Technical Issues in the Measurement of Vitamin D Status: Variability & Standard Reference Material

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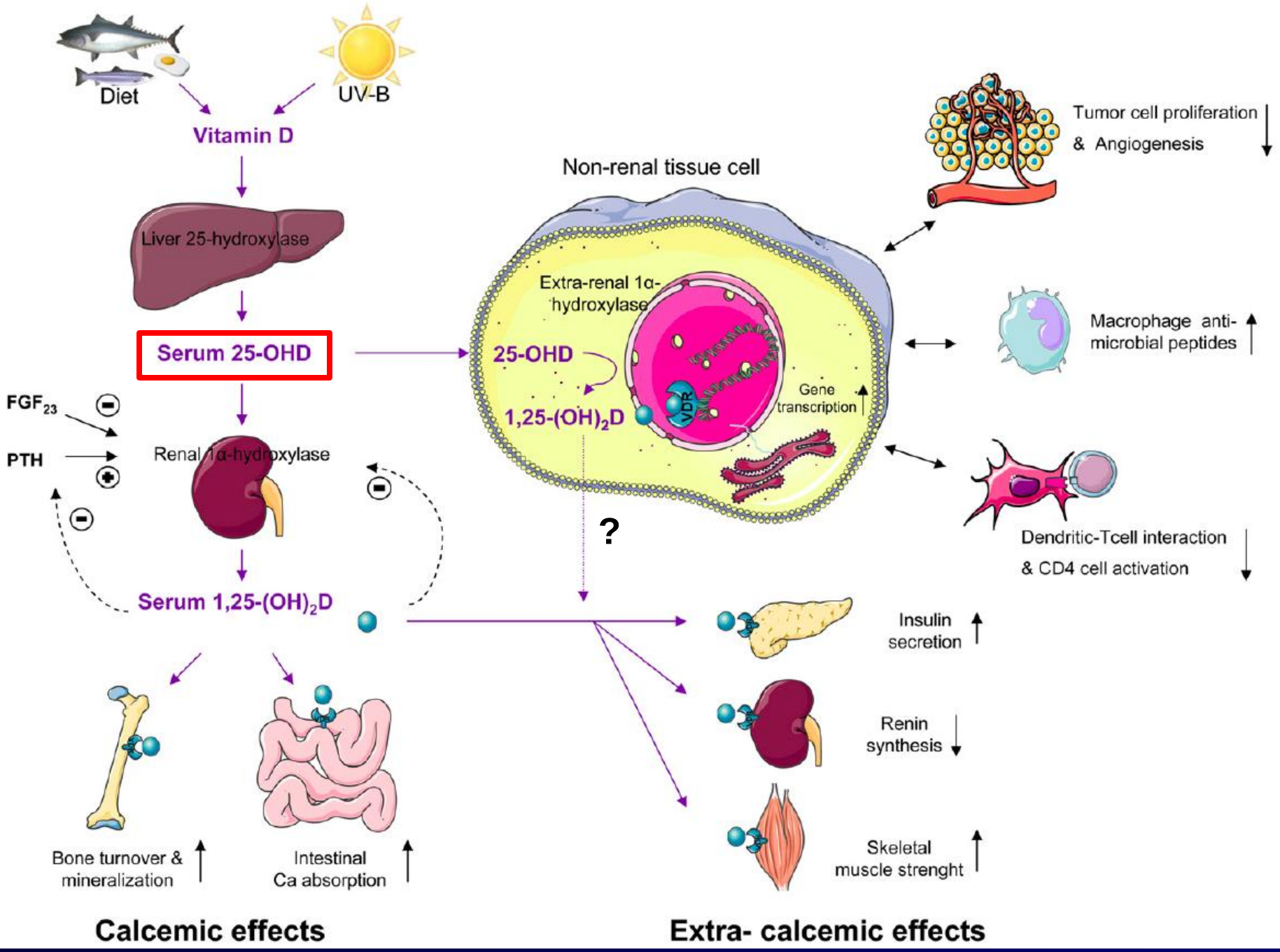
Technical Aspects in the Measurement of Vitamin D Status

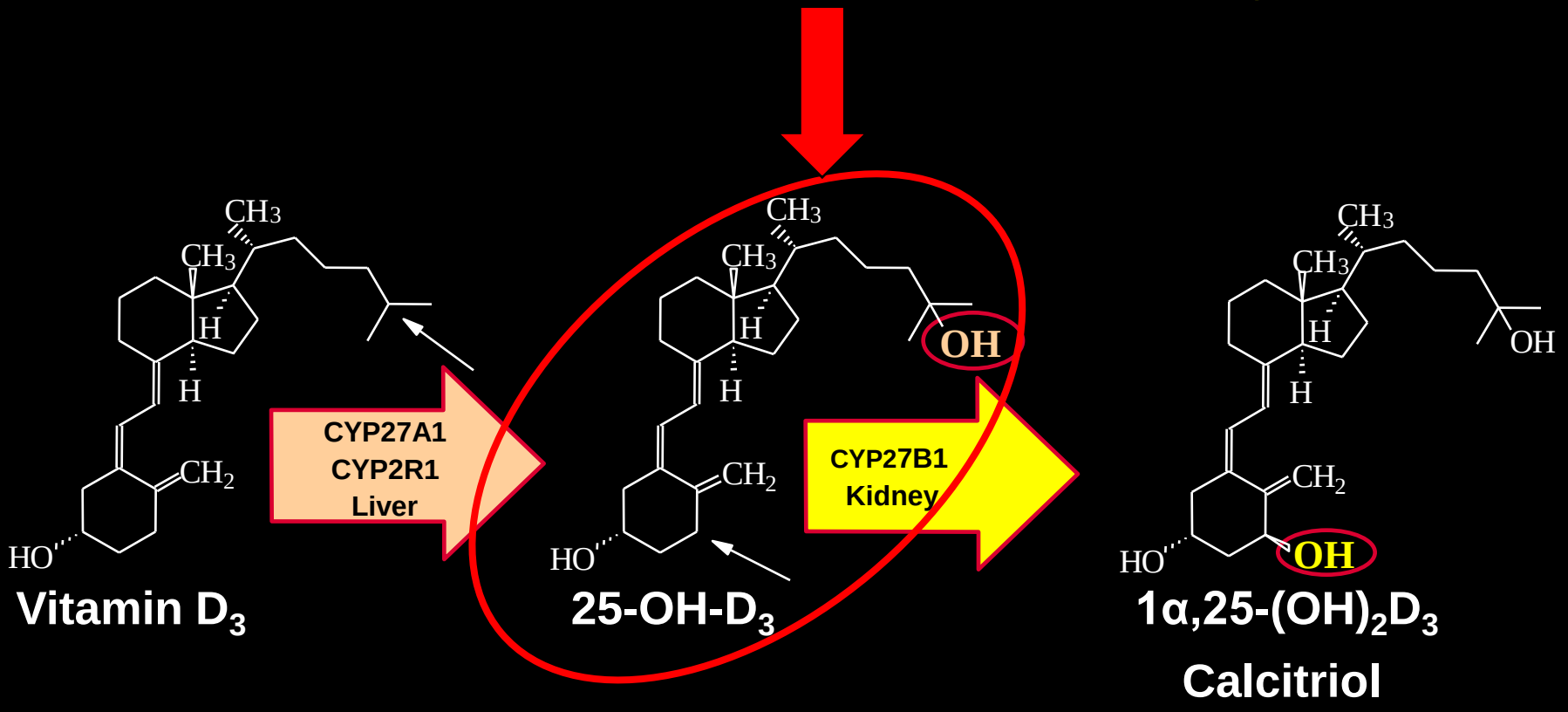
Talk Overview:

- What should we be measuring: 25-OH-D or 1,25-$(OH)_2D$?
- Brief overview of current methods
  - Antibody-based Methods
  - LC-based Methods
- Current controversies in vitamin D assay
  - What is the Normal range for 25-OH-D?
  - Performance Characteristics of Current 25-OH-D Assay
  - Standard Reference Material
  - Are Vitamin D_2 and Vitamin D_3 biologically equivalent ?
  - Frequency of 25-OH-D assay?



Janssens W et al (2009) Am J Respir Crit Care Med 179:630-6.

