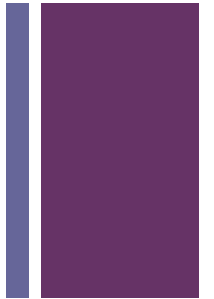


A review of the B's and C

Kirsten Hoad
 PathWest Laboratory Medicine WA – RPH
 Member of the AACB Vitamins Working Party

+ RCPA QAP Vitamins Program



1999

Vitamin A (retinol)

Vitamin E (tocopherol)

B-Carotene

Total carotenoids

2008

Vitamin B1

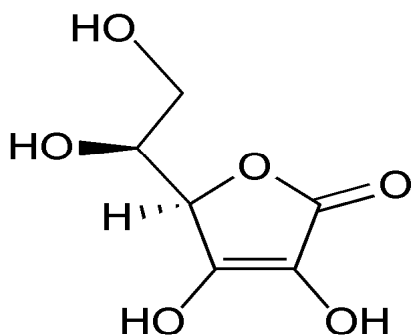
Vitamin B6

2010

Vitamin C



+ Vitamin C

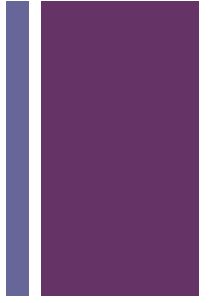


Ascorbic acid

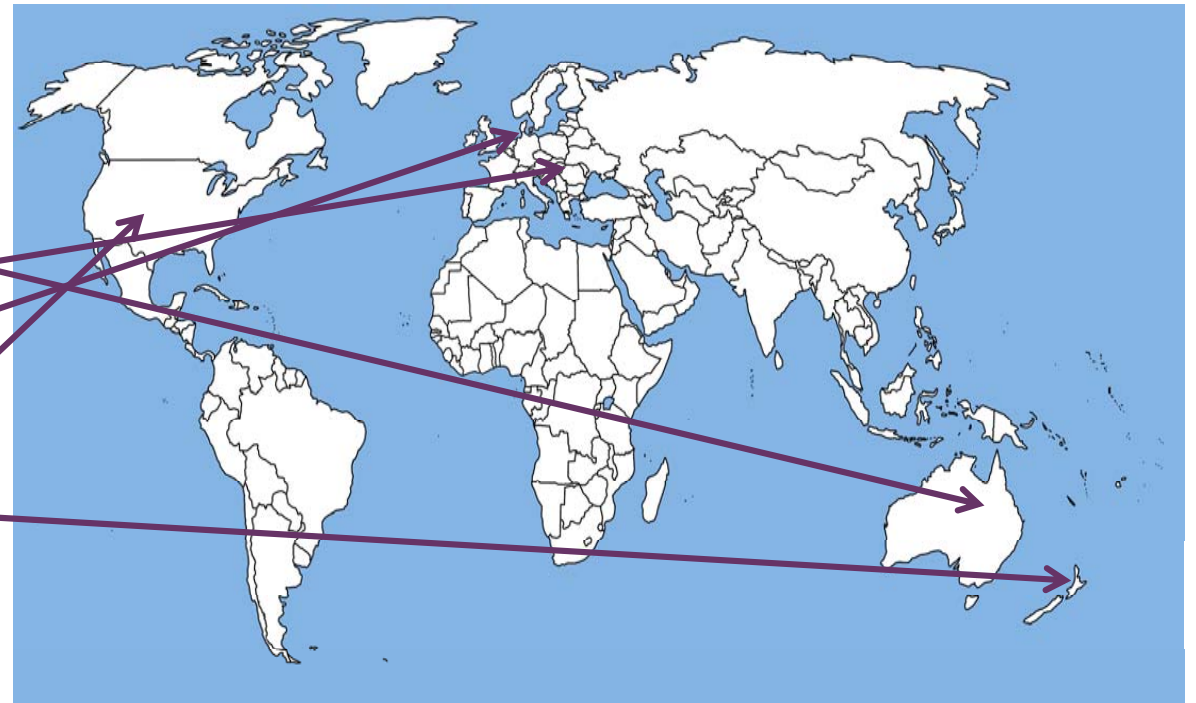
Vitamin C (ascorbic acid) anti-oxidant water soluble vitamin.

Analysis of vitamin C is most commonly performed by either HPLC (UV or ECD) or by spectrophotometric methods.

