Vitamins

Ronda Greaves
Chair: AACB Vitamins working party

HCMC - 20th June 2009

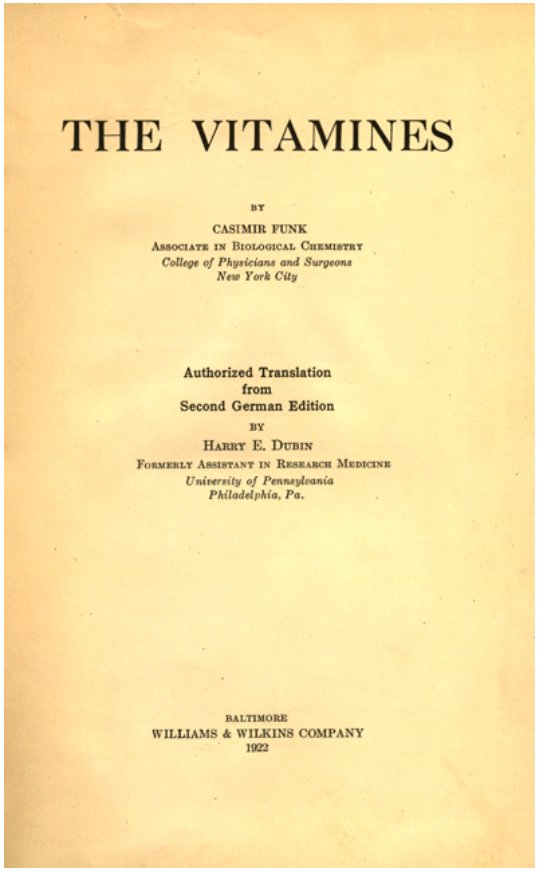
Outline

- Discovery and definition
- Vitamin A and E
- Vitamin D
- External QA vitamin programs

Discovery and definition

1912: "Vitamine"

- Casimir Funk
- 1884-1967
- **Vital amine**
- Describes a growth factor present in food which was essential for life.



THE VITAMINES

BY

CASIMIR FUNK

ASSOCIATE IN BIOLOGICAL CHEMISTRY

College of Physicians and Surgeons

New York City

Authorized Translation
from
Second German Edition

BY

HARRY E. DUBIN

FORMERLY ASSISTANT IN RESEARCH MEDICINE

University of Pennsylvania

Philadelphia, Pa.

BALTIMORE

WILLIAMS & WILKINS COMPANY

1922

- Funk, C. (1912) J. *State Med. 20,* 341-368

1916: "*Vitamine A & B*"

Dr Vitamin

THE DIETARY FACTORS OPERATING IN THE PRODUCTION OF POLYNEURITIS.*

By E. V. McCOLLUM and CORNELIA KENNEDY.

(*From the Laboratory of Agricultural Chemistry of the University of Wisconsin, Madison.*)

(Received for publication, February 29, 1916.)

- Elmer Vernon McCollum
- It became clear that there was more than one growth factor
- McCollum divided them into two classes:
  - 'fat-soluble A'
  - 'water-soluble B'.

http://www.mc.vanderbilt.edu/biolib/hc/nutrition/nh3.html

Vitamins today!

- ***Vitamin:***
- ***A*** ***Retinyl acetate***
- ***B1*** ***Thiamine nitrate***
- ***B2*** ***Riboflavine***
- ***B3*** ***Nicotinamide***
- ***B5*** ***Pantothenic acid***
- ***B6*** ***Pyridoxine HCl***
- ***B12*** ***Cyanocobalamin***
- ***B9*** ***Folic acid***
- ***C*** ***Ascorbic acid***
- ***D3*** ***Cholecalciferol***
- ***E*** ***d-α-tocopheryl acid succinate***
- ***Biotin***
- ***Trace metals / elements***

http://www.blackmores.com.au/Products/Detail.aspx?ProductId=2275

Definition of a vitamin

- An organic compound required as a nutrient.
- It cannot be synthesized in adequate amounts.
- Therefore it must be obtained from the diet.
- What is considered a vitamin varies between organisms.

- NB: Vitamin D is needed in the human diet only in certain circumstances

Water & Fat soluble vitamins

WATER

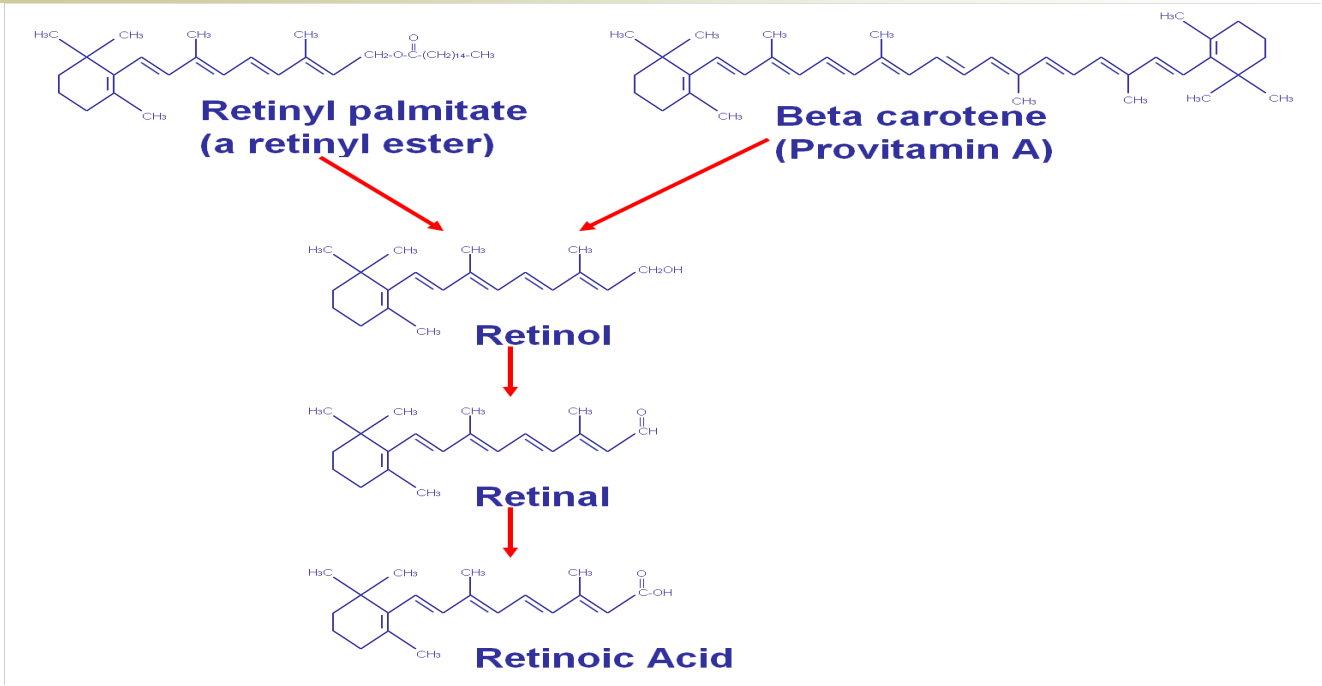
- B group vitamins
  - Vitamin B1 - thiamine
  - Vitamin B2 - ribofavine
  - Vitamin B6
  - Vitamin B12
  - Vitamin B9 - folate
- Vitamin C