Metabolism of Vitamin D_3



Vitamin D_3

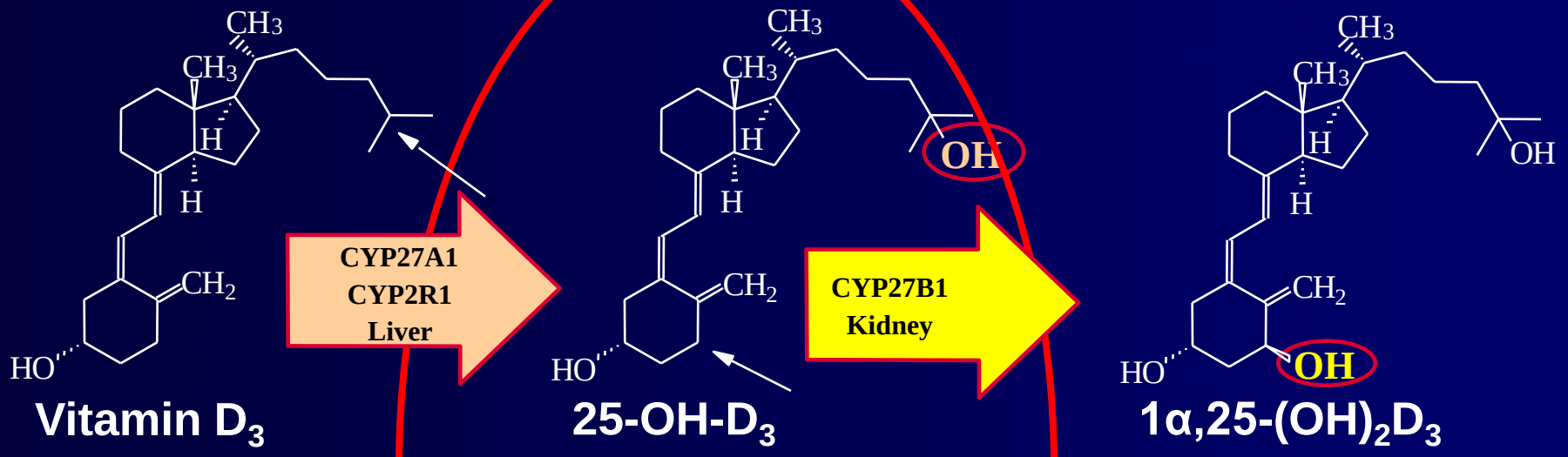
25-OH-D_3

1α,25-$(OH)_2D_3$

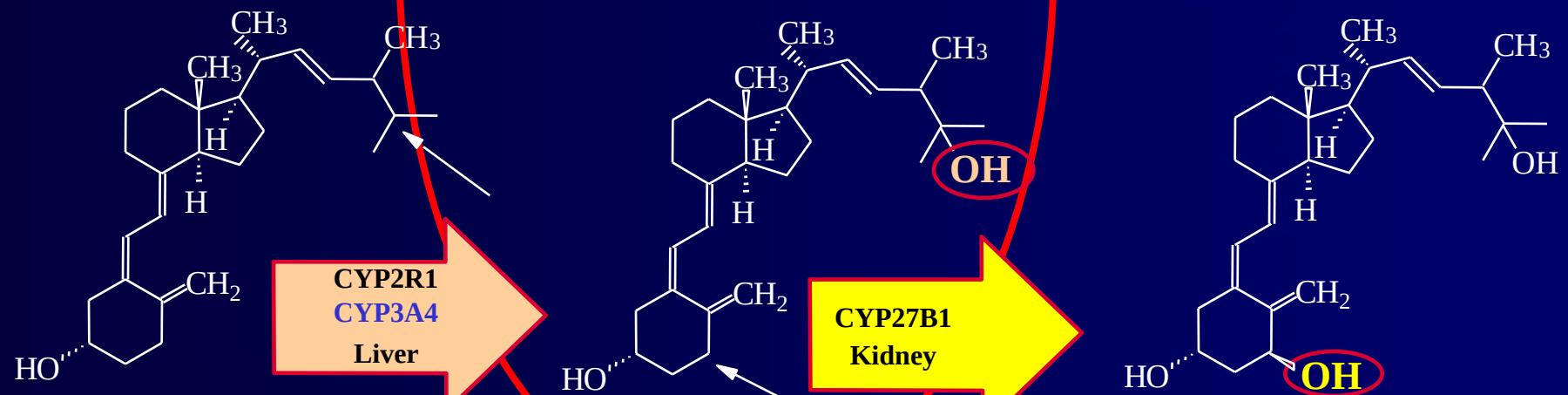
Calcitriol

Similar pathway exists for vitamin D_2

Metabolism of Vitamins D_3 and D_2



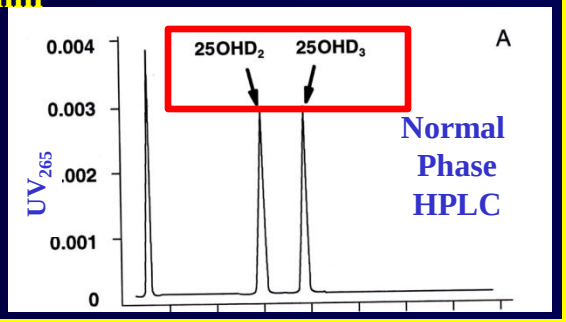
Vitamin D_3 | 25-OH-D_3 | 1α,25-$(OH)_2D_3$



Vitamin D_2 | 25-OH-D_2 | 1α,25-$(OH)_2D_2$

HPLC

Extraction → Minicolumn → UV_{265nm} Detection



RIA Kits

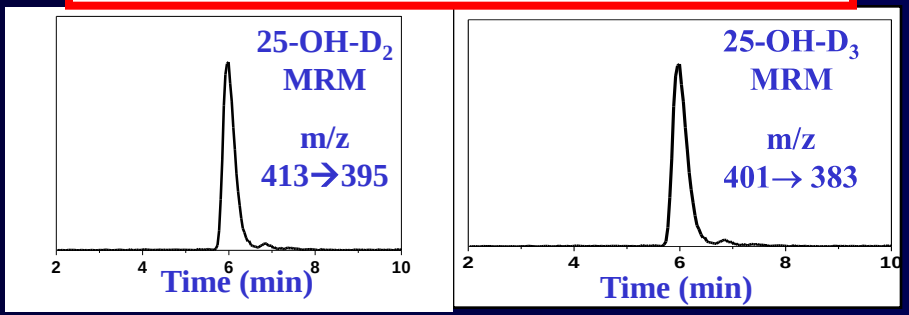
DiaSorin RIA, ^{125}I-ligand
-ACN extraction, primary & secondary Ab
-Co-specific for 25-OH-D_2 and 25-OH-D_3

IDS RIA, ^{125}I-ligand
-ACN extraction, primary & secondary Ab
-discriminates against 25-OH-D_2 (0.75)

LC-MS/MS

Extraction → Minicolumn → MRM using specific fragment

25-OH-D_2 + 25-OH-D_3= total 25-OH-D



EIA/Chemiluminescence

DiaSorin Liaison, chemiluminescence
-whole serum, w/antibody coated particles
-Detects 25-OH-D_2 & 25-OH-D_3,180 smpls/h

IDS EIA on New Dedicated Instrument
-no extraction, biotin labeled ligand,
-avidin-labeled horse radish peroxidase
-discriminates against 25-OH-D_2 (0.75)

Roche E170 Analyzer.
Automated electro-chemiluminescence method
-detects ONLY 25-OH-D_3

Current Controversies with 25-OH-D assay

(a) What is the normal range?

(b) Performance Characteristics of Current 25-OH-D Assays

- **i) Vitamin D External Quality Assessment Scheme (DEQAS)**
- **ii) Measurement of Total 25-OH-D in samples**
- **iii) Measurement of 25-OH-D_2 content**
- **iv) Pediatric samples**

(c) Are Vitamin D_2 and Vitamin D_3 biologically equivalent ?
Are separate assays of 25-OH-D_2 and 25-OH-D_3 clinically useful?