+ Vitamin C sub-program

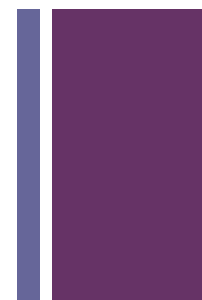


Participants (13)	Country
7	Australia
1	Austria
1	Netherlands
2	New Zealand
2	USA

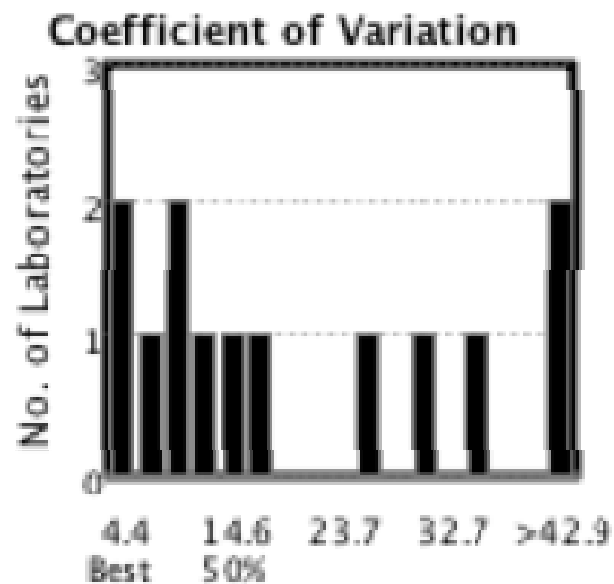


+ Vitamin C: End of cycle 22 report

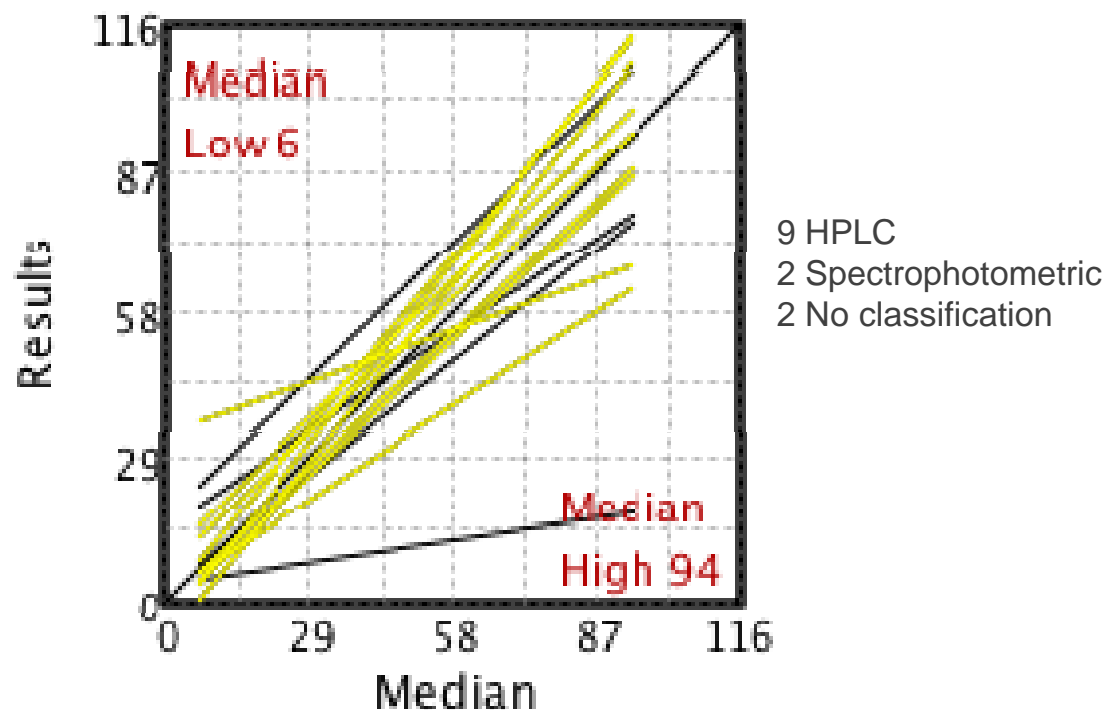
(www.rcpaqap.com.au/chempath)



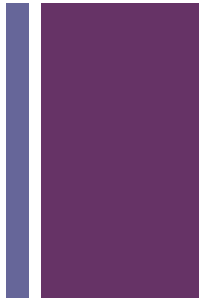
Intra-laboratory precision Analysis



Accuracy Analysis Regression Lines (9) HPLC



+ Vitamin B sub-program



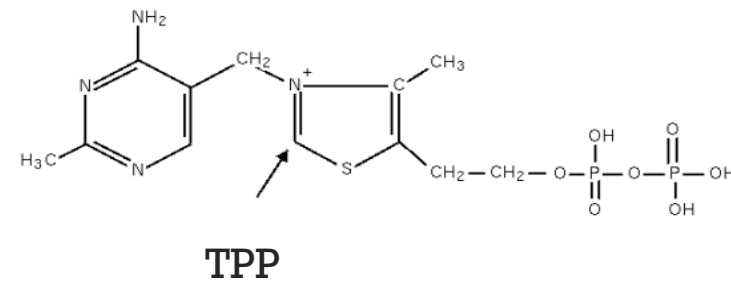
Currently vitamins B1 and B6 are offered.