FAT

- Carotenoids
- Vitamin A
- Vitamin D!
- Vitamin E
- Vitamin K
- Coenzyme Q10

Vitamin A & E

Vitamin A

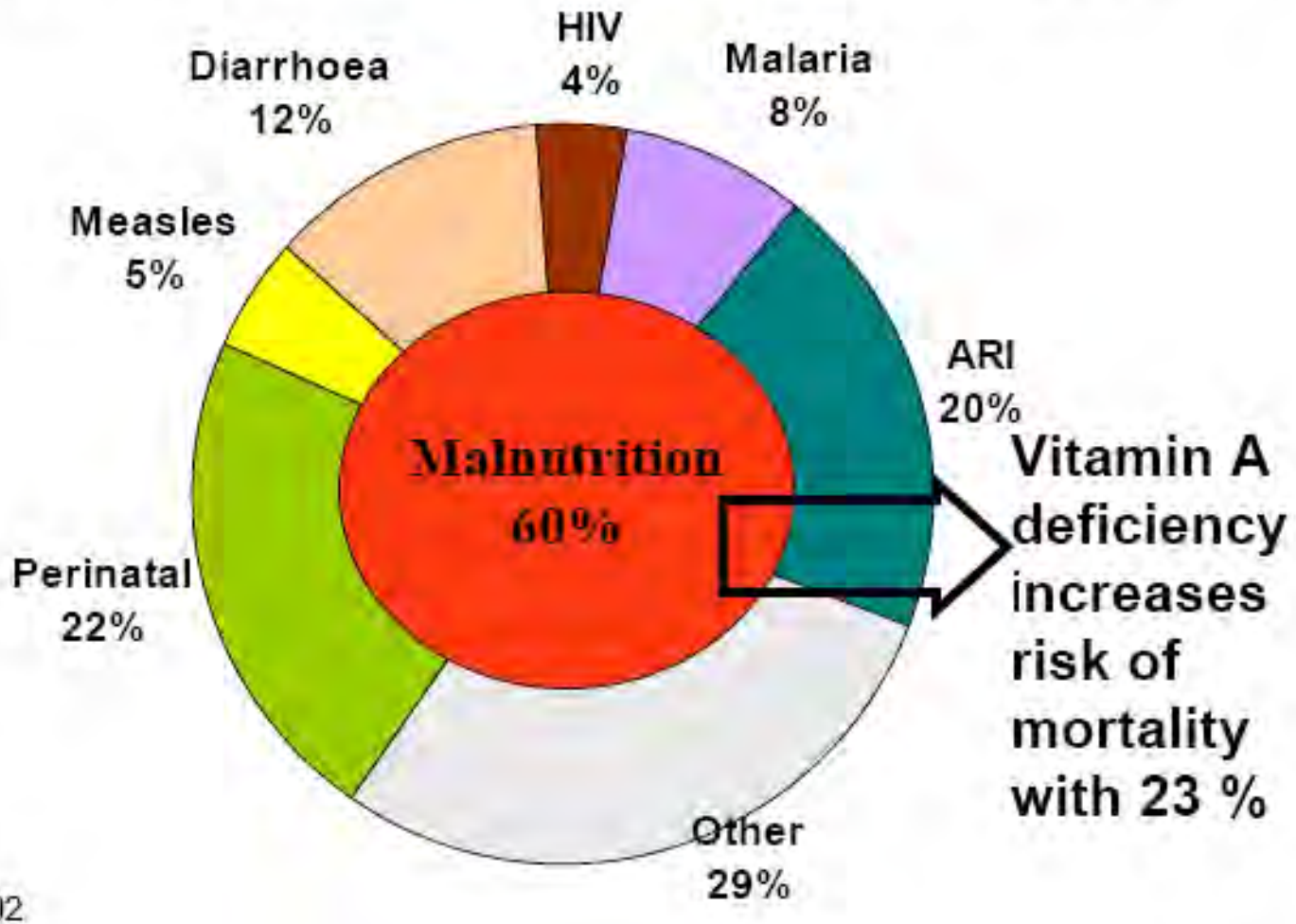


- Retinol carried in plasma by retinol binding protein
- Prevents night blindness:
  - Retinal complexes to opsin to form rhodopsin = dim light vision
  - When retinal is depleted in the retina, opsin is destabilised & catabolised = permanent destruction.

