

Vitamins



Ronda Greaves

Chair: AACB Vitamins working party

HCMC - 20th June 2009

[Outline]

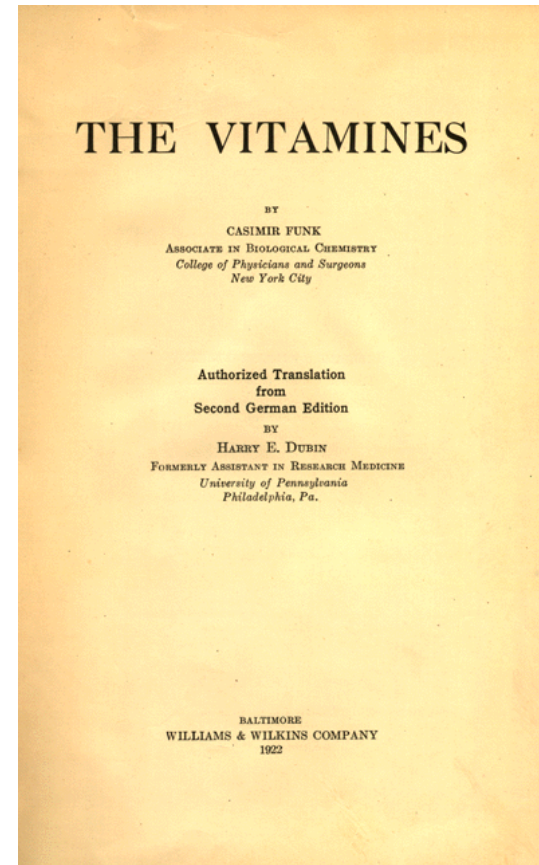
- n Discovery and definition
- n Vitamin A and E
- n Vitamin D
- n External QA vitamin programs



Discovery and definition

1912: “Vitamine”

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





Dr Vitamin

[1916: “*Vitamine A & B*”

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.)

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

Vitamins today!

n **Vitamin:**

n **A** *Retinyl acetate*

n **B1** *Thiamine nitrate*

n **B2** *Riboflavine*

n **B3** *Nicotinamide*

n **B5** *Pantothenic acid*

n **B6** *Pyridoxine HCl*

n **B12** *Cyanocobalamin*

n **B9** *Folic acid*

n **C** *Ascorbic acid*

n **D3** *Cholecalciferol*

n **E** *d- α -tocopheryl acid succinate*

n **Biotin**

n **Trace metals / elements**



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

Definition of a vitamin

- n An organic compound required as a nutrient.
- n It cannot be synthesized in adequate amounts.
- n Therefore it must be obtained from the diet.
- n What is considered a vitamin varies between organisms.
- n NB: Vitamin D is needed in the human diet only in certain circumstances

Water & Fat soluble vitamins

WATER

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

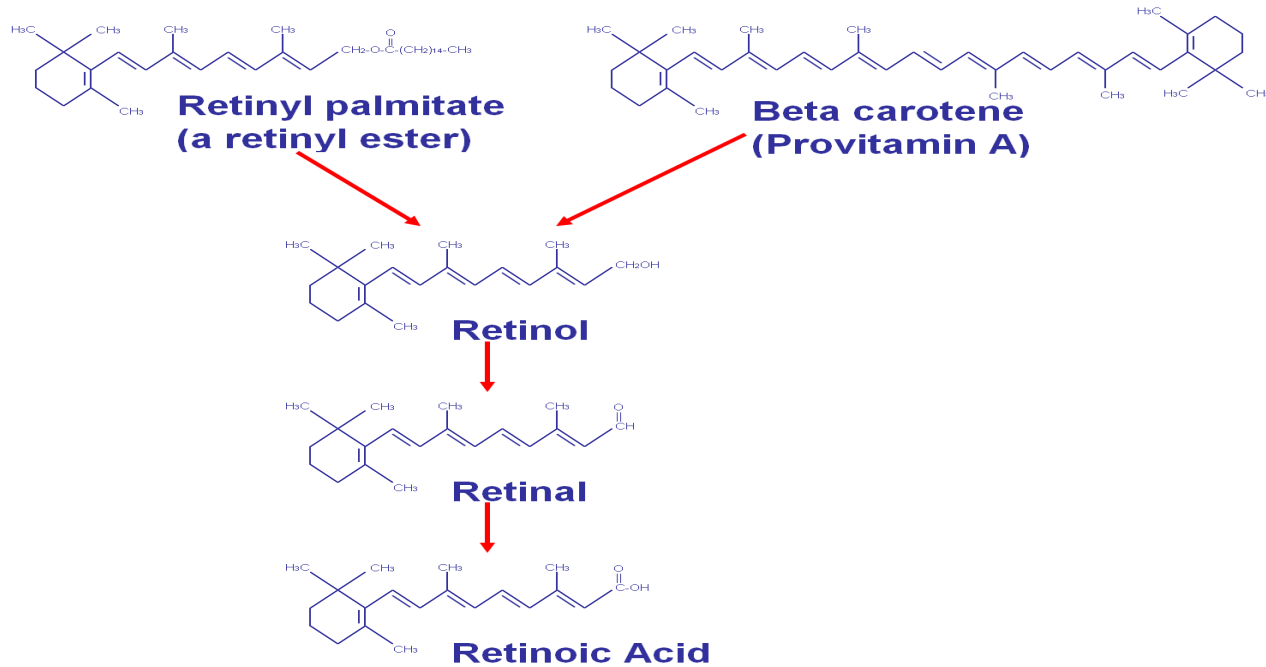
FAT

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

A decorative graphic consisting of a thin gold circle on the left side. A horizontal bar with a gold-to-white gradient extends from the circle across the top of the page. The text 'Vitamin A & E' is centered within the white portion of this bar. A large black left square bracket is positioned to the left of the bar, and a large gold right square bracket is positioned to the right of the bar.