(d) Can we avoid use of 25-OH-D assay?
Suggested frequency of 25-OH-D Testing

Plasma 25-OH-D Ranges

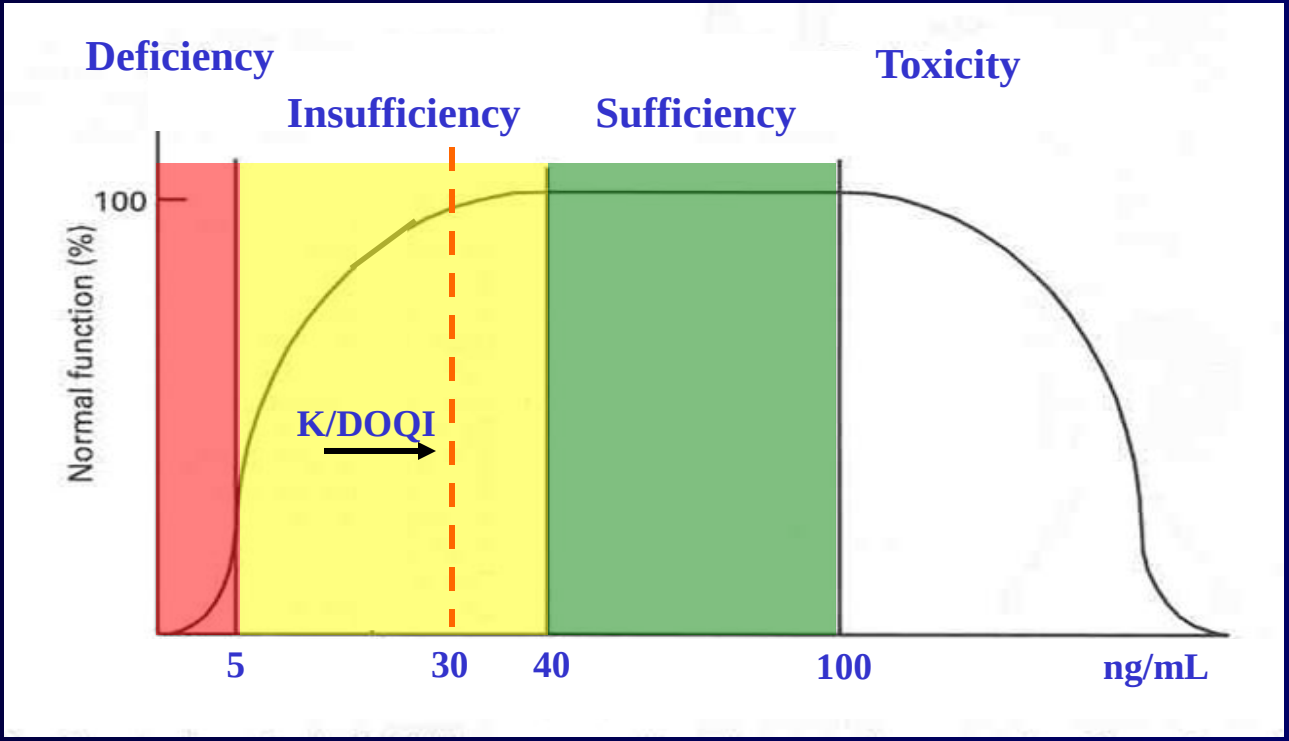
Observed Normal ranges

Jones (1978)- HPLC Assay = 9.1 - 23.9 ng/mL (Winter)

Hollis (1997) - RIA Assay = 9.9 - 41.5 ng/mL
(Chapter 38- 'Vitamin D' 1st Edition)

Hollis (2005)- CLIA Assay = 9.5 - 52.0 ng/mL
(Chapter 58 'Vitamin D' 2nd Edition)

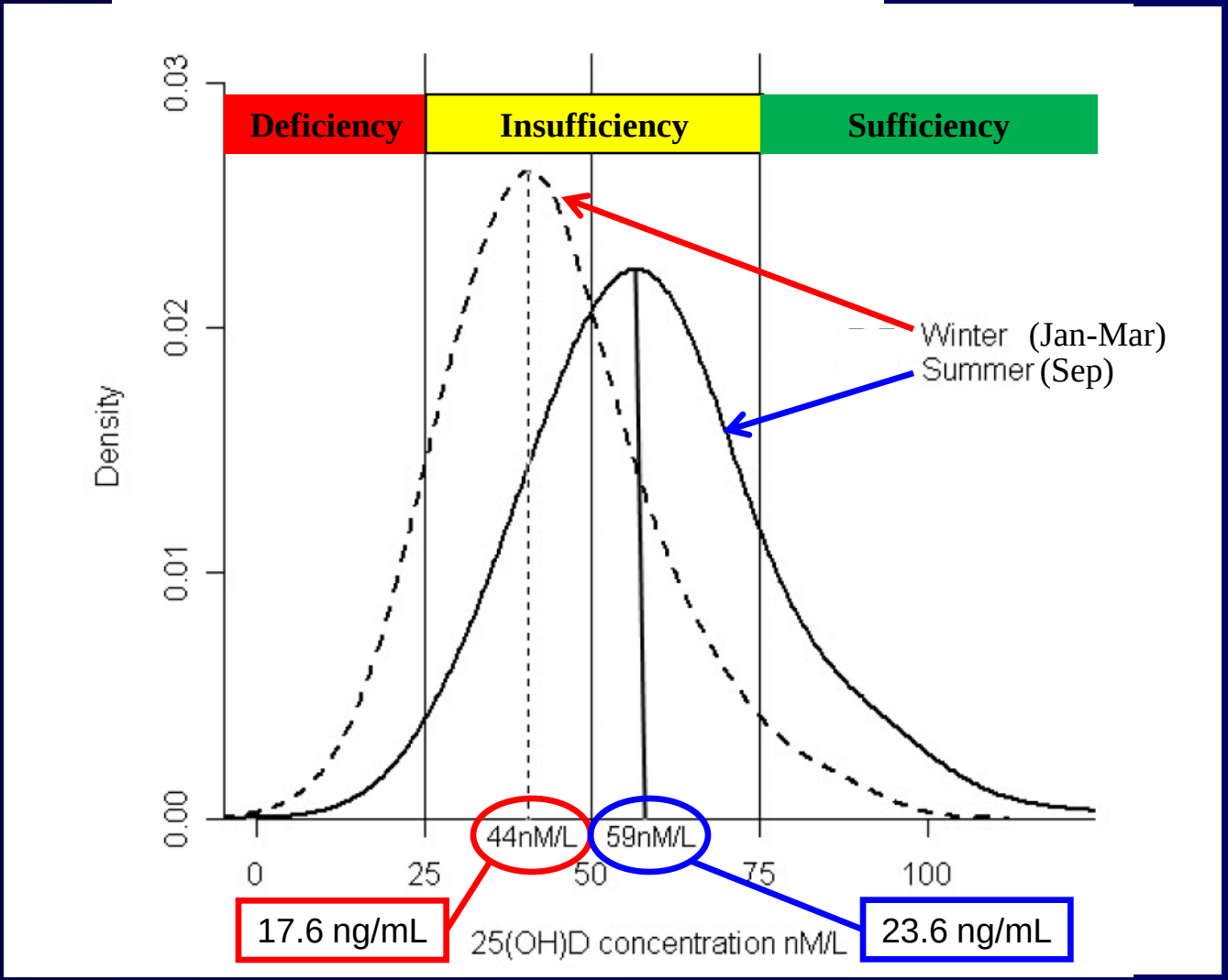
Proposed Target ranges



Seasonal variance of 25-(OH) vitamin D in the general population of Estonia, a Northern European country at Latitude 59° N

Mart Kull Jr*[1,2], Riina Kallikorm[1,2], Anu Tamm[2] and Margus Lember[1,2]

BMC Public Health 2009, **9**:22



Prevention and Treatment of vitamin D insufficiency and vitamin D deficiency

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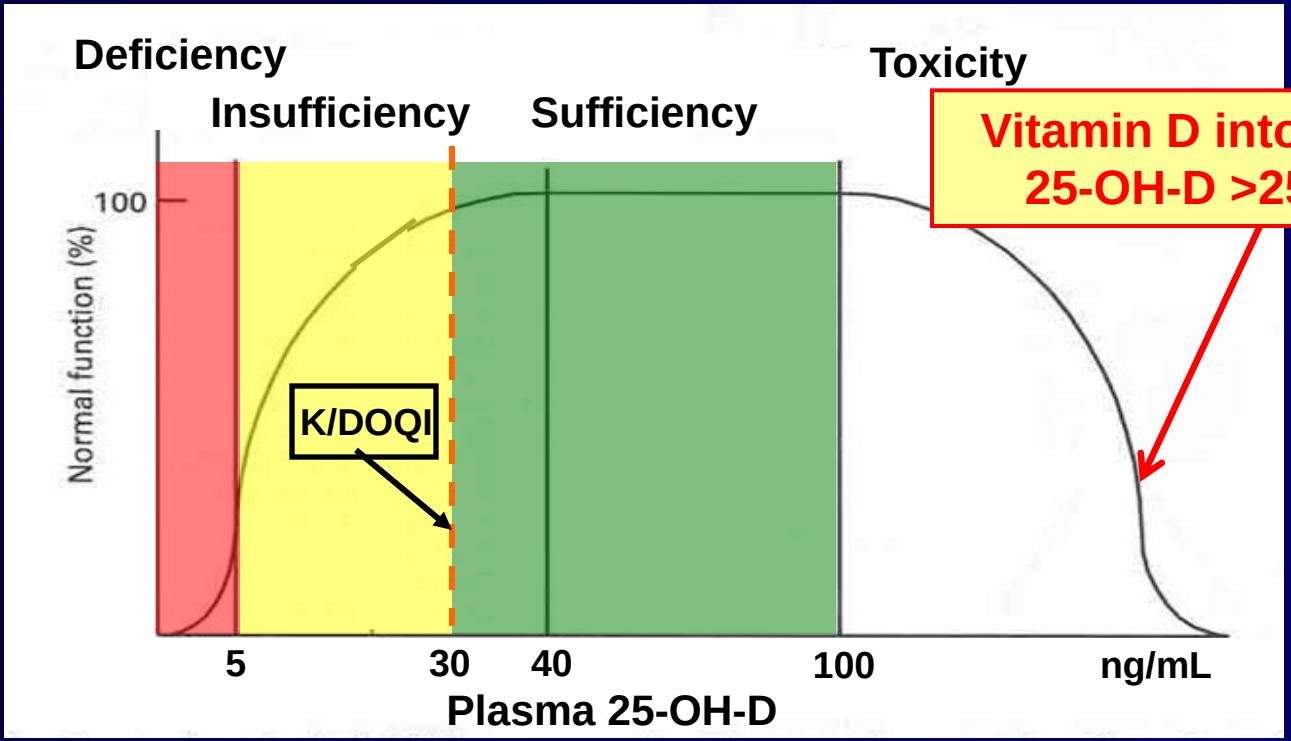
SUGGESTED THRESHOLD = 30 ng/mL or 75 nmol/L