Vitamin A: Deficiency

- Listed by WHO as a major health issue especially in developing countries
- Main cause of preventable childhood blindness
- Increased risk of morbidity and mortality
- Affects the most vulnerable - pre school children and pregnant women
- Worldwide public health problem – 254 million pre-school children vitamin A deficient

Causes of death among pre-school children in non-Industrialized countries, 2000



Ref.;WHO 2002

http://www.euro.who.int/ppt/nut/vad.pdf

Vitamin A deficiency in Viet Nam

- <0.3 µmol/L associated with symptoms
- Supplementation associated with immunisation program
- Supplementation Schedule:
  6-11, 12-17, 18-23, 24-29, 30-36 months of age
- Classified by WHO (2007) as sub-clinical for vitamin A deficiency

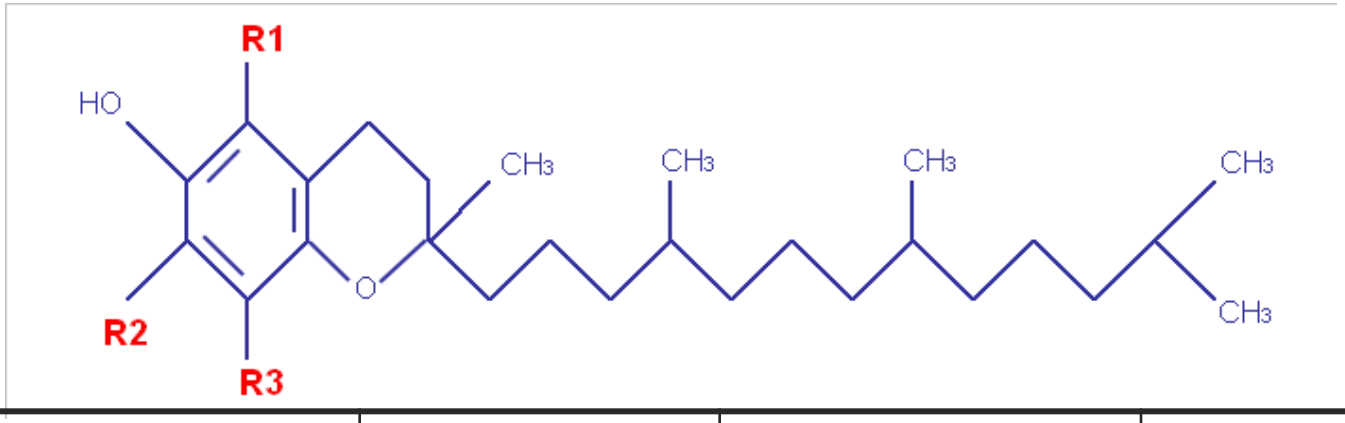
http://www.who.int/vaccines/globalsummary/immunization/countryprofileresult.cfm?C='vnm

Vitamin A: Toxicity

- Levels not well defined
- Acute:
  - 20 – 100x the RDI
- Chronic: Daily intakes of:
  - 25 000 IU for 6 years
  - 100,000 IU for 6 months
  - Serum levels may be in RI
- Fasting retinyl ester concentrations >10% of total circulating vitamin A
- Vitamin A (retinol esters) could be a biomarker for toxicity

Am J Clin Nutr 2006;83:191–201

Vitamin E

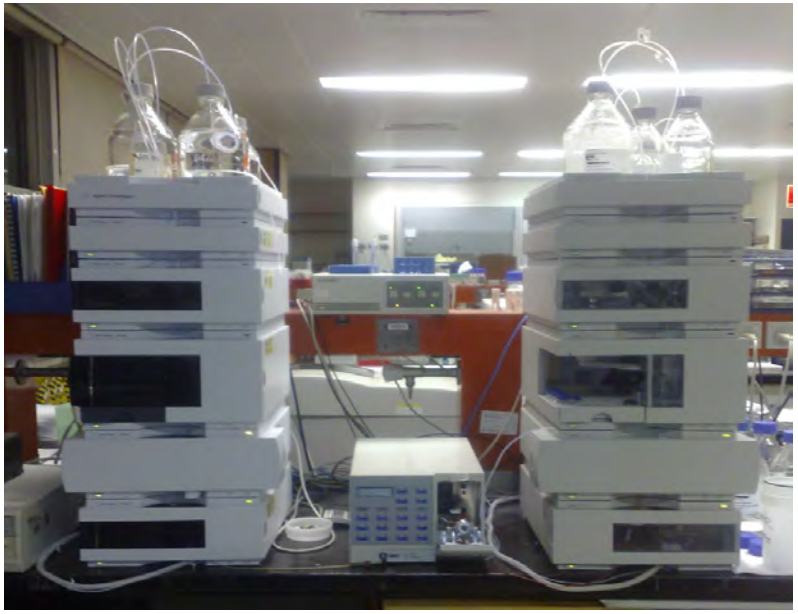


Compound	R^1	R^2	R^3
a-Tocopherol	CH_3	CH_3	CH_3
b-Tocopherol	CH_3	H	CH_3
g-Tocopherol	H	CH_3	CH_3
d-Tocopherol	H	H	CH_3

Also 4 x Tocotrienols (3 double bonds in the phytyl side chain)

Vitamin A & E analysis

Agilent HPLC 1200 & 1100 series



Degasser
Quaternary pump
Autosampler
Column Oven
UV/Vis detector
EzChrome software

Sample preparation

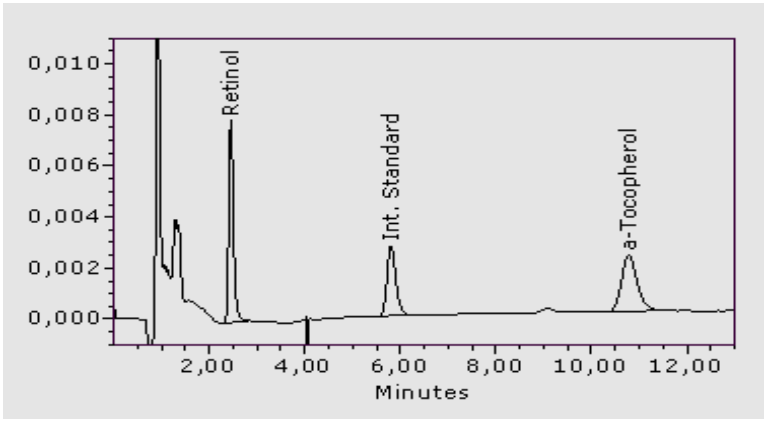
- Protein precipitation
- Liquid extraction with hexane