Vitamin A & E

Vitamin A

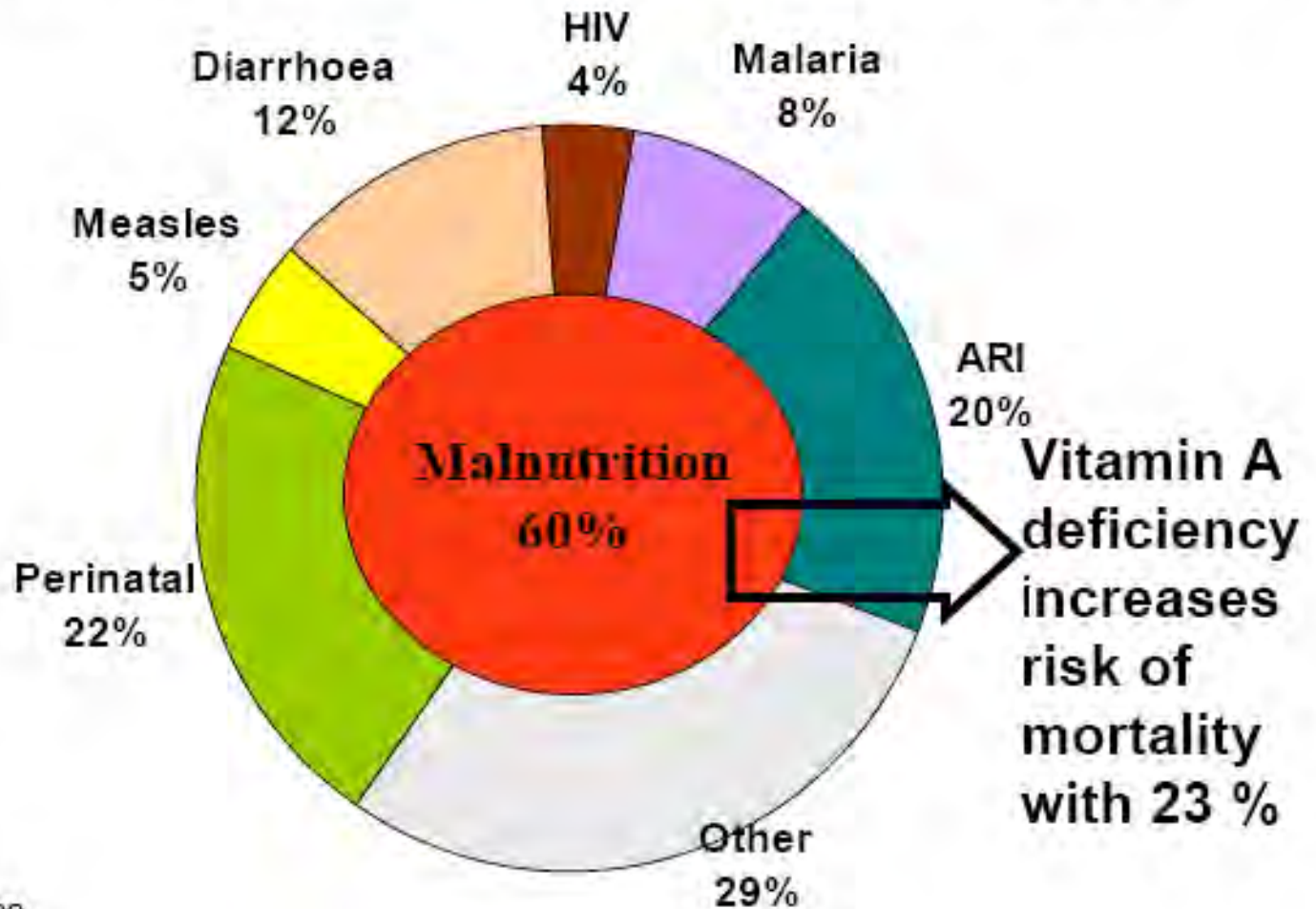


- n Retinol carried in plasma by retinol binding protein
- n Prevents night blindness:
 - i Retinal complexes to opsin to form rhodopsin = dim light vision
 - i When retinal is depleted in the retina, opsin is destabilised & catabolised = permanent destruction.

[Vitamin A: Deficiency]

- n Listed by WHO as a major health issue especially in developing countries
- n Main cause of preventable childhood blindness
- n Increased risk of morbidity and mortality
- n Affects the most vulnerable - pre school children and pregnant women
- n 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

Vitamin A deficiency in Viet Nam

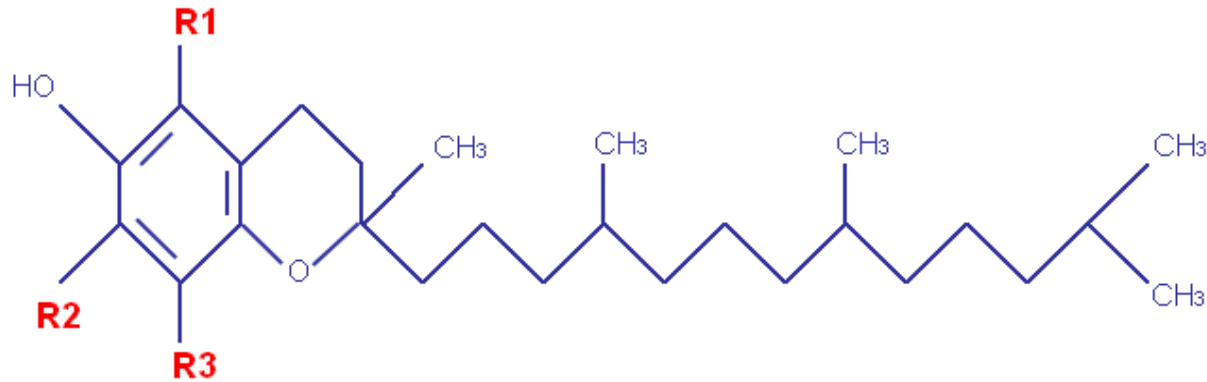
- n <0.3 $\mu\text{mol/L}$ associated with symptoms
- n Supplementation associated with immunisation program
- n Supplementation Schedule:
6-11, 12-17, 18-23, 24-29, 30-36 months of age
- n Classified by WHO (2007) as sub-clinical for vitamin A deficiency