Adapted from Hollis

Current Controversies with 25-OH-D assay

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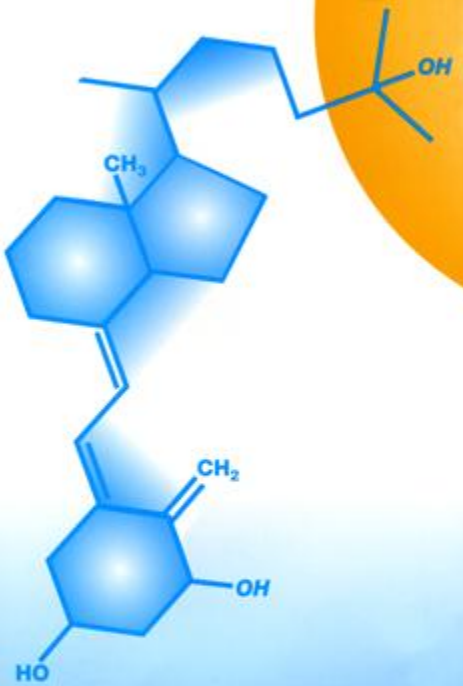
Suggested frequency of 25-OH-D Testing



DEQAS

D · E · Q · A · S
Vitamin D External Quality Assessment Scheme

Vitamin D External Quality Assessment Scheme

An International Programme for monitoring the accuracy and precision of 25 Hydroxyvitamin D and 1,25 Dihydroxyvitamin D Assays

Experience in clinical chemistry laboratories suggests that participation in an external quality assessment scheme (EQAS) is a prerequisite for improved analytical performance. The 25 hydroxyvitamin D EQAS (DEQAS) was launched in 1989 after several surveys[1,2] revealed serious inconsistencies among laboratories measuring the analyte. The scheme was expanded in1997 to include 1,25 dihydroxyvitamin D.

The widespread use of commercial assays coupled with the need for accreditation has stimulated considerable interest in DEQAS which has over 180 participants in 23 countries.

Endocrine Laboratory
Charing Cross Hospital
Fulham Palace Road
London W6 8RF · UK
E-mail administrator@deqas.org
Website www.deqas.org

1. Mayer, E. and Schmidt-Gayk, H. (1984) Interlaboratory Comparison of 25-Hydroxyvitamin D Determination. Clin.Chem. 30, 1199-1204
2. Carter, G.D. and Short, F. (1988) 25 Hydroxyvitamin D: Results of a n*ational Quality Assessment. J.Endocrinol. 117, suppl: 112
3. Healey, M.J.R. (1979) Outliers in Clinical Chemistry Quality-Control Schemes. Clin.Chem. 25,675-677

ASSAY METHOD PERFORMANCE

LC-BASED versus ANTIBODY-BASED

DIFFERENT STRENGTHS & WEAKNESSES

OPERATOR PERFORMANCE

LEVEL OF EXPERIENCE WITH VITAMIN D

What is the 'Gold Standard' to judge 25-OH-D Assays by?

1) Gas Chromatography-Mass Spectrometry (GC-MS)

Extraction; lengthy purification; derivatization; GC; detection of fragments

- **Seamark DA, Trafford D, Makin HLJ (1980) The estimation of vitamin D and some metabolites in human plasma by mass fragmentography. Clinica Chimica Acta 106:51-62**

2) HPLC with UV Detection

Extraction; clean-up on LC-1; separation 25-OH-D_2 & 25-OH-D_3 on LC-2; UV

- **Eisman JA, Shepard RM, DeLuca HF(1977) Determination of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 in human plasma using high pressure liquid chromatography. Analytical Biochemistry 80: 298-305.**
- **Jones G (1978) Assay of vitamins D_2 & D_3, and 25-hydroxyvitamins D_2 & D_3 in human plasma by high-performance liquid chromatography. Clinical Chemistry 24: 287-298.**

3) All-Laboratory Trimmed Mean (ALTM) from 650+ labs

Adopted by DEQAS as appropriate tool for comparison of data in lieu of GC-MS

4) Vitamin D Council claims it is the Diasorin RIA assay!

John Cannell promotes the Diasorin assay as a check for the blood spot assay

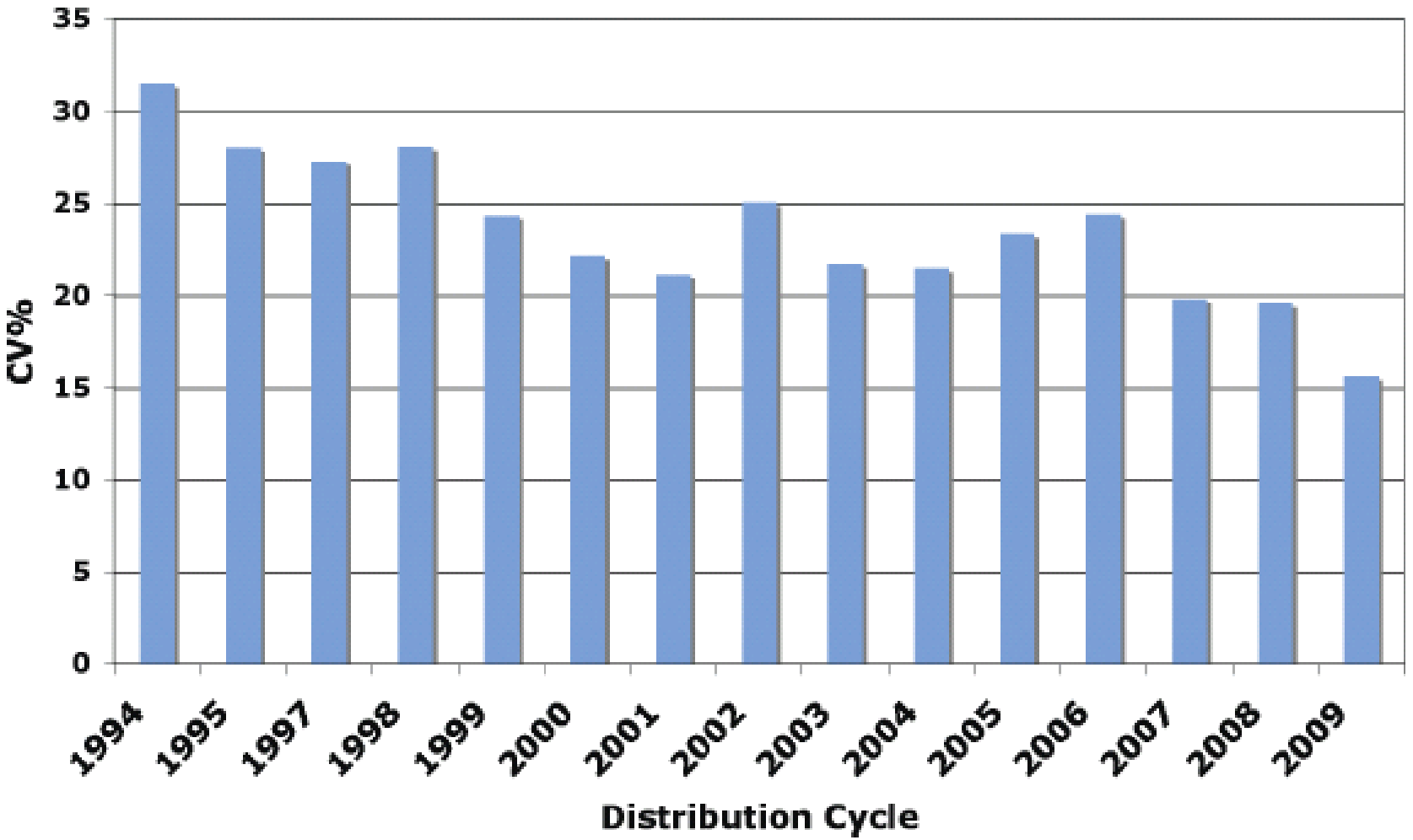


FIGURE 4 Imprecision of 25-OHD results (all participants) from 1994 to 2009*.