The material for this sub-program is sourced from SKML.

Lyophilised whole blood matrix.

Country	Participants (13)
Australia	7
France	1
New Zealand	1
South Africa	1
USA	3

+ Vitamin B1



Forms:

- Thiamine pyrophosphate or diphosphate (TPP) (80%)
- Thiamine mono (TMP) and tri-phosphate
- free thiamine.

Erythrocytes: contain 80% of the blood TPP, small amounts of thiamine and TMP.

Plasma: contains mainly thiamine and TMP.

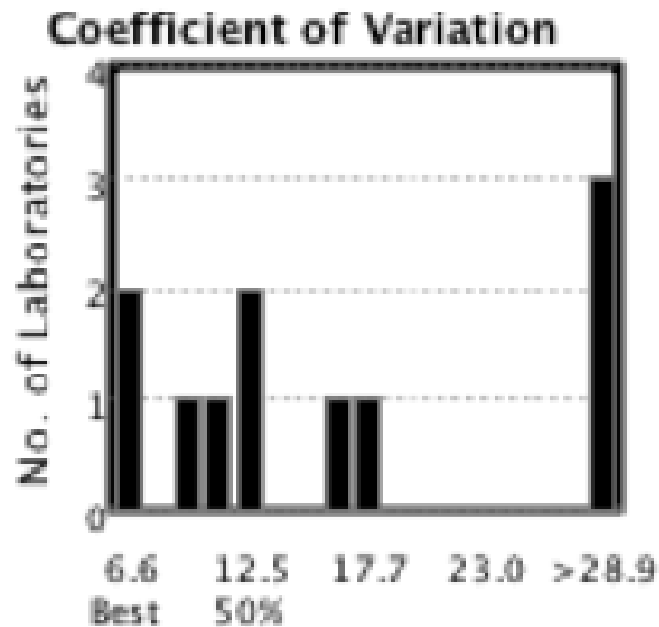
Measurement: RBC transketolase activity, HPLC with fluorescence detection.

+ TPP: End of cycle 22 report

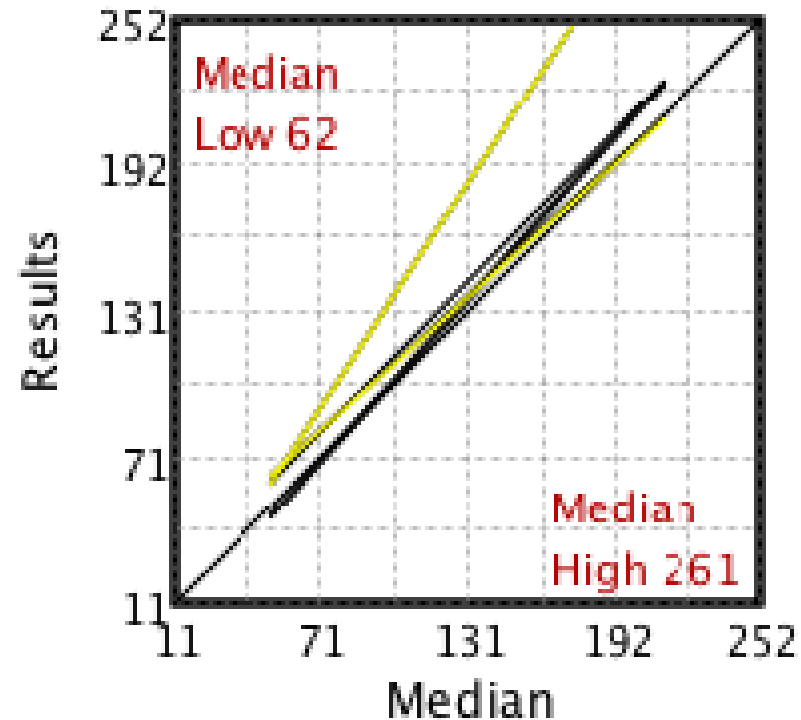
(www.rcpaqap.com.au/chempath)

11 Participants (HPLC)

Intra-laboratory Precision Analysis



Accuracy Analysis Regression Lines (2) In-house



Reagent Source:

- 8 Chromsystems
- 2 In-house
- 1 Not specified

+ Vitamin B6

Occurs in 6 biologically interconvertible 3-hydroxy-2-methylpyridine compounds.

Pyridoxal-5-phosphate is the primary biologically-active form.

Fluorometric, enzymatic and microbiological methods have been employed in the past

HPLC with FD is currently the method of choice.