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

Vitamin A: Toxicity

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

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

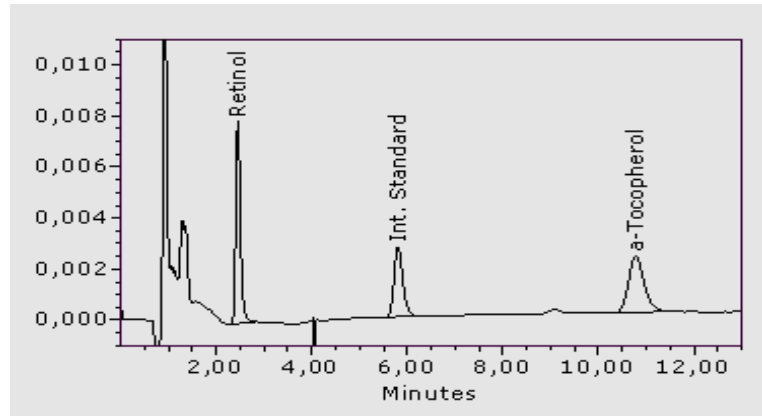
- n Protein precipitation
- n Liquid extraction with hexane

HPLC

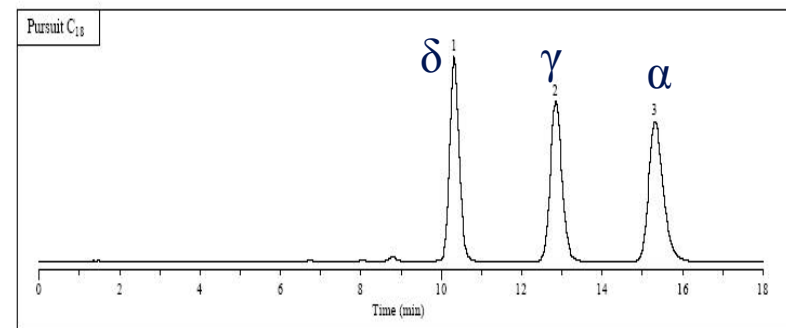
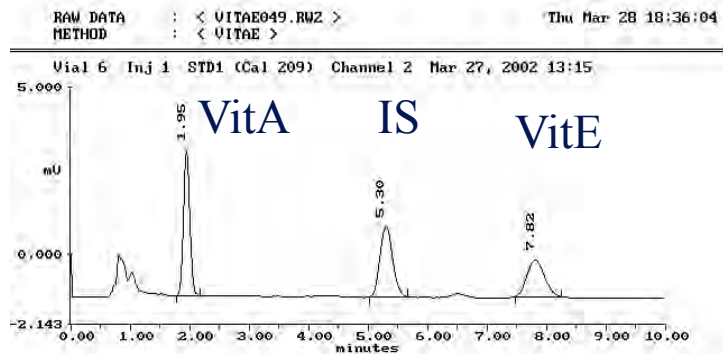
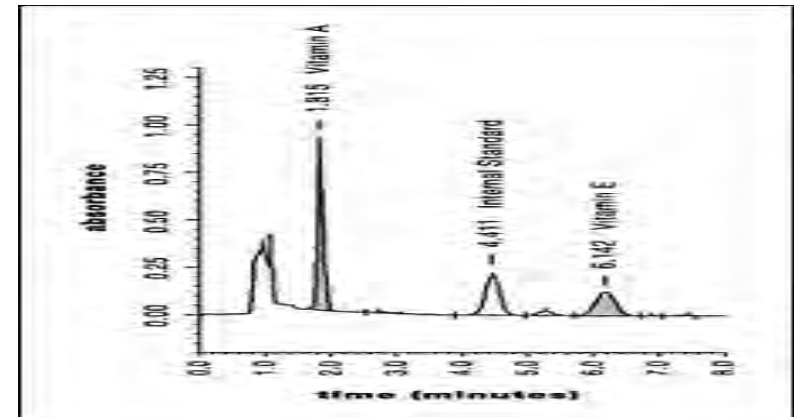
- n Reverse phase C18
- n Isocratic/gradient
- n Vitamin A: 325 nm
- n Vitamin E: 292 nm