HPLC

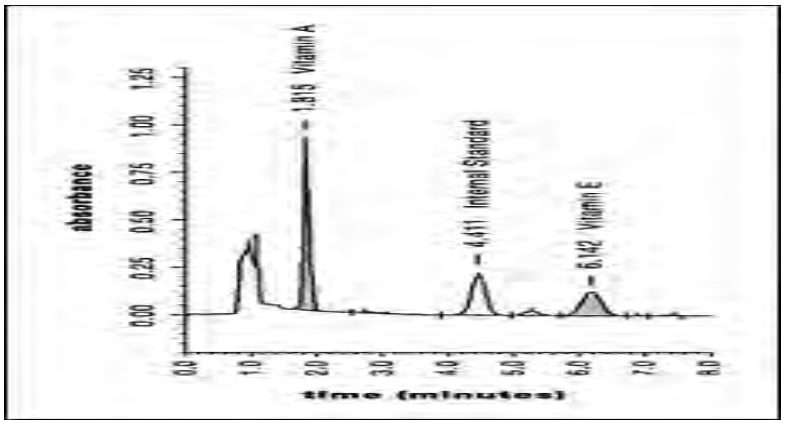
- Reverse phase C18
- Isocratic/gradient
- Vitamin A: 325 nm
- Vitamin E: 292 nm