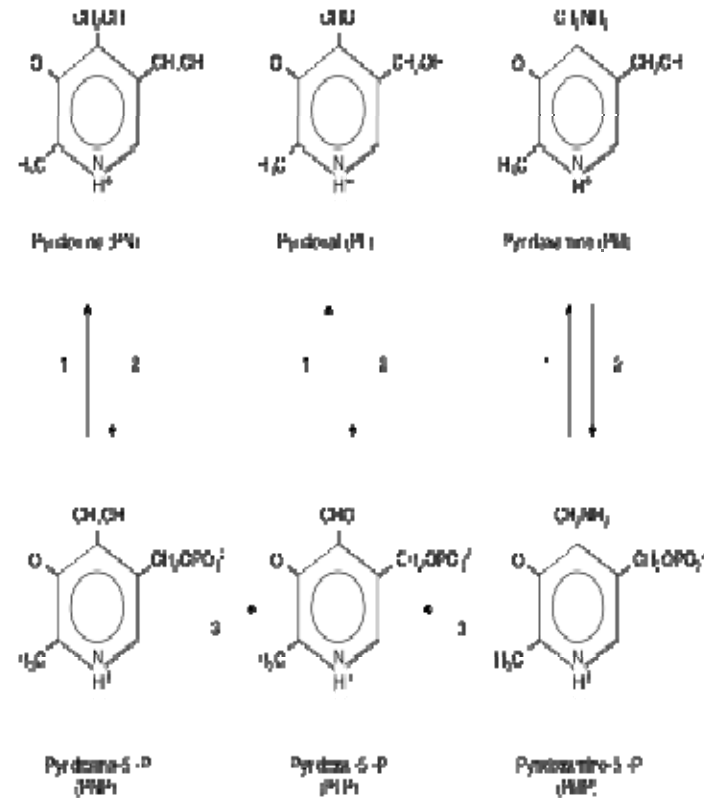


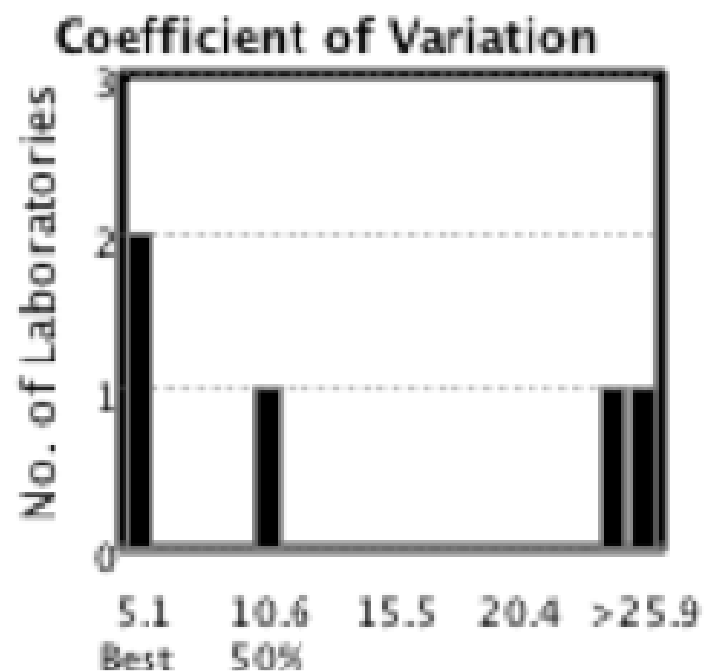
FIGURE 10.1 Interconversion of vitamin B6 vitamers: (1) Phosphorylation; (2) Oxidation; and (3) Pyridoxamine phosphorylation.

+ Pyridoxal Phosphate: End of cycle 22 Report

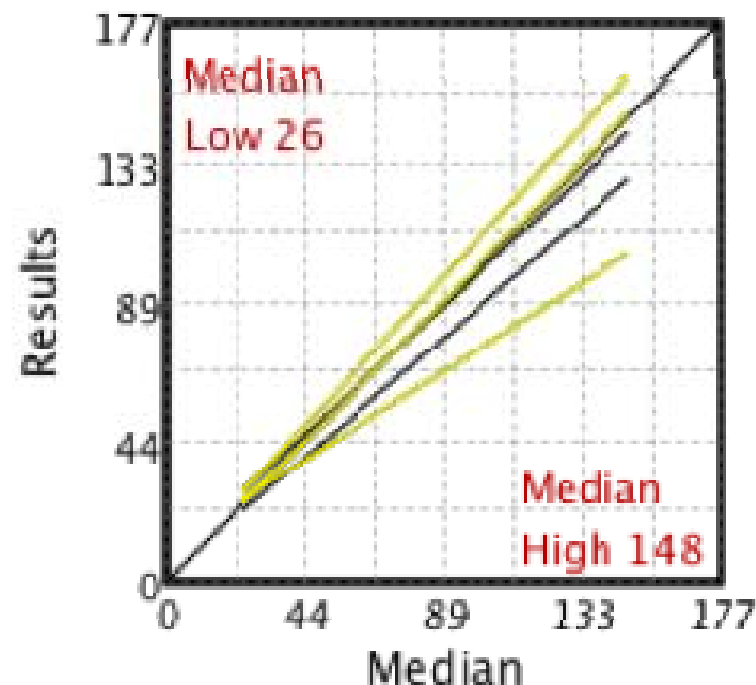
(www.rcpaqap.com.au/chempath)

