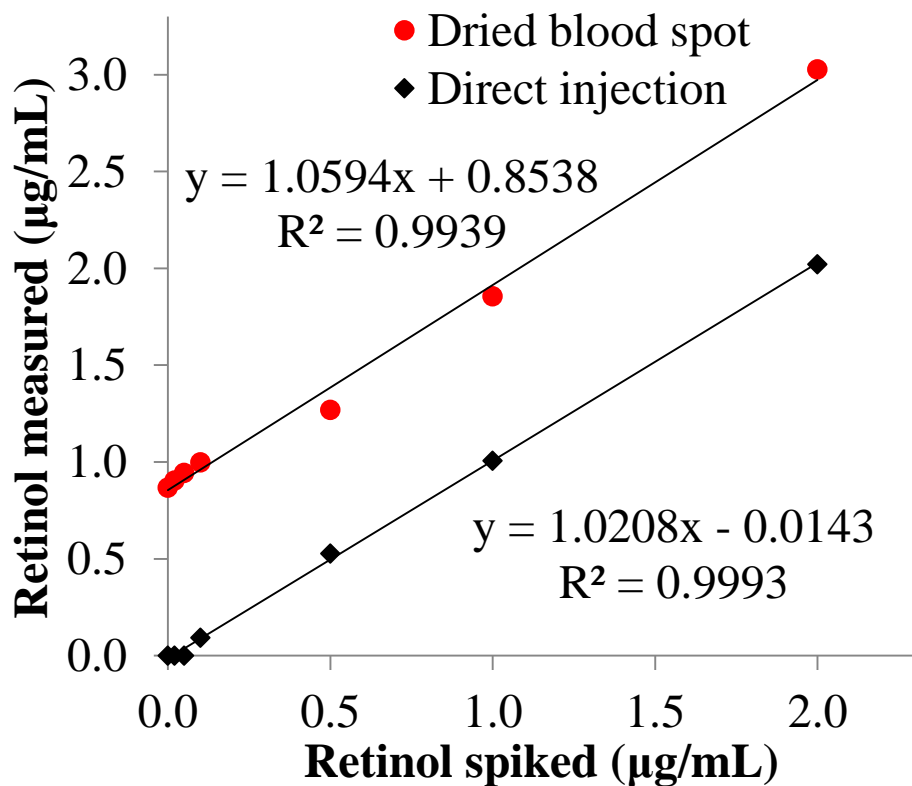


Stability of vitamin A in whole blood on paper



Method validation

Linearity



Precision

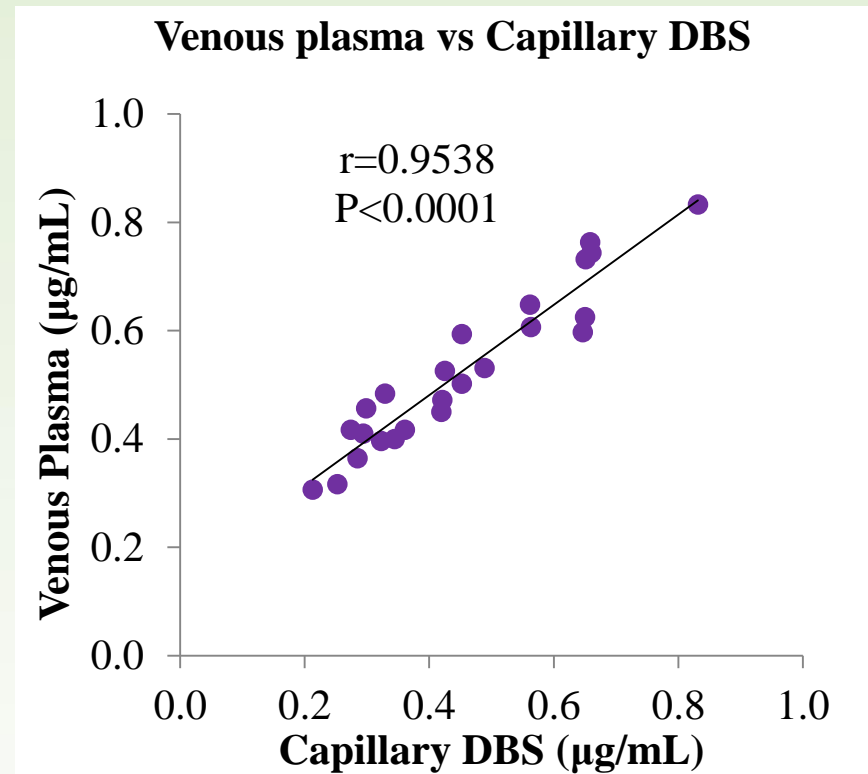
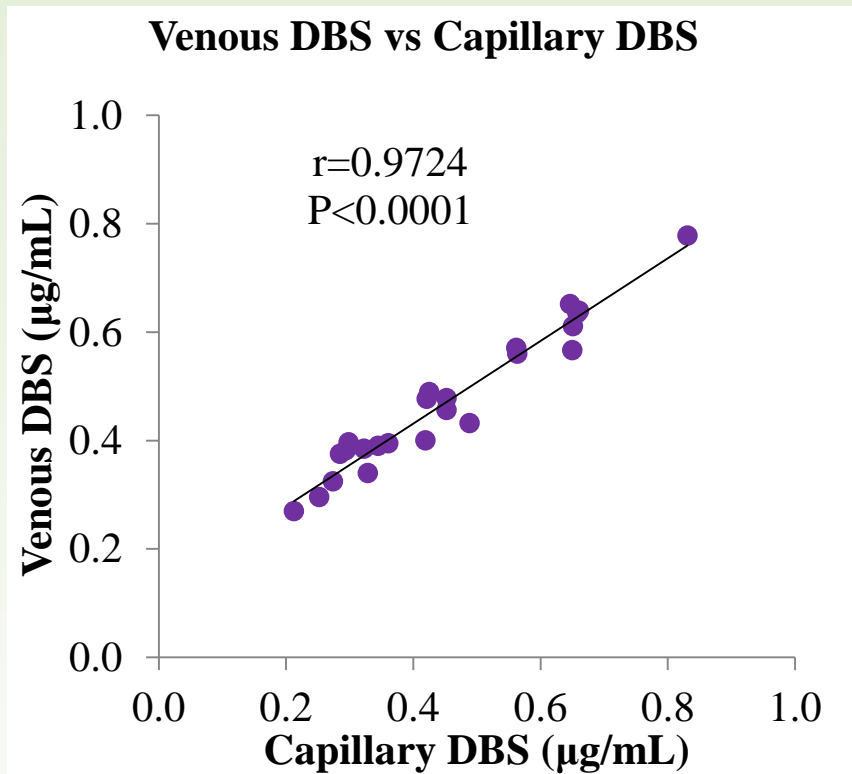
Intra-day and inter-day errors

	Intra-day (n=6)	Inter-day (n=13)
DBS retinol (µg/mL)	2.02 ± 0.03	2.13 ± 0.13
CV%	1.7	6.2

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

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)