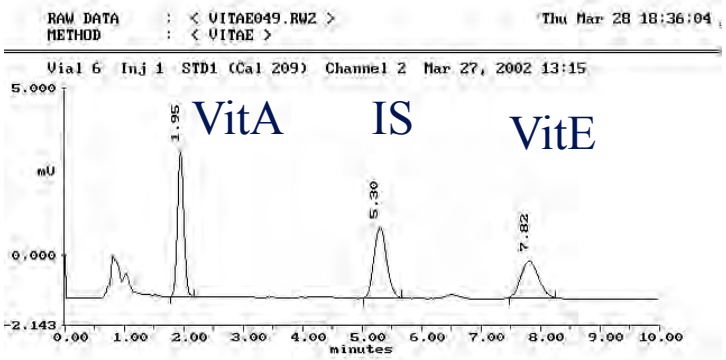
Commercial Chromatograms

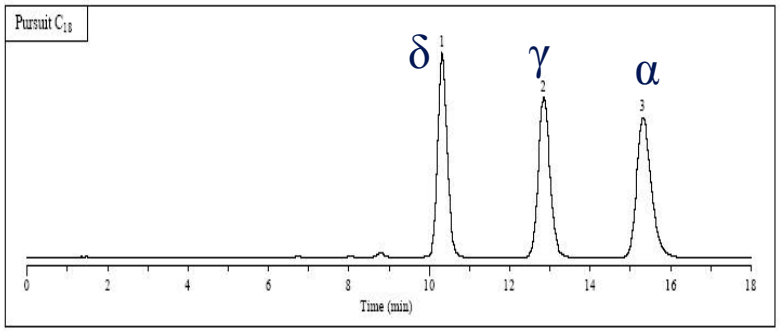
Chromsystems



Bio-Rad







Recipe: http://www.recipe.de/

Varian application note

Commercial sources

Standards

- Bio-Rad
- Chromsystems
- Sigma
- NIST
- Other

Commercial Kits

- Bio-Rad
- Chromsystems
- Recipe
- Other

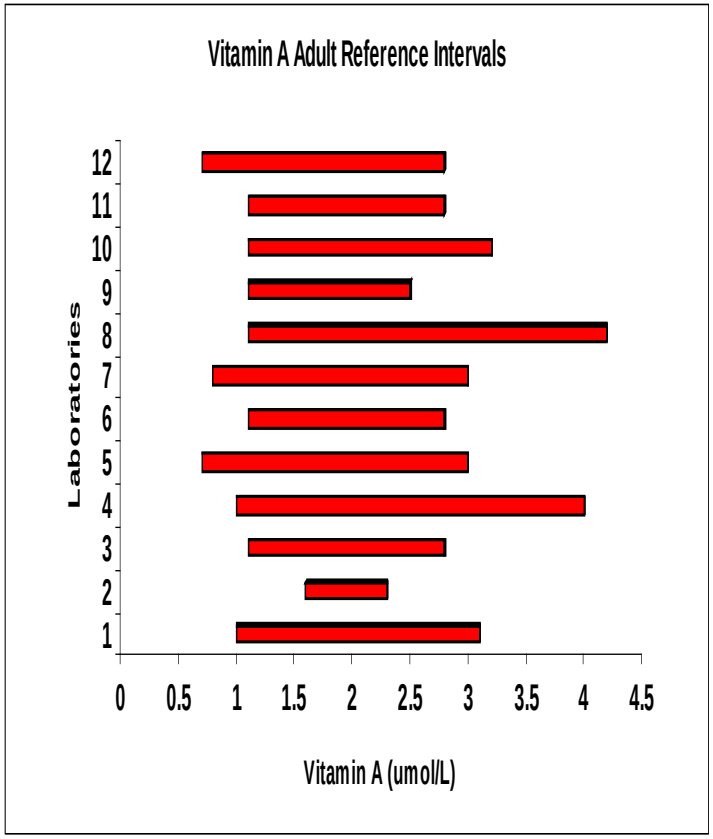
Internal QC

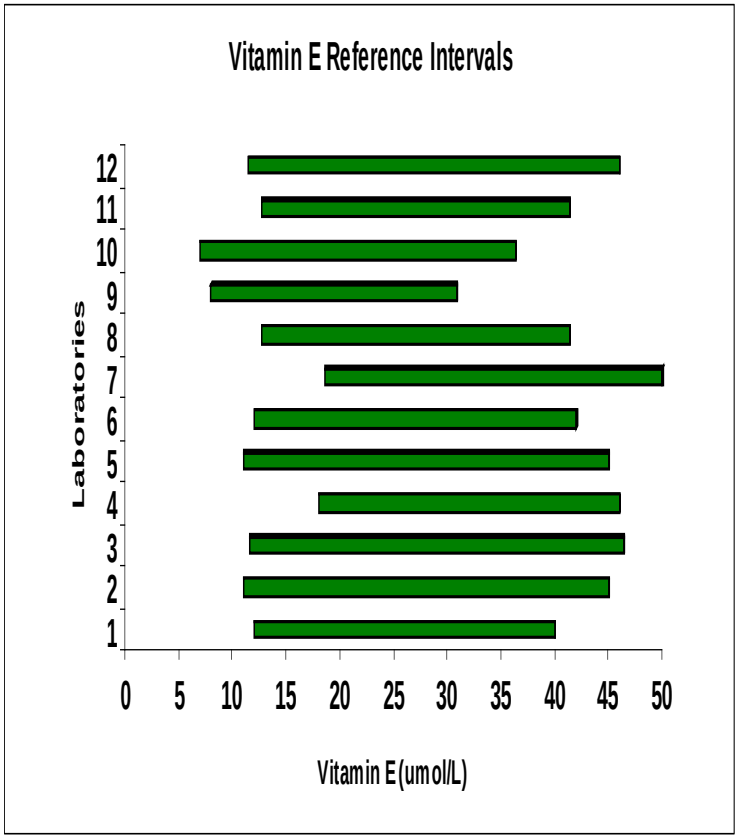
- Bio-Rad
- Chromsystems
- Recipe
- In house
- Other