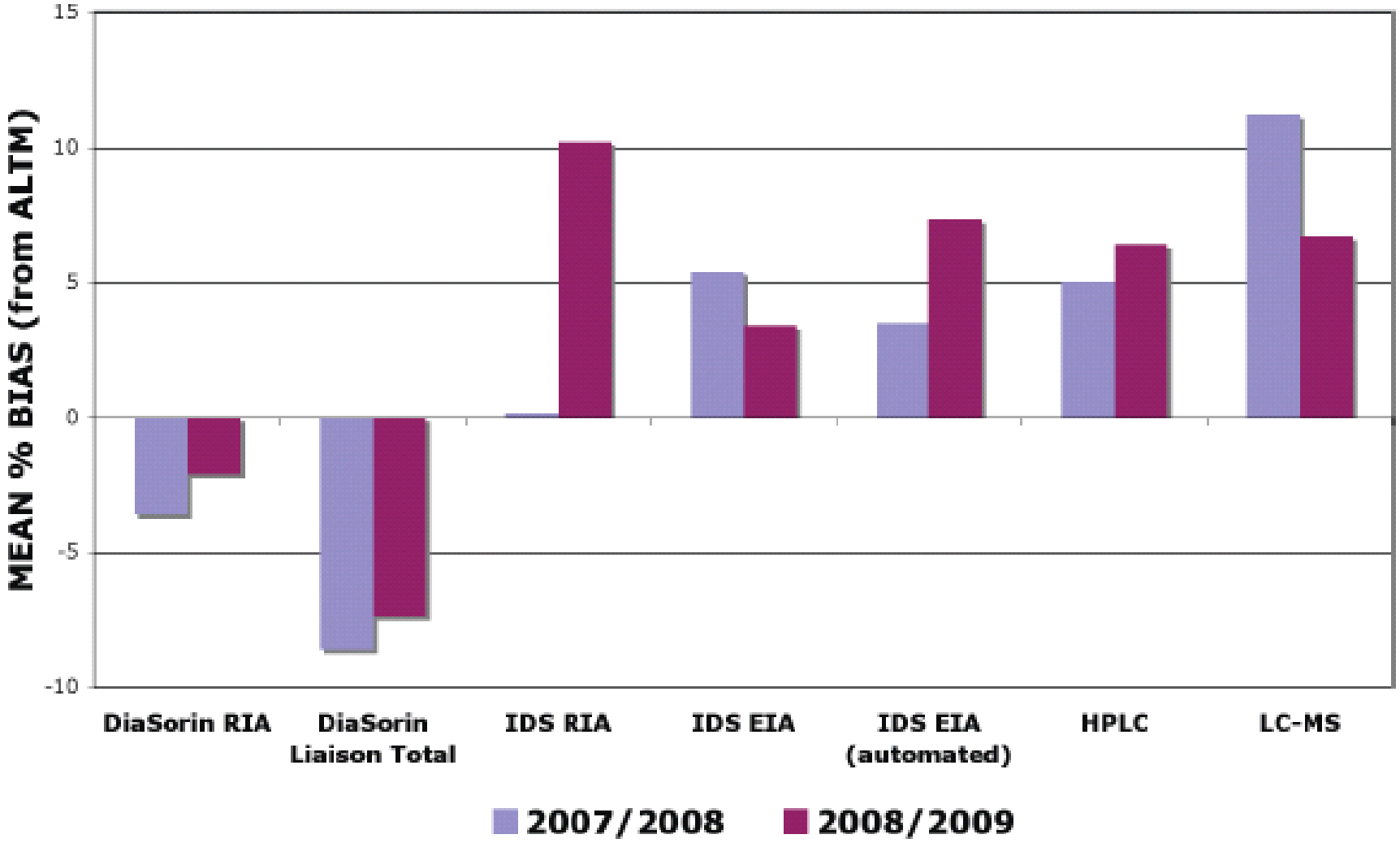
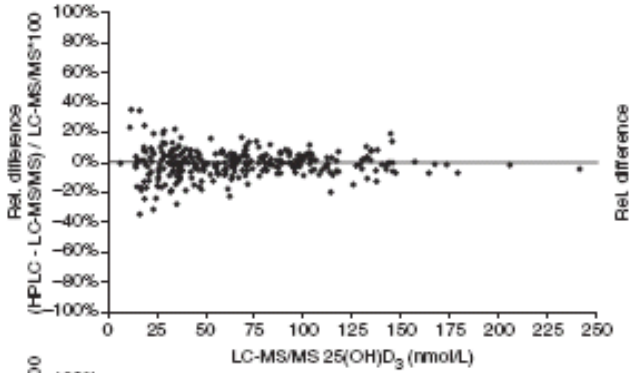


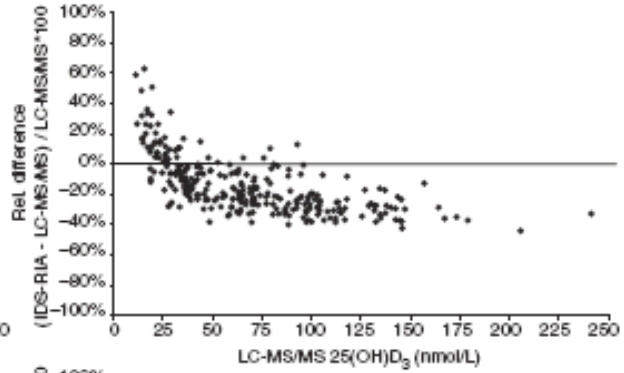
FIGURE 5 Relative performance of 25-OHD methods in the last two distribution cycles.

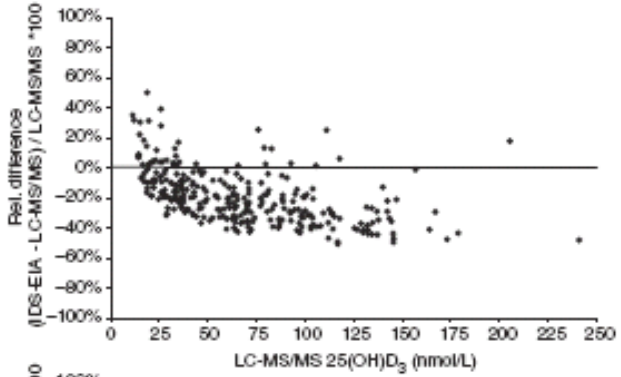
Accuracy and clinical implications of seven 25-hydroxyvitamin D methods compared with liquid chromatography–tandem mass spectrometry as a reference

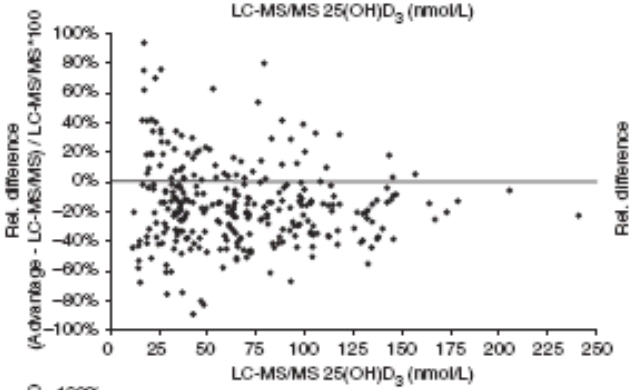
Heinz Jürgen Roth[1], Heinrich Schmidt-Gayk[1], Holger Weber[2] and Christoph Niederau[3]

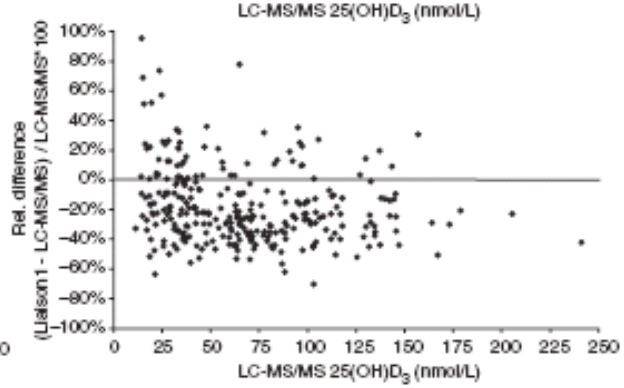
Ann Clin Biochem 2008; 45: 153–159.