5 Participants (HPLC, Chromsystems)

Intra-laboratory Precision Analysis



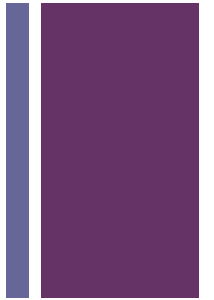
Accuracy Analysis
Regression Lines (3)



Calibrator Source:

3 Chromsystems
2 Commercial

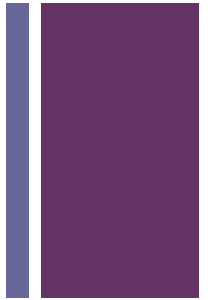
+ Vitamin B1 & B6 Questionnaire



Circulated at the end of first cycle, 2010:

- Sample collection
- Storage
- Assay methodology
- Calibration
- Performance characteristics
- Other analytes for inclusion in the program

+ Vitamin B Questionnaire: B1



9/12 responses (75%).

1/9 microbiological assay for total thiamine.

8/9 HPLC-FD for TPP.

Sample collection – whole blood (EDTA).

Protection of samples from light exposure.

Storage of samples at -20°C.

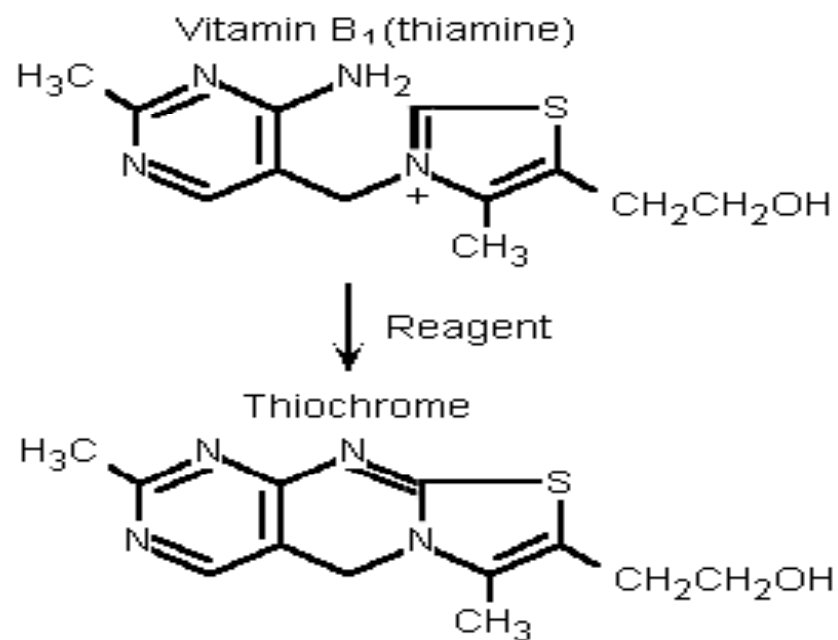
+ Vitamin B Questionnaire: TPP

Sample extraction and clean-up
(protein precipitation)

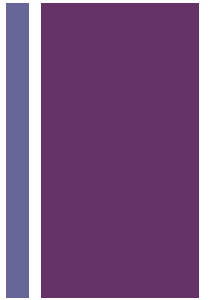
Pre-column oxidation

HPLC (6/8 isocratic chromatography)