Columns

- Alltech
- Phenomenex
- Varian
- Waters
- Other

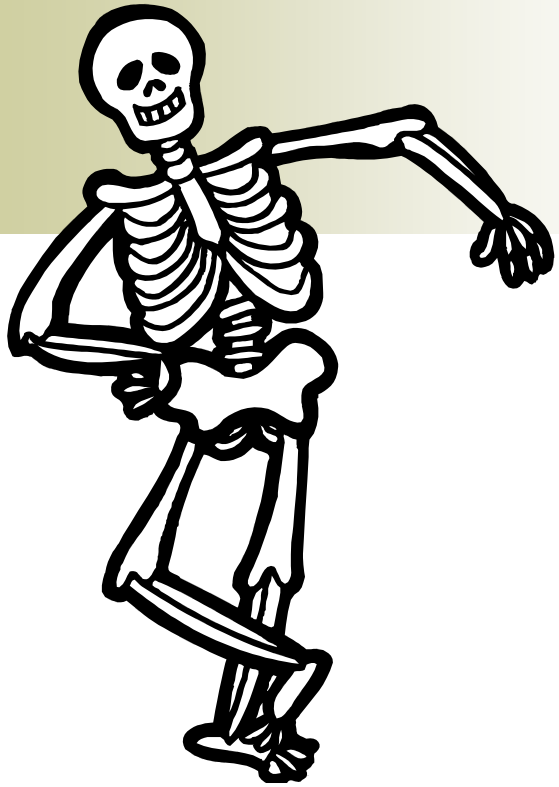
Adult reference intervals



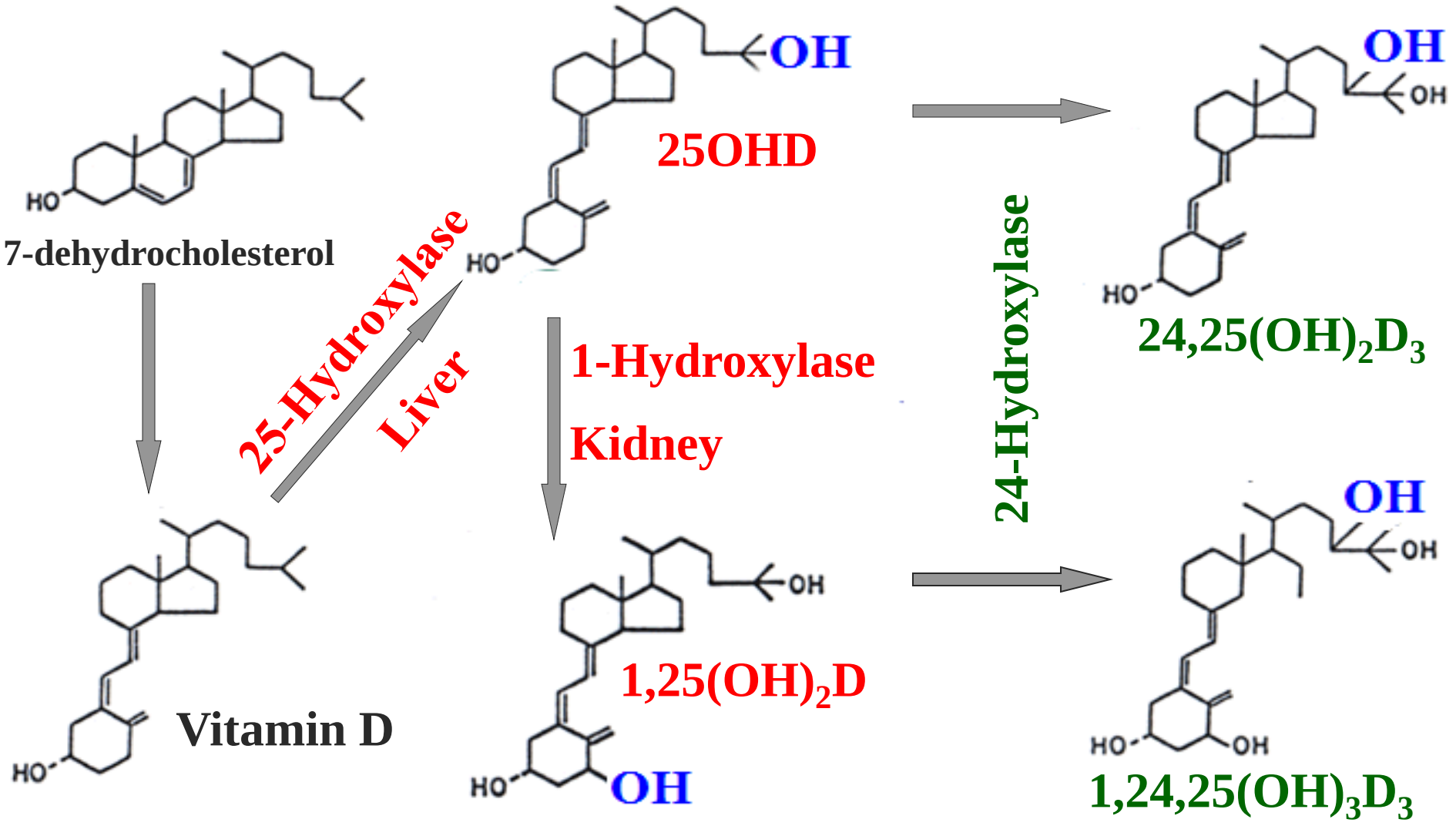


Results from 2007 RCPA-QAP vitamin A & E questionnaire

Vitamin D

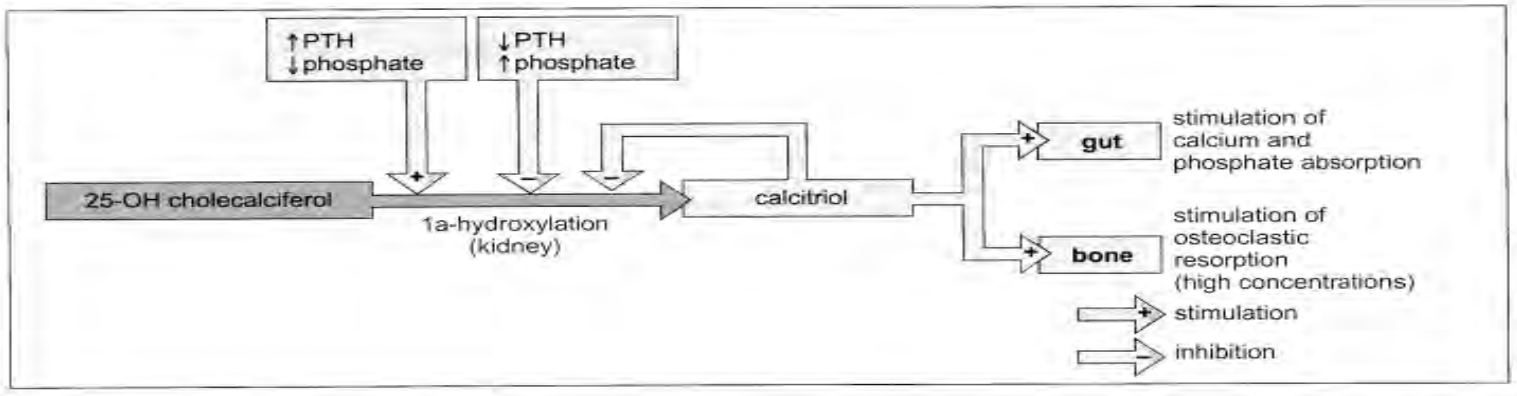


Vitamin D Metabolism



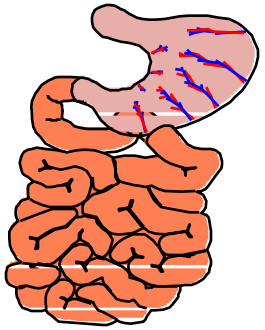
1,25 di OH Vitamin D

- Regulates absorption of calcium
- Important for bone growth & development
- Two forms:
  - Vitamin D2 = ergocalciferol, is ingested in foods
  - Vitamin D3 = cholecalciferol, is produced in the skin on exposure to sunlight.
- These forms are converted in the liver to 25 OH vitamin D and then in the kidney to the active 1,25 di OH vitamin D.



1,25 Vitamin D (Calcitriol)