Commercial Chromatograms

Chromsystems



Bio-Rad



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

Varian application note

Commercial sources

Standards

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

Internal QC

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

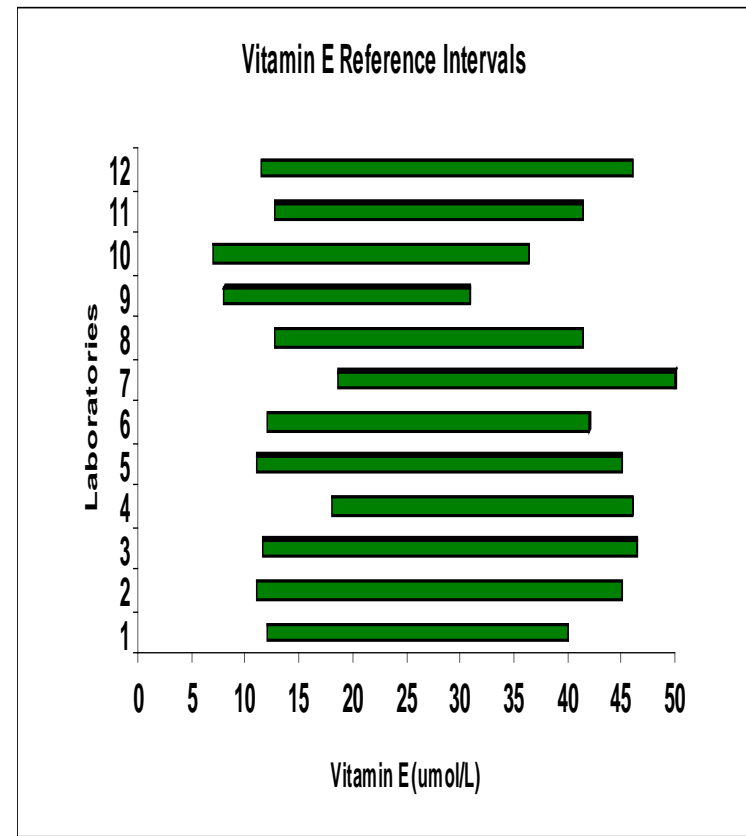
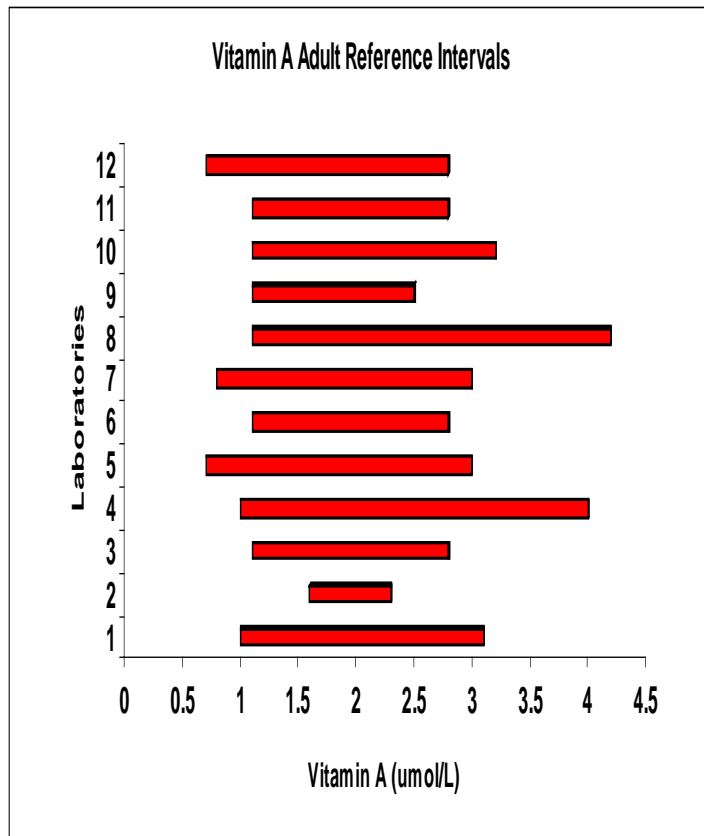
Commercial Kits

- n Bio-Rad
- n Chromsystems
- n Recipe
- n Other

Columns

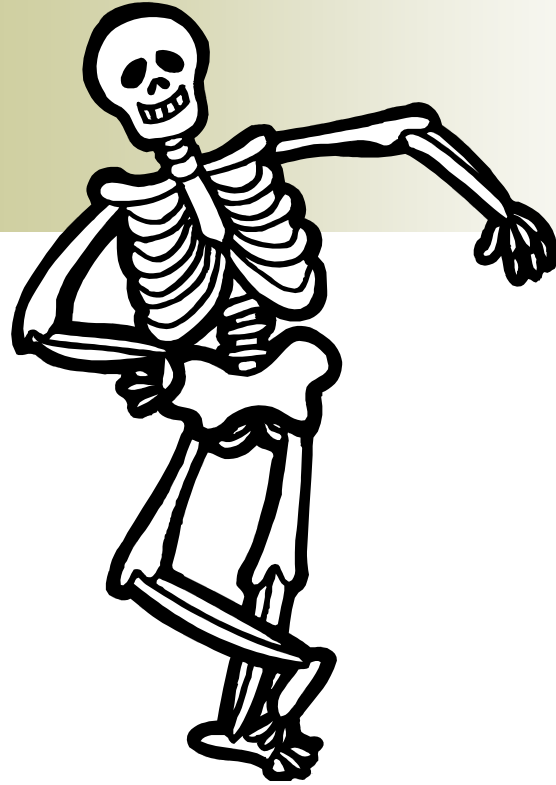
- n Alltech
- n Phenomenex
- n Varian
- n Waters
- n Other