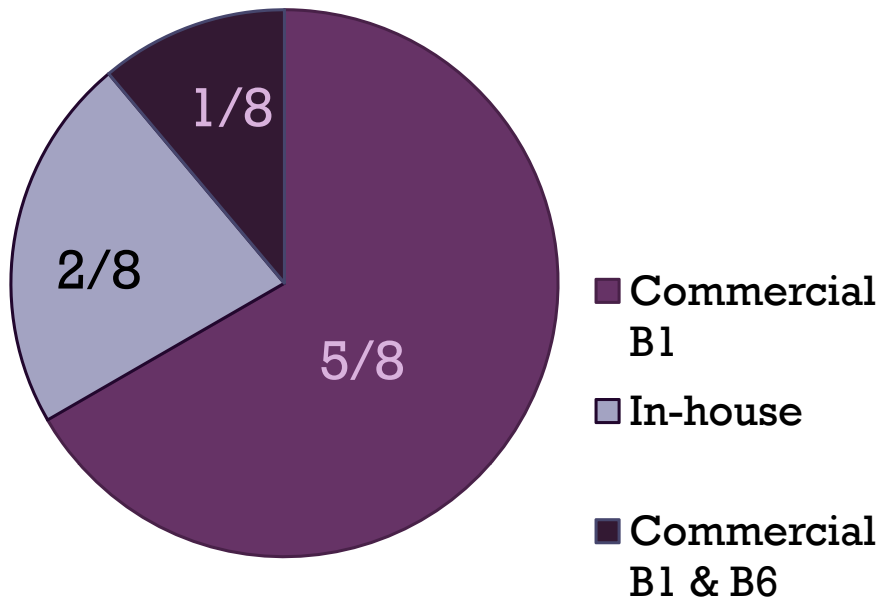
Fluorescence detection



+ Vitamin B Questionnaire: TPP



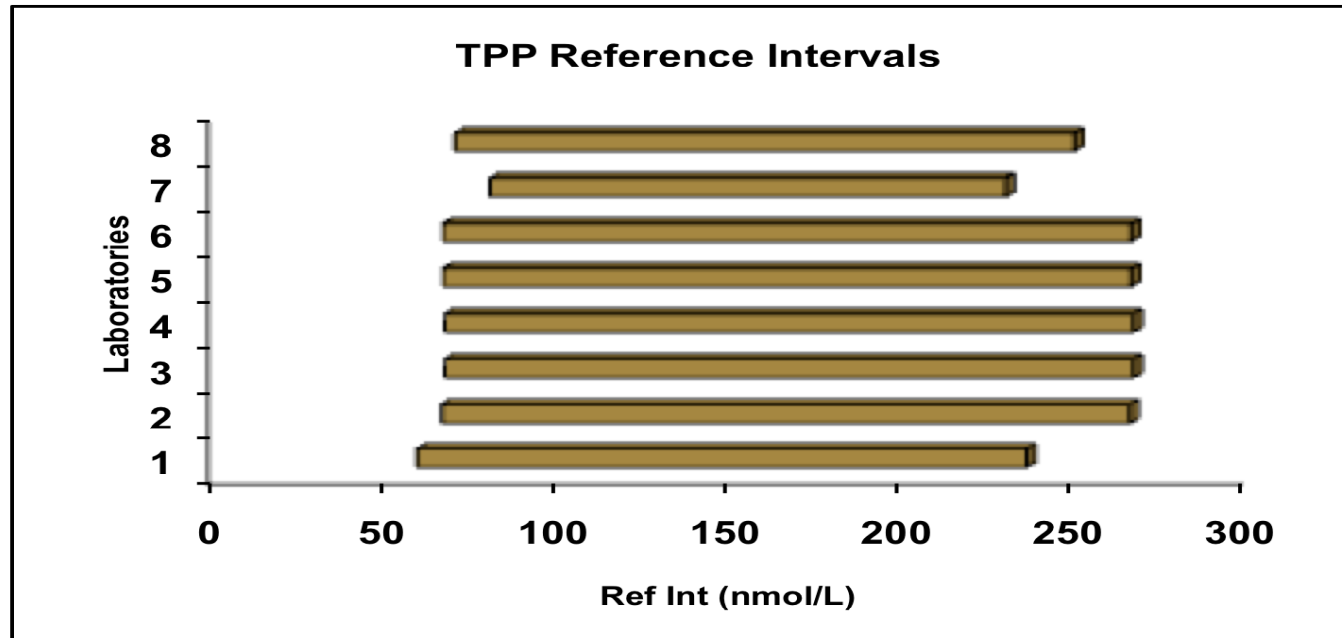
TPP assays



Calibrator use separated along lines of commercial and in-house assays.

1/8 (Commercial B1/B6 kit) uses an internal standard.

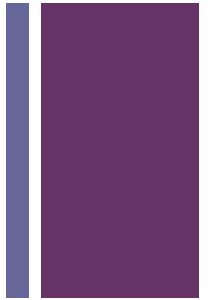
+ Vitamin B Questionnaire: TPP



Reference intervals for the whole blood TPP assays.

Laboratories 7 & 8 use in-house assays.

+ Vitamin B Questionnaire: B6



All 9 respondents measure B6, but only 5 registrants in the first cycle for 2010.

5/8 labs assay plasma or serum vitamin B6 **

3/8 use whole blood.

Samples light protected and stored frozen.

+ Vitamin B Questionnaire: B6



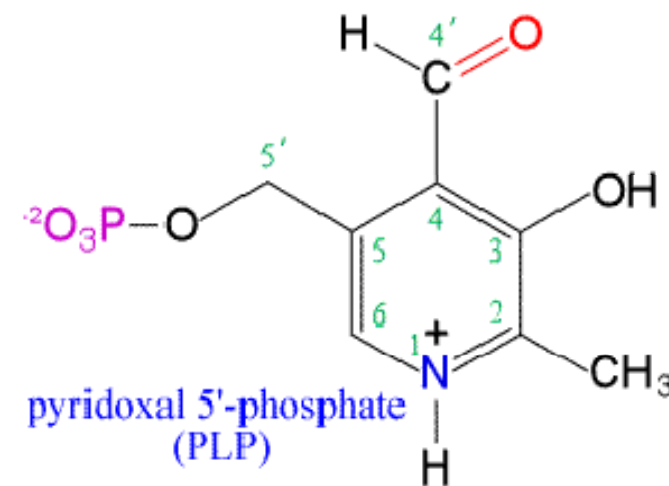
1/9 microbiological method (total serum pyridoxal, pyridoxal-5-P only on request).