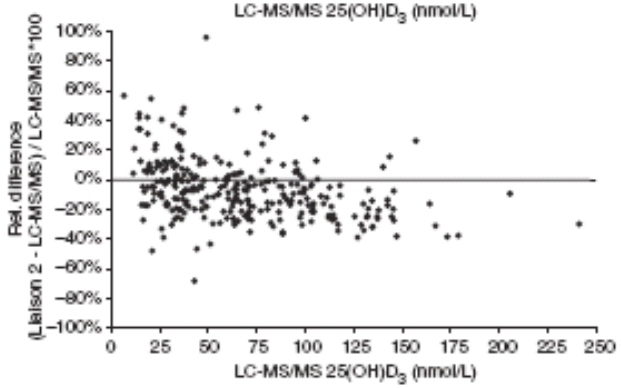


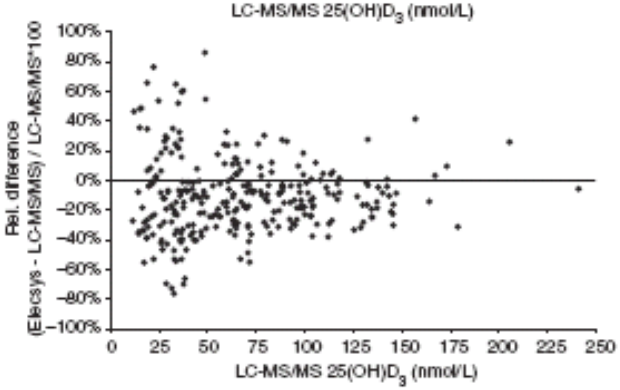






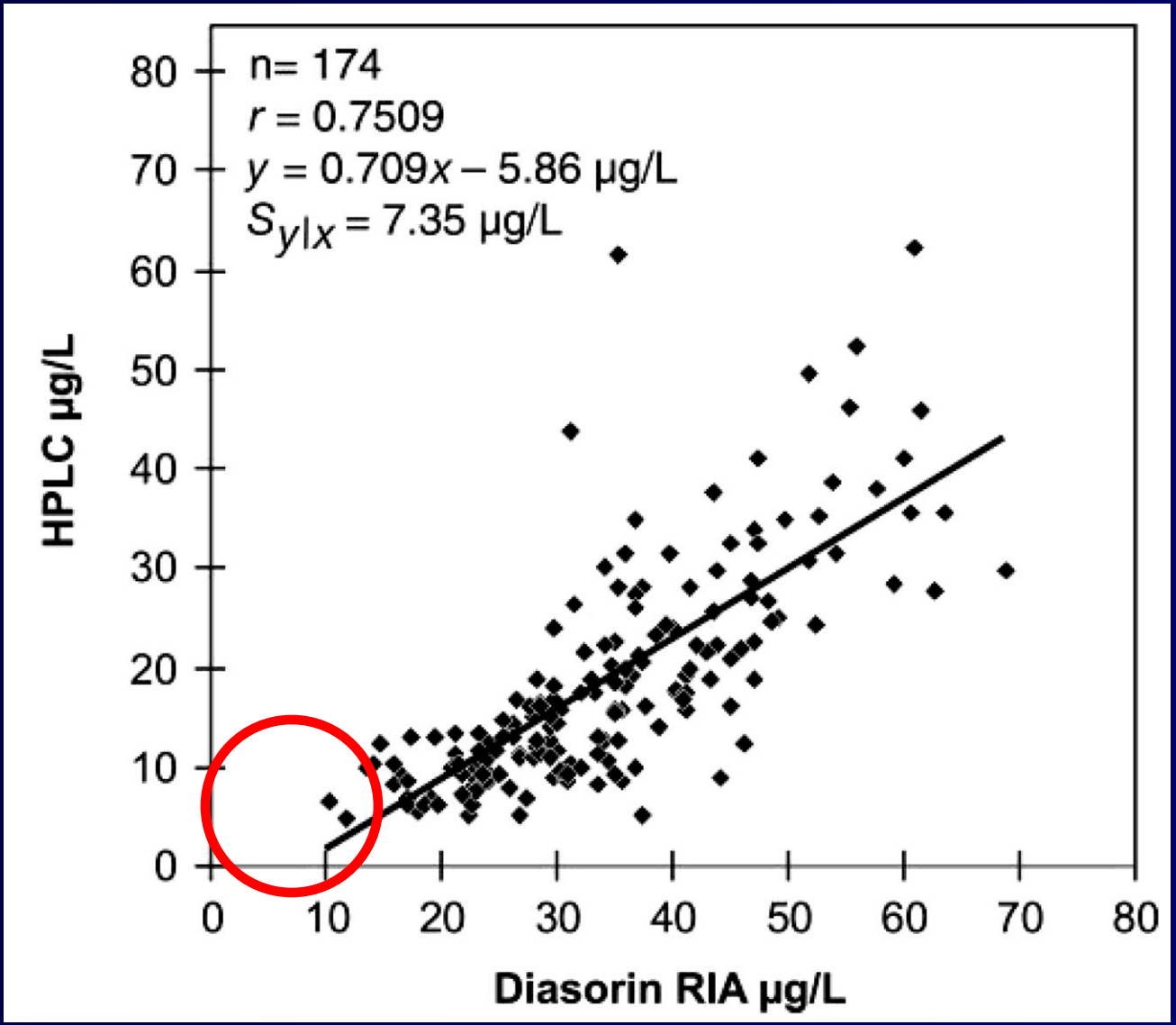






COMPARISON OF HPLC vs DIASORIN RIA

Lensmeyer et al (2006) Clin Chem 52:1120-6



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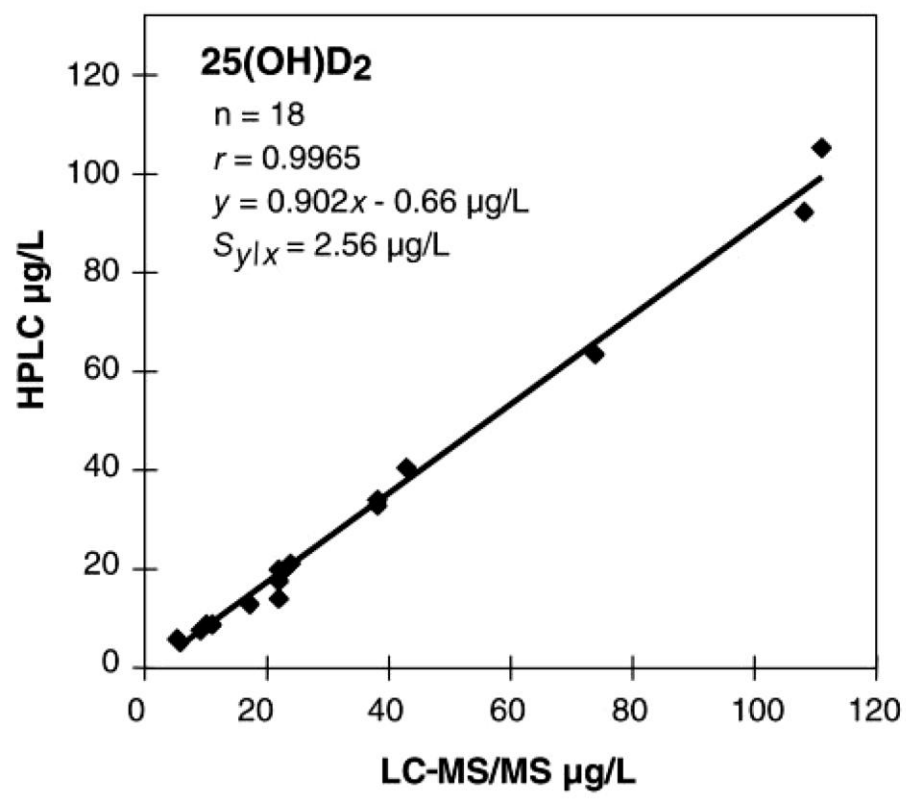
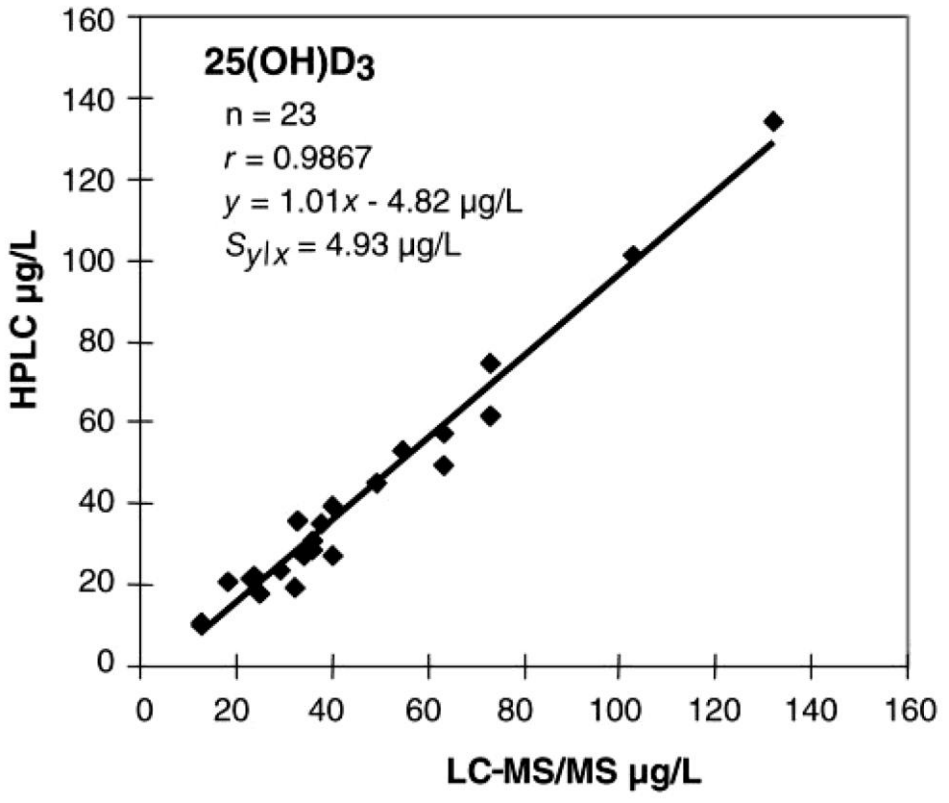
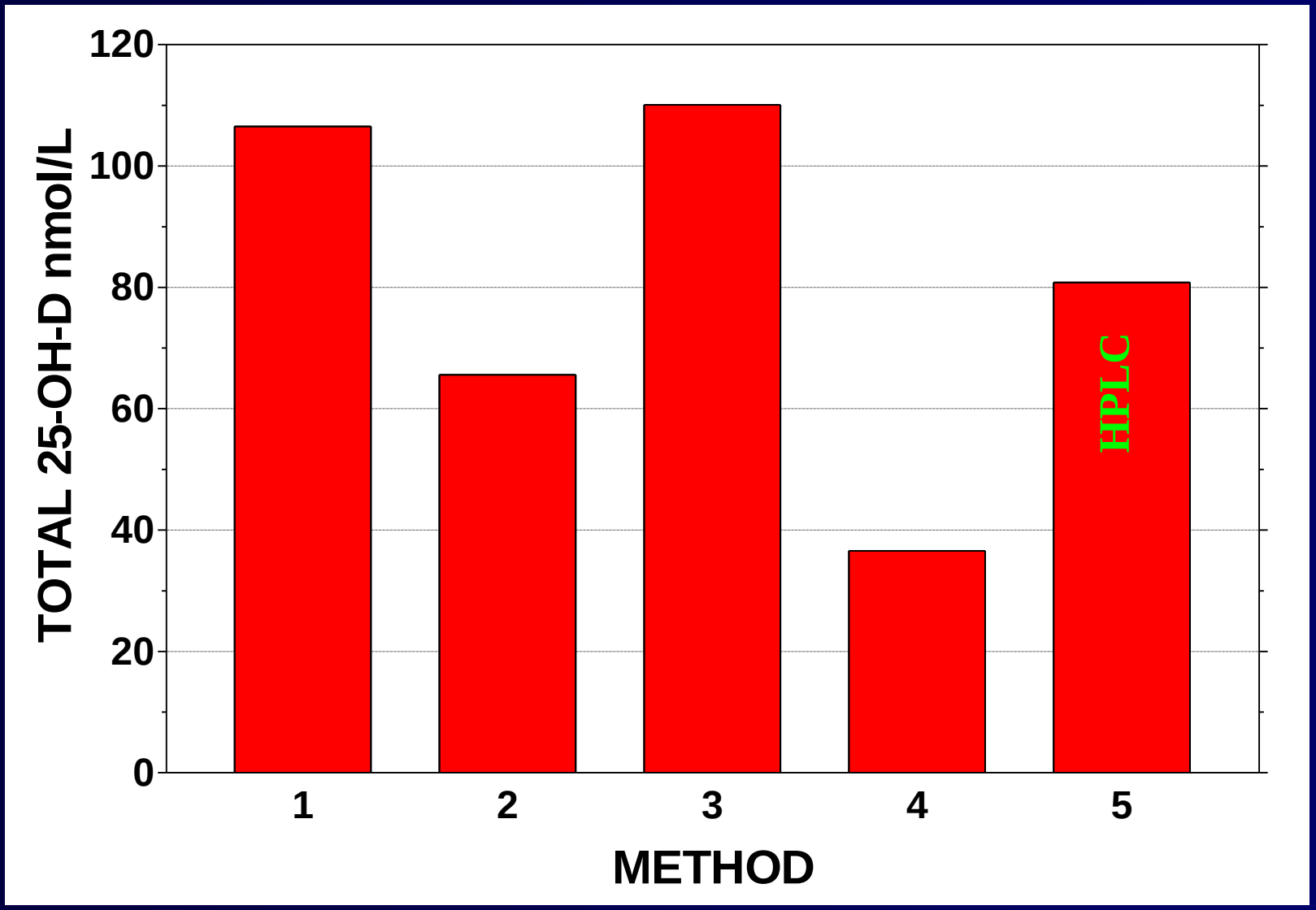
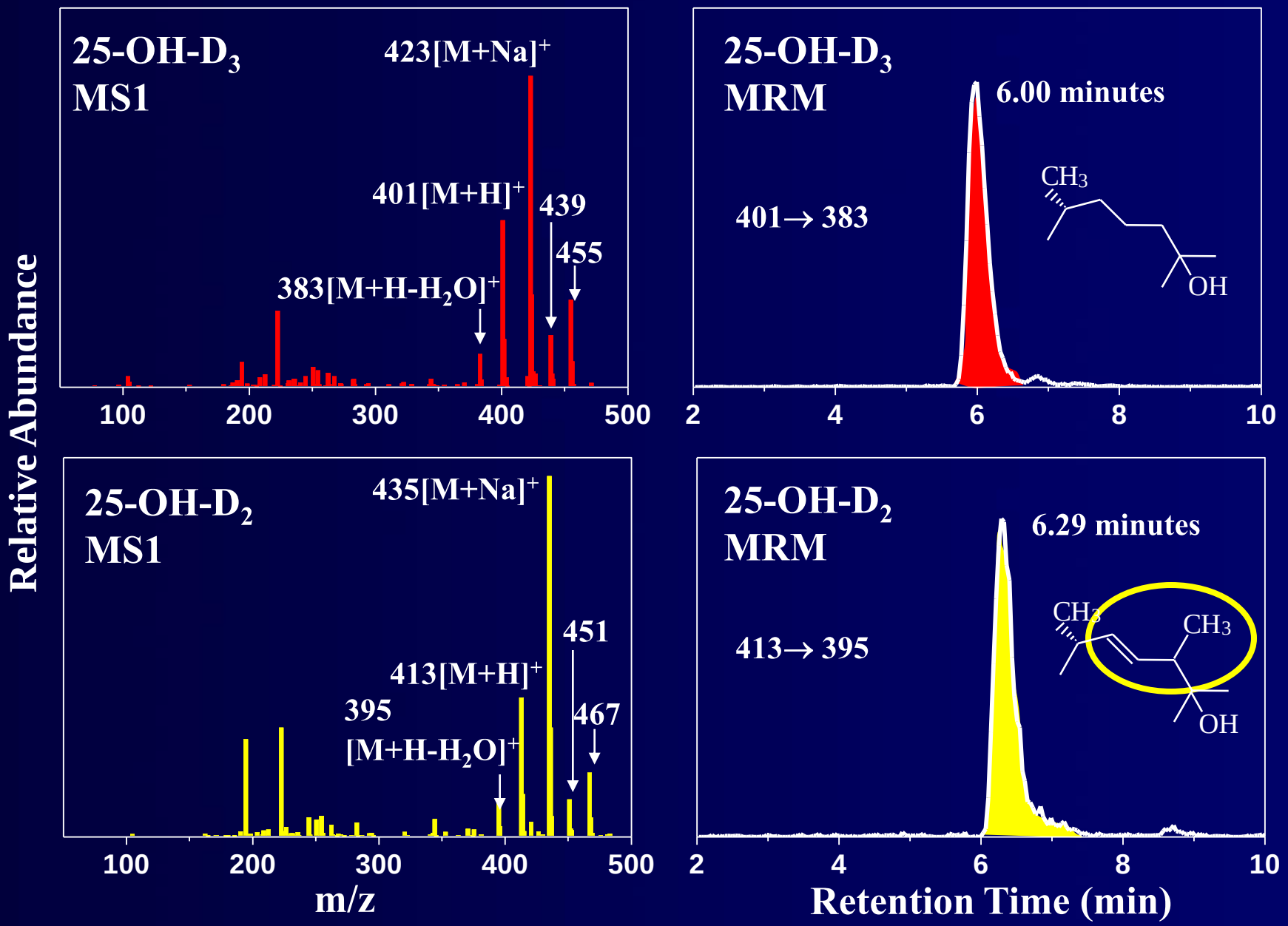




RECOVERY OF 25-OH-D_2–ENRICHED SAMPLES



LC-MS/MS analysis of 25-OH-D



0021-972X/06/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 91(8):3055–3061

doi: 10.1210/jc.2006-0710

C-3 Epimers Can Account for a Significant Proportion of Total Circulating 25-Hydroxyvitamin D in Infants, Complicating Accurate Measurement and Interpretation of Vitamin D Status

Ravinder J. Singh, Robert L. Taylor, G. Satyanarayana Reddy, and Stefan K. G. Grebe

Departments of Laboratory Medicine and Pathology (R.J.S., R.L.T., S.K.G.G.) and Medicine (S.K.G.G.), Mayo Clinic, Rochester, Minnesota 55905; and Epimer, LLC (G.S.R.), Providence, Rhode Island 02906

Context: We have recently introduced liquid chromatography-tandem mass spectrometry (LC-MS/MS) for 25-hydroxyvitamin D_2 ($25OHD_2$) and $25OHD_3$ testing. During subsequent clinical use, we identified significantly elevated results in some infants. We hypothesized this might represent assay interference caused by C-3 epimers of $25OHD_2$ or $25OHD_3$.