Adult reference intervals

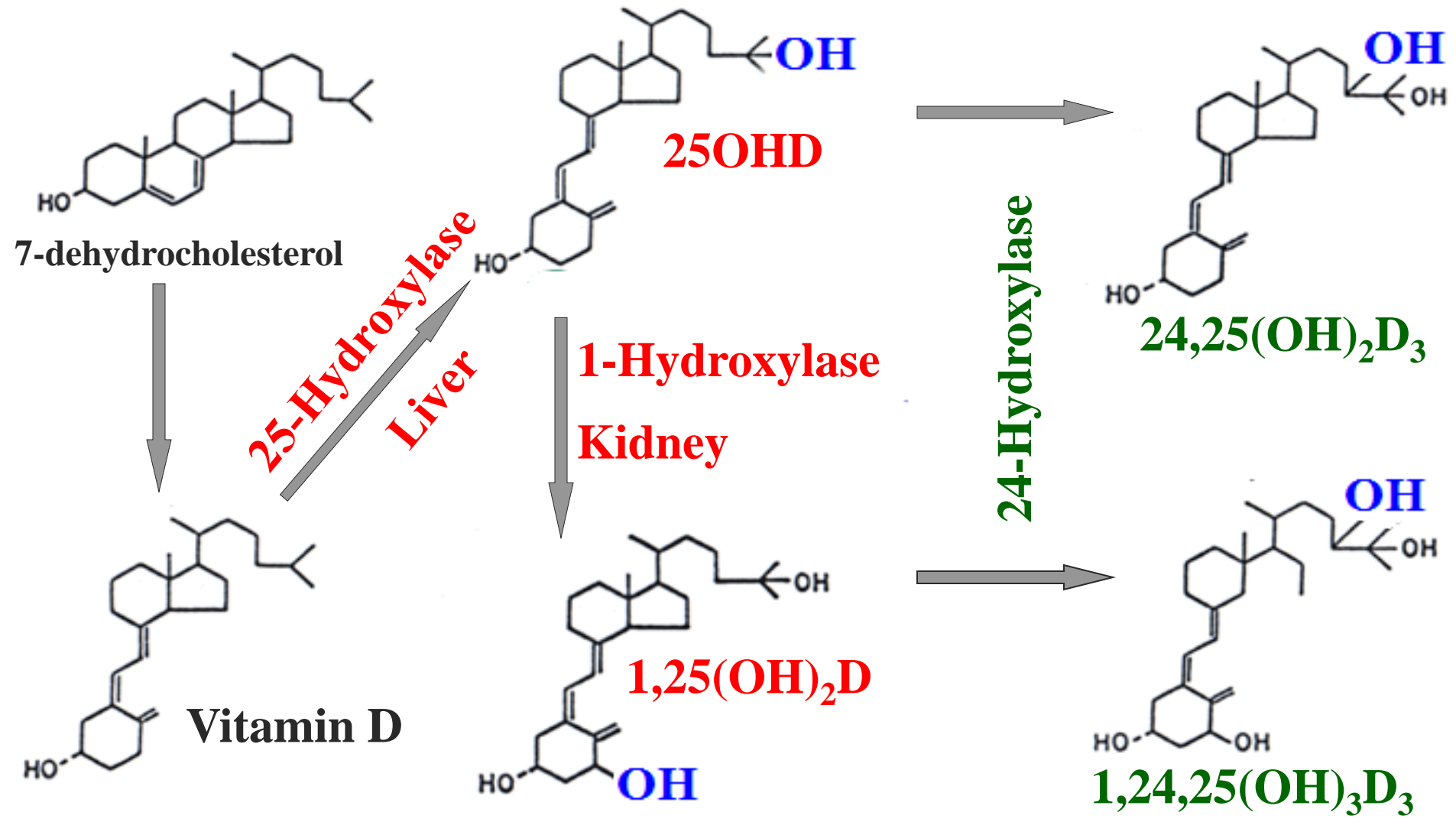


Results from 2007 RCPA-QAP vitamin A & E questionnaire

Vitamin D

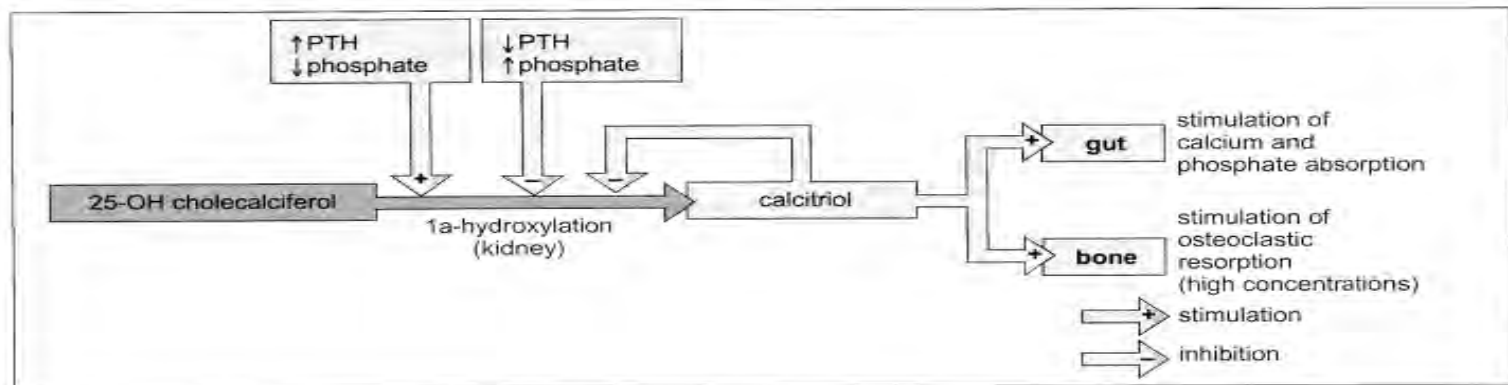


Vitamin D Metabolism

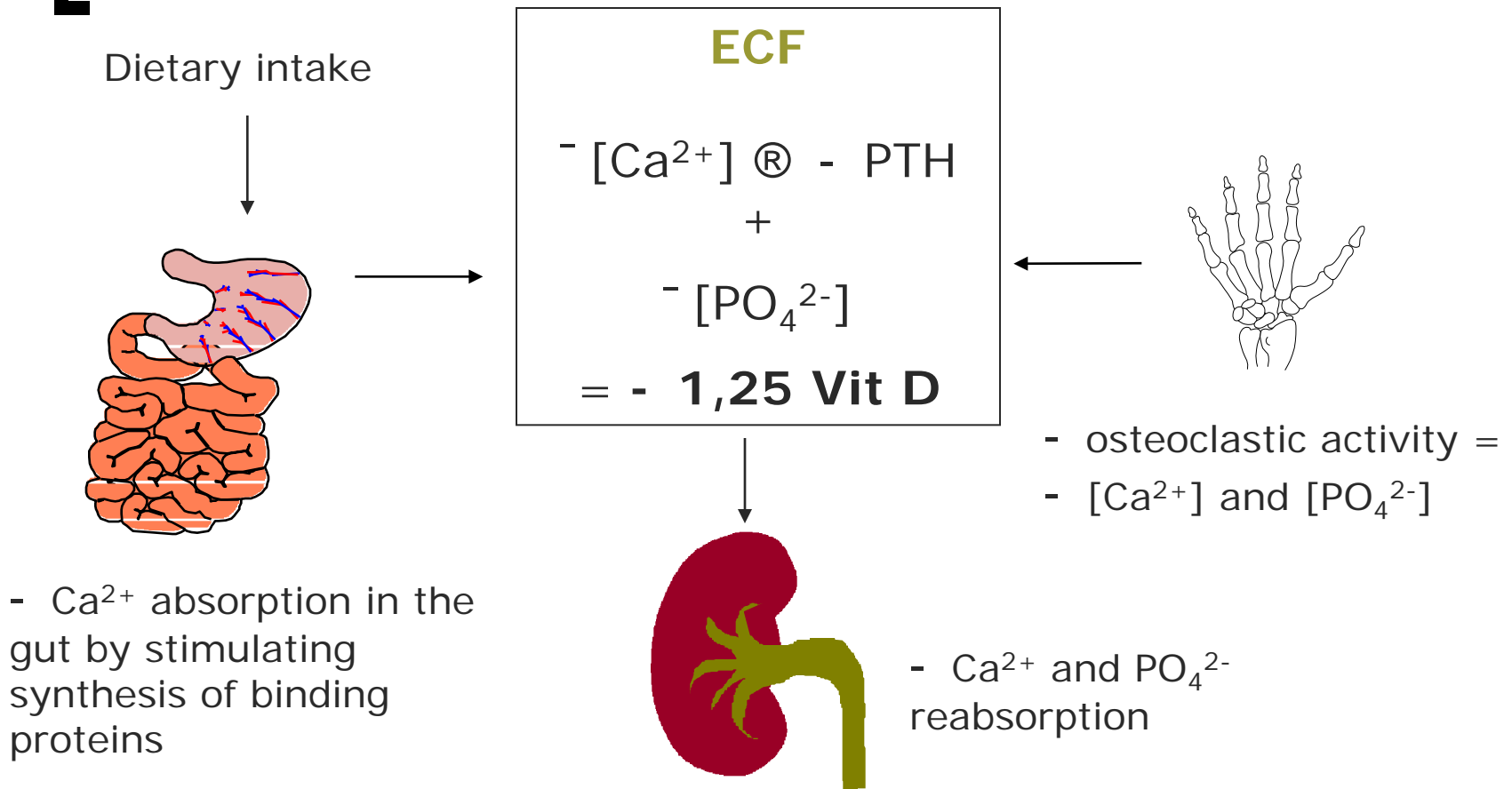


1,25 di OH Vitamin D

- n Regulates absorption of calcium
- n Important for bone growth & development
- n Two forms:
 - i Vitamin D2 = ergocalciferol, is ingested in foods
 - i Vitamin D3 = cholecalciferol, is produced in the skin on exposure to sunlight.
- n 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)



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

Vitamin D: Automated analysis

- n Roche Cobas e601
 - i 25 OH Vit D3 only



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



[Chromatography + MS (+MS)]

- n Gold standard
- n TAT a problem
- n Expertise required
- n 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?

JA Grant^{1,2}, MJ Whiting³, RF Greaves⁴, MJ Black⁵, AM Wootton²

¹Biochemistry Department, The Royal Melbourne Hospital, Parkville Vic 3050 Australia; ²School of Medical Sciences, RMIT University, Bundoora Vic 3083 Australia; ³SouthPath, Flinders Medical Centre, Bedford Park, SA 5042 Australia; ⁴Complex Biochemistry Department, The Royal Children's Hospital, Parkville Vic 3052 Australia; ⁵Clinical Biochemistry Department, Alfred Pathology Service, Melbourne Vic 3004 Australia.

Introduction

Vitamin D plays an important role in calcium homeostasis. Deficiency is associated with defects in bone mineralisation, and may predispose to a range of proinflammatory and immune disorders (1). Low levels are prevalent in many Australian populations and dietary supplementation is a major strategy in reducing fracture risk, particularly in the elderly (2). Accurate assessment of vitamin D status is essential, both to identify patients at risk and to monitor safe and effective treatment. A number of different vitamin D metabolites are present in serum as a result of hepatic hydroxylation of the parent compound. Of these, 25-hydroxyvitamin D (25OHD) is accepted as the best indicator of clinical vitamin D status (3) and is used routinely for this purpose.