8/9 use HPLC with fluorescence detection.

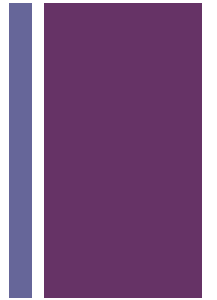
Measure P-5-P

Sample extraction

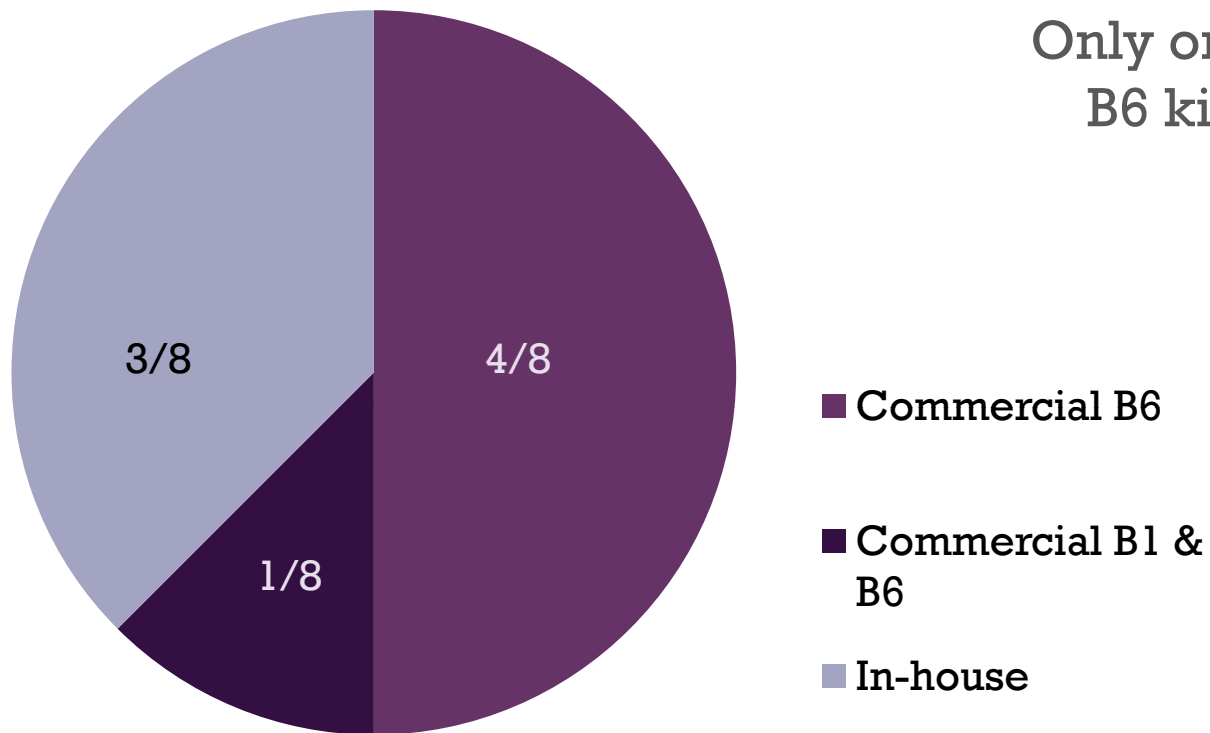
Pre-column derivatisation (C-4 aldehyde)