Dietary intake



- Ca^{2+} absorption in the gut by stimulating synthesis of binding proteins

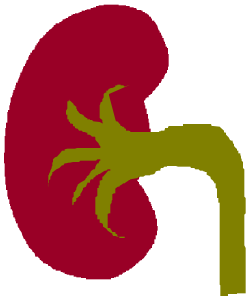
ECF

$^{-}[Ca^{2+}]$ ® - PTH

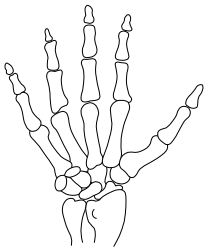
+

$^{-}[PO_4^{2-}]$

= - **1,25 Vit D**



- Ca^{2+} and PO_4^{2-} reabsorption



- osteoclastic activity =
- $[Ca^{2+}]$ and $[PO_4^{2-}]$

Overall effect – - plasma $[Ca^{2+}]$ and - plasma $[PO_4^{2-}]$

Vitamin D: Automated analysis

- Roche Cobas e601
  - 25 OH Vit D3 only

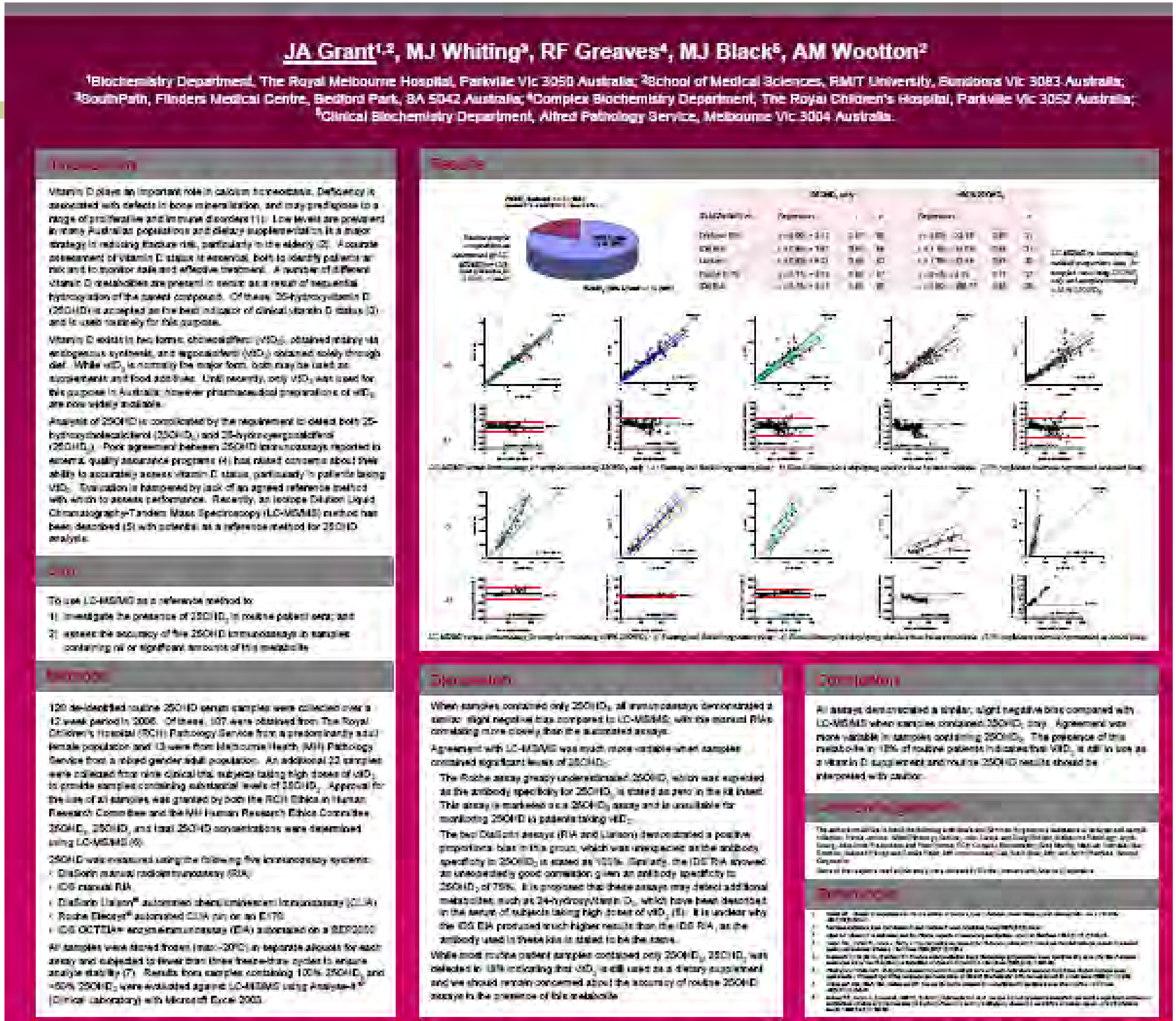
- Liasion
  - 25 OH Vit D3
  - >80% cross reactivity with 25 OH Vit D2

Chromatography + MS (+MS)

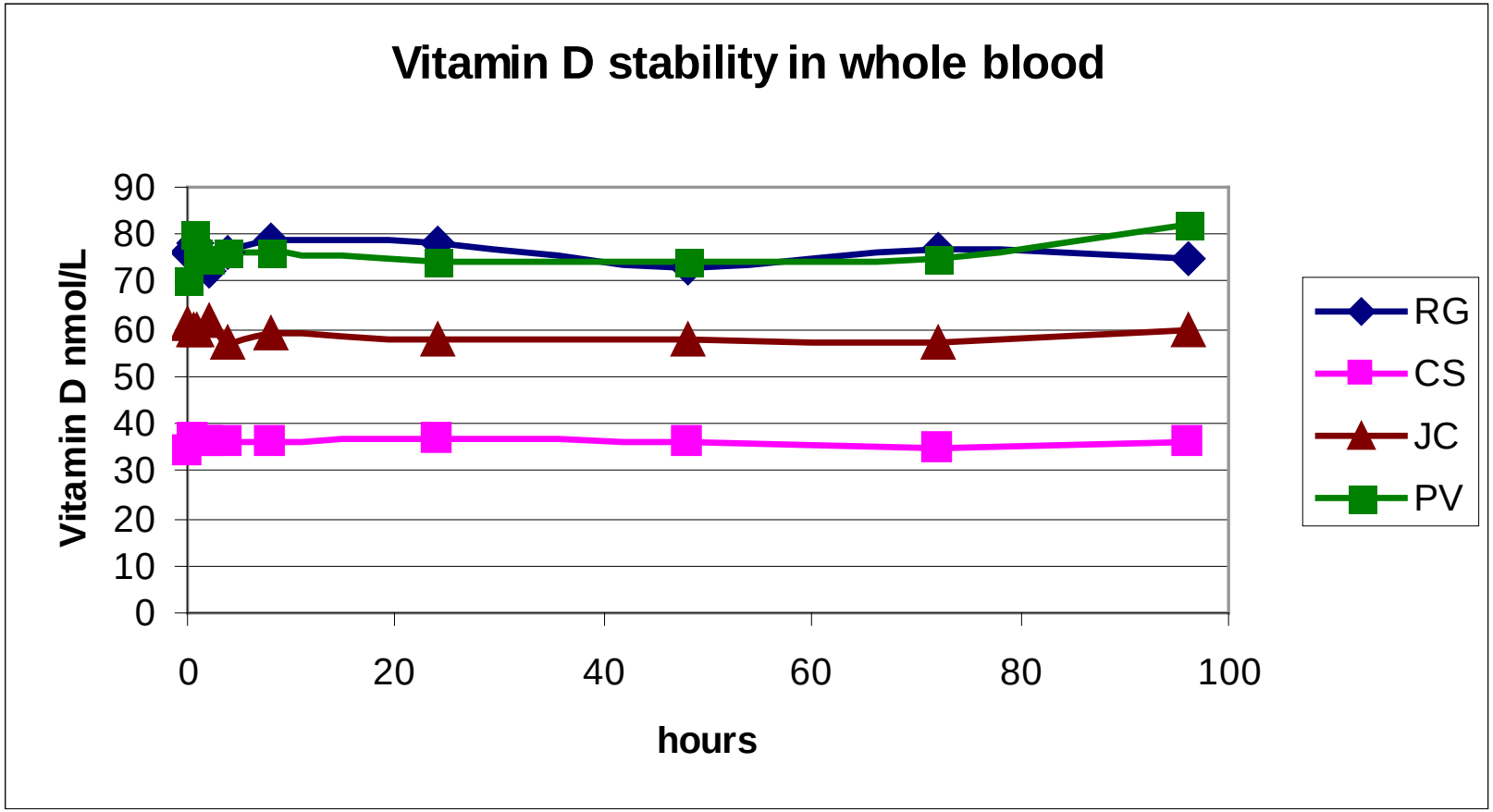
- Gold standard
- TAT a problem
- Expertise required
- Up front cost high