Vitamin D exists in two forms, cholecalciferol (vitamin D₃), obtained mainly via endogenous synthesis, and ergocalciferol (vitamin D₂) obtained solely through diet. While vitamin D₃ is normally the major form, both may be listed as supplements and food additives. Until recently, only vitamin D₂ was used for this purpose in Australia; however pharmaceutical preparations of vitamin D₃ are now widely available.

Analysis of 25OHD is complicated by the requirement to detect both 25-hydroxycholecalciferol (25OHD₃) and 25-hydroxyergocalciferol (25OHD₂). Poor agreement between 25OHD immunoassays reported in several quality assurance programs (4) has raised concerns about their ability to accurately assess vitamin D status, particularly in patients taking vitamin D₂. Evaluation is hampered by lack of an agreed reference method with which to assess performance. Recently, an isotopic dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been described (5) with externalists as a reference method for 25OHD analysis.

AIM

To use LC-MS/MS as a reference method to

- 1) investigate the presence of 25OHD₂ in routine patient sera; and
- 2) assess the accuracy of five 25OHD immunoassays in samples containing one or significant amounts of this metabolite.

Methods

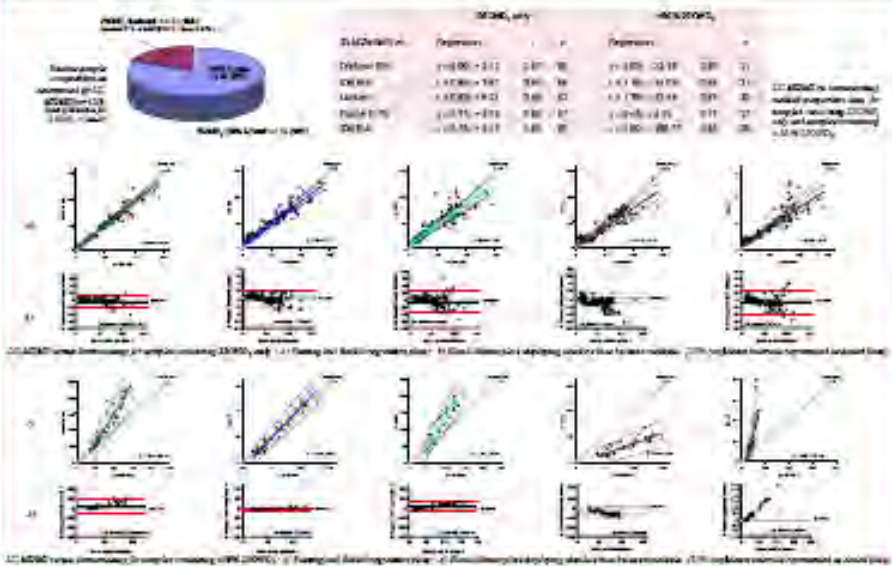
120 de-identified routine 25OHD serum samples were collected over a 12 week period in 2006. Of these, 107 were obtained from The Royal Children's Hospital (RCH) Pathology Service from a predominantly adult female population and 13 were from Melbourne Health (MH) Pathology Service from a mixed gender adult population. An additional 22 samples were collected from nine clinical trial subjects taking high doses of vitamin D₂ to provide samples containing substantial levels of 25OHD₂. Approval for the use of all samples was granted by both the RCH Ethics in Human Research Committee and the MH Human Research Ethics Committee. 25OHD₃, 25OHD₂, and total 25OHD concentrations were determined using LC-MS/MS (6).