+ Vitamin B Questionnaire: P-5-P

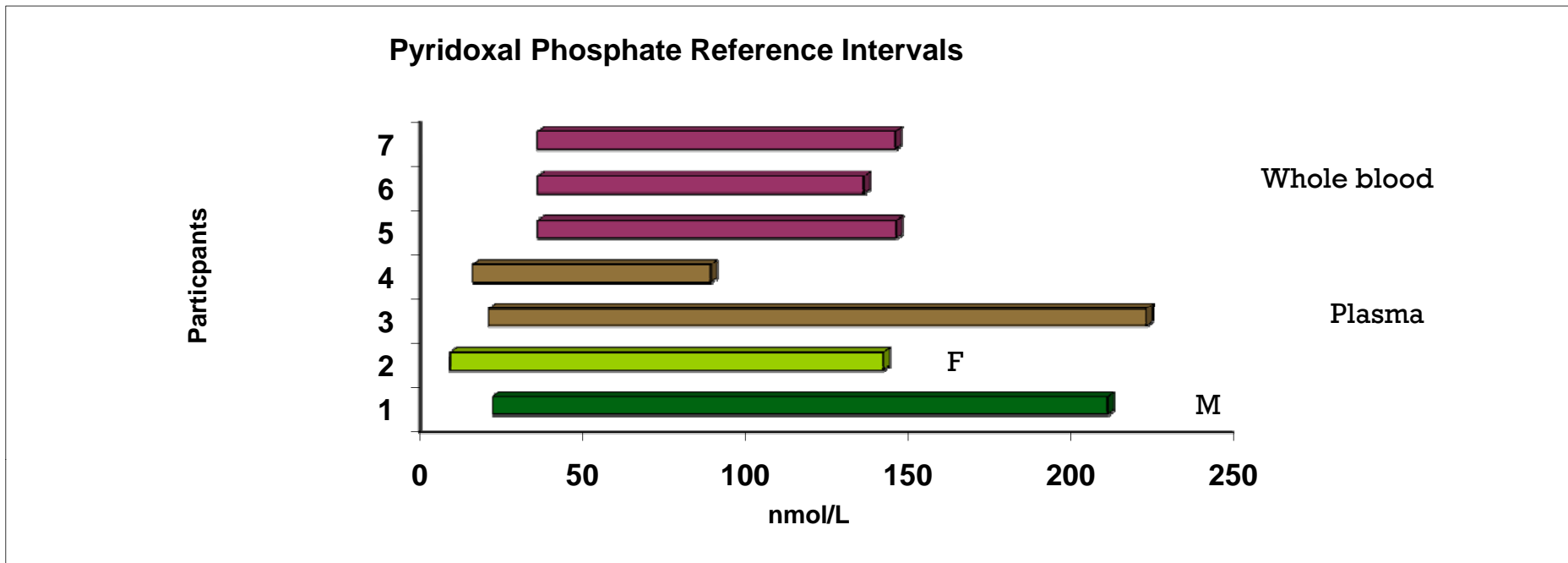
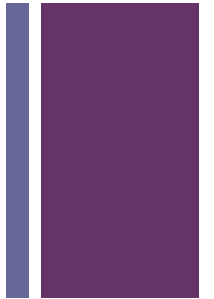


P-5-P assays



Only one laboratory (Chromsystems B1 & B6 kit) uses an internal standard.

+ Vitamin B Questionnaire: P-5-P



P-5-P Reference Intervals, whole blood and plasma.

+ Review: Accuracy - Calibration

The majority of labs use commercial calibrators.

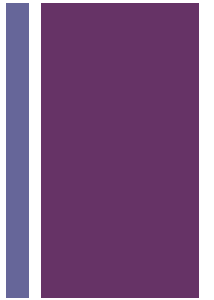
Investigation of the impact of a common calibrator on the dispersion of results.

Standard Reference Material if available.

Alternatively an commercial calibrator could be used.



+ Review: Precision - Internal standards



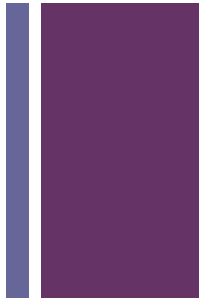
The lack of internal standardisation could potentially contribute to the imprecision observed.

An internal standard is added to all samples/calibrators in an assay and undergoes the same extraction and measurement procedure. This enables correction for variation in extraction and injection volume.

Desirable qualities:

- Similar chemical, physical and chromatographic properties to the analyte.
- Remains stable in the sample throughout extraction and separation.
- Does NOT occur naturally in humans.

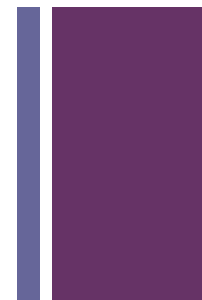
+ Review: Precision - Internal standards



The majority of methods for vitamins B1 and B6 use external standardisation.

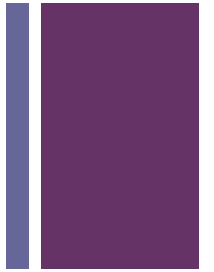
Lynch et al, J Chrom B 1997: thiamine pyrophosphate assay using **acetylneurine** as an internal standard.

+ Summary



- **Vitamin C:** Pilot cycle successfully commenced in 2010. It is a small but important niche program. Initial performance suggests considerable variation in results but is expected to improve.
- **Vitamins B1 & B6:** Review of the performance prompted circulation of a survey of participants. Although the majority of labs use very similar assays and conditions there are 2 aspects that suggest potential directions for the working party to investigate to enable further improvement of performance: calibration and internal standardisation.

+ Vitamins Working Party



L-R: Gerald Woollard, Wendy Carter, Janice Gill, Trevor Walmsley, Ronda Greaves, Scott Briscoe, Kirsten Hoad (May 2010)