300,000.00 AUD	=	4,168,904,855.85 VND
Australia Dollars		Vietnam Dong
1 AUD = 13,896.35 VND		1 VND = 0.0000719613 AUD

25-Hydroxyvitamin D - Which Assay?



2005 Vitamin D stability study

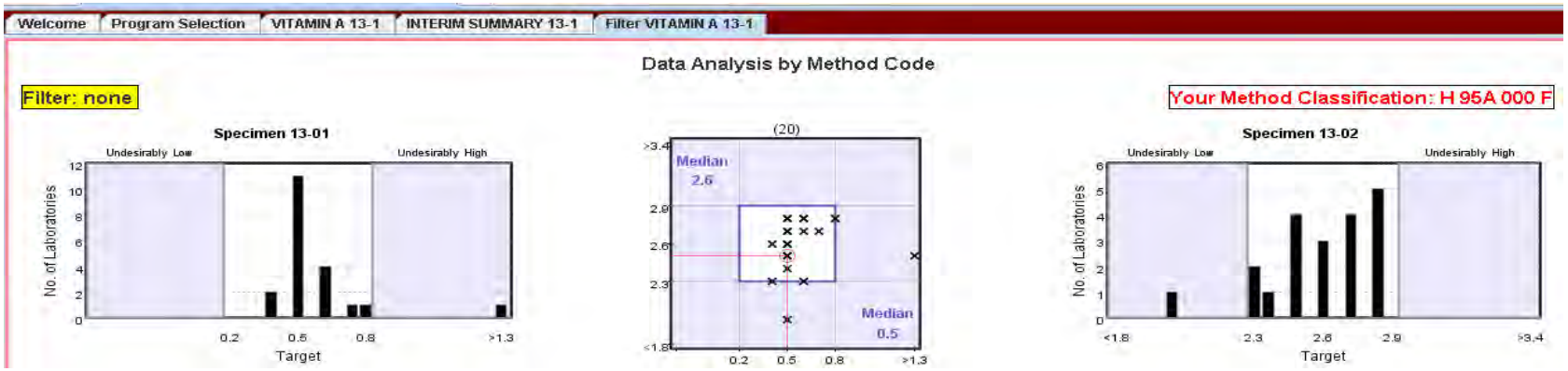


Conclusion: *Vitamin D is stable in whole blood stored at room temperature in sunlight for up to 96 hours.* Presented at the AACB ASM in 2005

External QA vitamin programs

RCPA-QAP Vitamin program

- Countries:
  - Australia
  - New Zealand
  - South Africa
  - Thailand
  - USA
  - Singapore
  - Israel
- Analytes:
  - **Vitamin A**
  - Vitamin E
  - Vitamin B1
  - Vitamin B6
  - Beta carotene
  - Total carotenoids



RCPA QUALITY ASSURANCE PROGRAMS PTY LIMITED

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VITAMIN QUESTIONNAIRE

July 2006

The AACB Vitamin Working Party assists the QAP with the Vitamin Quality Assurance Program by providing expert advice, reviewing the program and suggesting future direction.

At this year's meeting a number of suggestions for vitamins to be added to the program were proposed. To assess the demand for new vitamins, the Vitamin Working Party and the QAP would appreciate your feedback on the following questions. Please return to the QAP Office by **30 July 2006**.

Laboratory Name: _____ **Lab. No.**

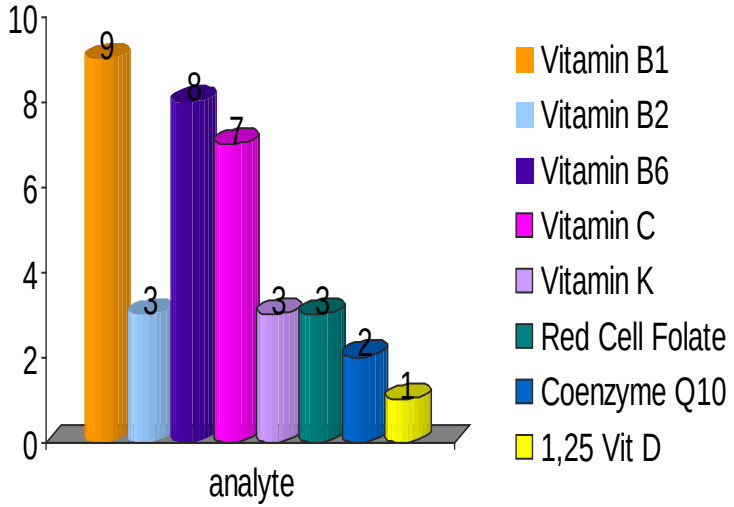
Your Name: _____

Questionnaire sent to labs participating in the Vitamin and Endocrine Programs

RCPA-QAP vitamin program

Response to questionnaire:

- Total respondents = 66
- Strongest interest in Vitamins B1, B6 & C
- EQA material for Vit B1 & B6 available from SKML
- Decision to pilot Vitamin B1/B6 program 2008. Program in operation from 2009
- EQA material for Vit C not currently available. Under development. Proposed pilot 2010.
- Coenzyme Q10 under consideration.



Leading world wide EQA program

Members of the AACB vitamins working party 2009

- Chris Salonikas
- Lisa Jolly
- Kirsten Hoad
- Ronda Greaves
- Trevor Walmsley
- Lambro Johnson
- Gerald Woollard
- Scott Briscoe (not pictured)

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