25OHD was evaluated using the following five immunoassay systems:

- DiaSorh automated radioimmunoassay (RIA)
- IDS manual RIA
- DiaSorh Liaison[®] automated chemiluminescent immunoassay (CLIA)
- Roche Diagnostics[®] automated CLIA run on an E176
- IDS OC720E enzyme immunoassay (EIA) automated on a BEP2000

All samples were stored frozen (max -20°C) in separate aliquots for each assay and subjected to fewer than three freeze-thaw cycles to ensure analysis stability (7). Results from samples containing 100% 25OHD₃ and >50% 25OHD₂ were evaluated against LC-MS/MS using Analyse-it[®] (Clinical Laboratory) with Microsoft Excel 2003.

Results



Discussion

When samples contained only 25OHD₃, all immunoassays demonstrated a similar, slight negative bias compared to LC-MS/MS, with the manual RIA coming more closely than the automated assays.

Agreement with LC-MS/MS was much more variable when samples contained significant levels of 25OHD₂:

The Roche assay greatly underestimated 25OHD₂, which was reported as the antibody specificity for 25OHD₂ is stated as zero in the kit insert. This assay is marketed as a 25OHD₃ assay and is unsuitable for monitoring 25OHD in patients taking vitamin D₂.

The two DiaSorin assays (RIA and Liaison) demonstrated a positive proportional bias in this group, which was unexpected as the antibody specificity to 25OHD₂ is stated as 100%. Similarly, the IDS RIA showed an unexpectedly good correlation given an antibody specificity to 25OHD₂ of 75%. It is proposed that these assays may detect additional metabolites, such as 24-hydroxyvitamin D₃, which have been detected in the serum of subjects taking high doses of vitamin D₂ (8). It is unclear why the IDS RIA produced much higher results than the IDS RIA, as the antibody used in these kits is stated to be the same.

While most routine patient samples contained only 25OHD₃, 25OHD₂ was detected in 18% indicating that vitamin D₂ is still used as a dietary supplement and we should remain concerned about the accuracy of routine 25OHD assays in the presence of this metabolite.

Conclusions

All assays demonstrated a similar, slight negative bias compared with LC-MS/MS when samples contained 25OHD₃ only. Agreement was more variable in samples containing 25OHD₂. The presence of this metabolite in 18% of routine patients indicates that vitamin D₂ is still in use as a vitamin D supplement and routine 25OHD results should be interpreted with caution.

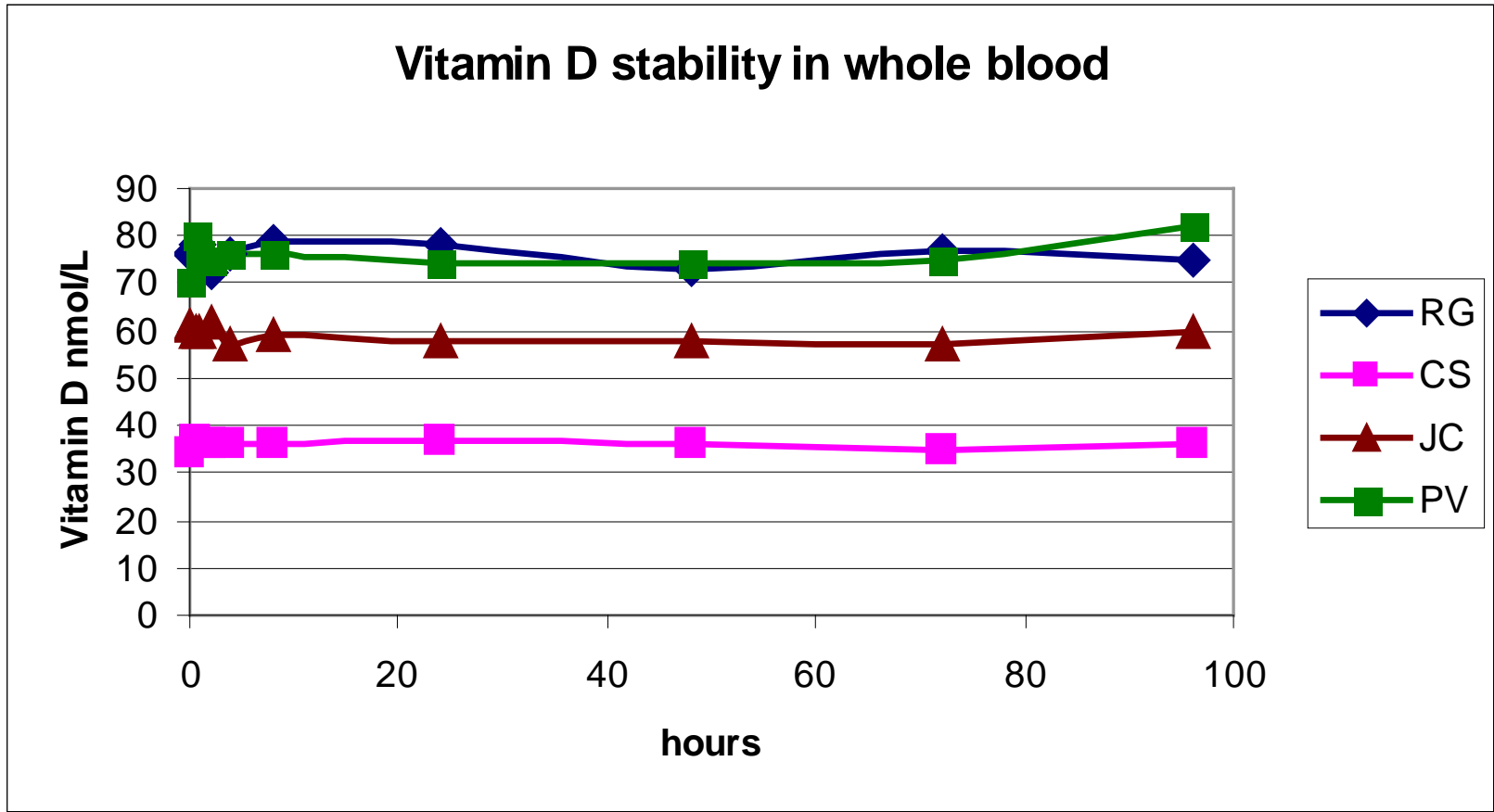
References

1. Holm M, Wimalawansa S. In: Vitamin D: A Practical Approach, 2nd ed. Oxford: Blackwell, 2005: 1-15.
2. Holm M, Wimalawansa S. In: Vitamin D: A Practical Approach, 2nd ed. Oxford: Blackwell, 2005: 16-25.
3. Holm M, Wimalawansa S. In: Vitamin D: A Practical Approach, 2nd ed. Oxford: Blackwell, 2005: 26-35.
4. Holm M, Wimalawansa S. In: Vitamin D: A Practical Approach, 2nd ed. Oxford: Blackwell, 2005: 36-45.
5. Holm M, Wimalawansa S. In: Vitamin D: A Practical Approach, 2nd ed. Oxford: Blackwell, 2005: 46-55.
6. Holm M, Wimalawansa S. In: Vitamin D: A Practical Approach, 2nd ed. Oxford: Blackwell, 2005: 56-65.
7. Holm M, Wimalawansa S. In: Vitamin D: A Practical Approach, 2nd ed. Oxford: Blackwell, 2005: 66-75.
8. Holm M, Wimalawansa S. In: Vitamin D: A Practical Approach, 2nd ed. Oxford: Blackwell, 2005: 76-85.

References

1. Holm M, Wimalawansa S. In: Vitamin D: A Practical Approach, 2nd ed. Oxford: Blackwell, 2005: 1-15.
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8. Holm M, Wimalawansa S. In: Vitamin D: A Practical Approach, 2nd ed. Oxford: Blackwell, 2005: 76-85.

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

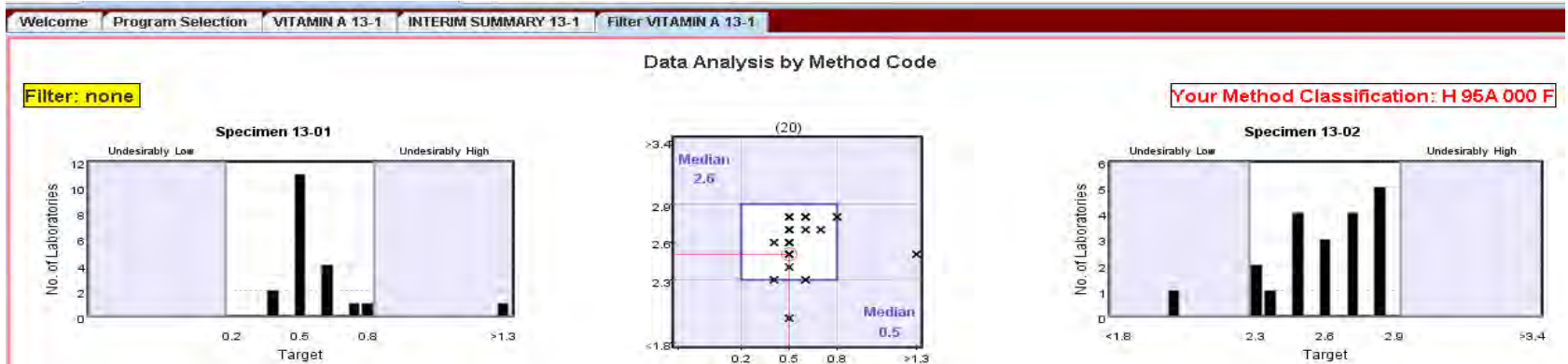
RCPA-QAP Vitamin program

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

ABN 32 003 520 072

RCPA-AACB CHEMICAL PATHOLOGY QAP GROUP

Flinders Medical Centre Bedford Park South Australia 5042 AUSTRALIA

• Tel: +61 8 8374 0797 • Fax: +61 8 8374 0695 • Email: Chempath.QAP@fmc.sa.gov.au

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.

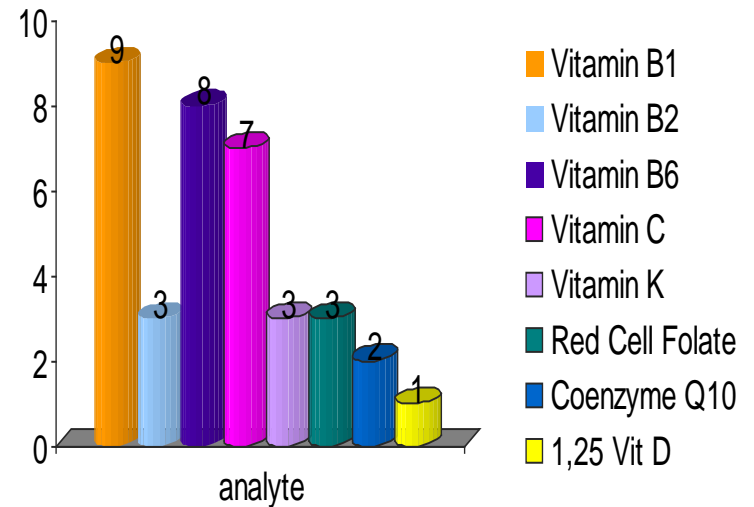
Your Name: _____

Questionnaire sent to labs participating in the
Vitamin and Endocrine Programs

RCPA-QAP vitamin program

Response to questionnaire:

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



Leading world wide EQA program

Members of the AACB vitamins working party 2009



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

Ronda.greaves@rch.org.au