Objective: Our aims were to 1) determine the prevalence of C-3 epimers of $25OHD_2$ or $25OHD_3$ in human serum, and 2) identify the patient populations that might be affected.

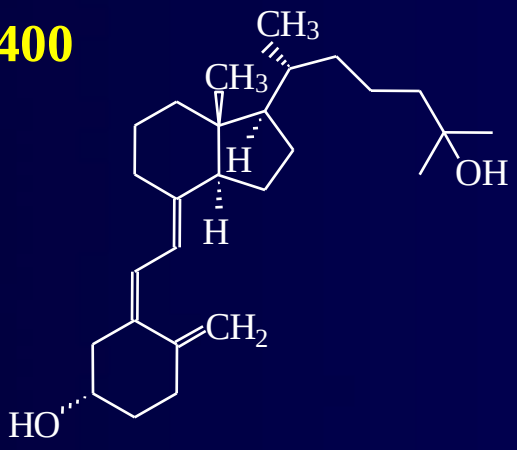
Study Design: We modified our LC-MS/MS method to allow detection of C-3 epimers. We retested specimens from four patient groups with the new method and an extracted RIA: 1) children less than 1 yr old, 2) children 1–18 yr old, 3) adults aged 20–87 yr with liver disease, and 4) adults aged 19–91 yr without liver disease.

Results: In 172 children from group 1 with detectable $25OHD_2$ or $25OHD_3$, we identified C-3 epimers in 39 (22.7%). The epimers contributed 8.7–61.1% of the total 25-OHD. There was an inverse relationship between patient age and epimer percentage ($r = 0.48$; $P < 0.002$). The RIA gave accurate 25-OHD results that correlated with the modified LC-MS/MS method. No C-3 epimers were detected in any of the other groups.

Conclusions: Significant concentrations of C-3 epimers of $25OHD_2$ or $25OHD_3$ are commonly found in infants. This can lead to overestimation of 25-OHD levels. Measurements in children less than 1 yr should therefore be performed with an assay that allows accurate detection of 25-OHD in the presence of its C-3 epimers. ***(J Clin Endocrinol Metab* 91: 3055–3061, 2006)**

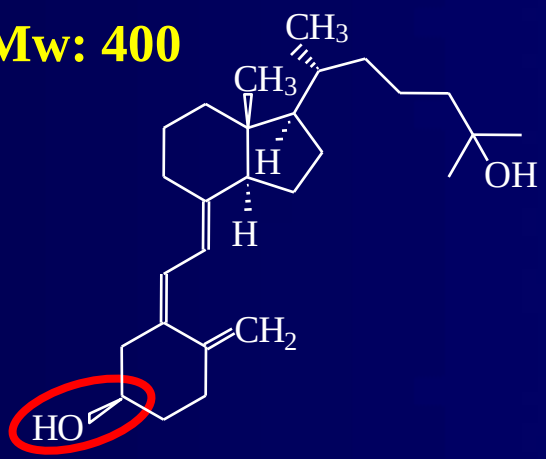
Comparison of 25-OH-D Structures

Mw: 400



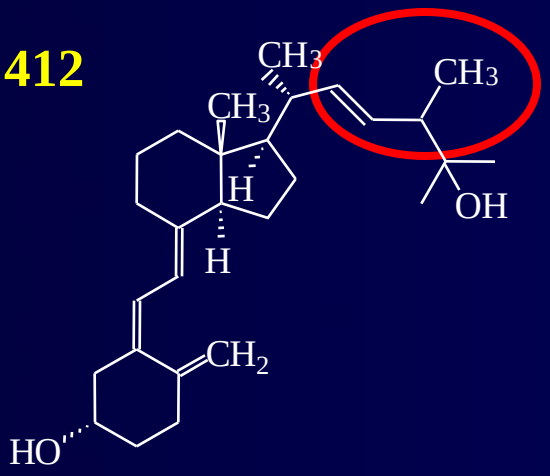
25-OH-D_3

Mw: 400



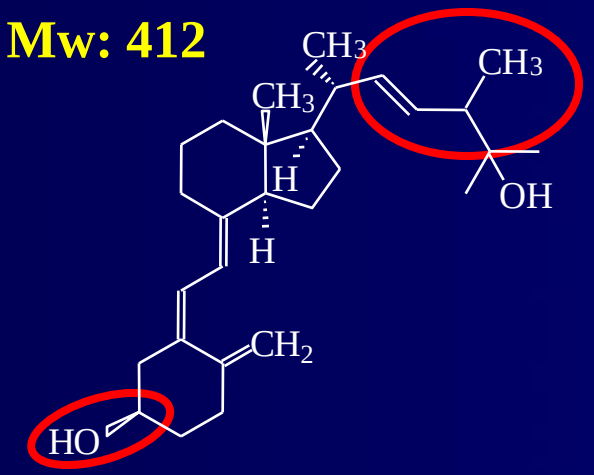
3-epi-25-OH-D_3

Mw: 412



25-OH-D_2

Mw: 412



3-epi-25-OH-D_2

Development of SRM 972

Level 1

65 ± 15 nmol/L 25-hydroxyvitamin D_3 ("normal")

Level 2

Blend of "normal" serum and horse serum to obtain approximately half the level of 25-hydroxyvitamin D_3 in the "normal" pool (35 ± 5 nmol/L)

Level 3

"Normal" serum spiked with an equivalent amount of 25-hydroxyvitamin D_2

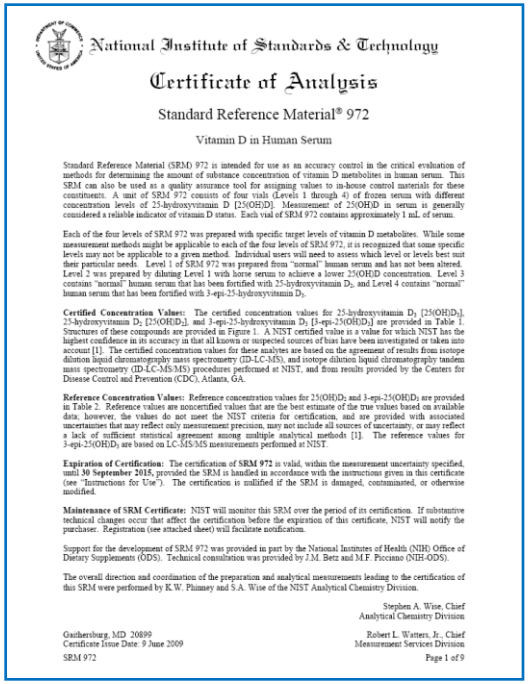
Level 4

"Normal" serum spiked with 3-epi-25-hydroxyvitamin D_3

(Courtesy of Karen Phinney, NIST)

SRM 972 Vitamin D in Human Serum

- Four levels, each containing 1.0 mL serum
- Certified and reference values for 25(OH)D_2, 25(OH)D_3, and 3-epi-25(OH)D_3
- Value assignment by isotope-dilution LC-MS and LC-MS/MS using data from NIST and CDC
- Metabolite concentrations reported in ng/g, ng/mL, and nmol/L
- COA does not provide data from other analytical techniques



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 972

Vitamin D in Human Serum

Standard Reference Material (SRM) 972 is intended for use as an accuracy control in the critical evaluation of methods for determining the amount of substance concentration of vitamin D metabolites in human serum. This SRM can also be used as a quality assurance tool for assigning values to in-house control materials for these constituents. A unit of SRM 972 consists of four vials (Levels 1 through 4) of frozen serum with different concentration levels of 25-hydroxyvitamin D [25(OH)D]. Measurement of 25(OH)D in serum is generally considered a reliable indicator of vitamin D status. Each vial of SRM 972 contains approximately 1 mL of serum.

Each of the four levels of SRM 972 was prepared with specific target levels of vitamin D metabolites. While some measurement methods might be applicable to each of the four levels of SRM 972, it is recognized that some specific levels may not be applicable to a given method. Individual users will need to assess which level or levels best suit their particular needs. Level 1 of SRM 972 was prepared from "normal" human serum and has not been altered. Level 2 was prepared by diluting Level 1 with horse serum to achieve a lower 25(OH)D concentration. Level 3 contains "normal" human serum that has been fortified with 25-hydroxyvitamin D_2 and Level 4 contains "normal" human serum that has been fortified with 3-epi-25-hydroxyvitamin D_3.

Certified Concentration Values: The certified concentration values for 25-hydroxyvitamin D_3 [25(OH)D_3], 25-hydroxyvitamin D_2 [25(OH)D_2], and 3-epi-25-hydroxyvitamin D_3 [3-epi-25(OH)D_3] are provided in Table 1. Structures of these compounds are provided in Figure 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified concentration values for these analytes are based on the agreement of results from isotope dilution liquid chromatography mass spectrometry (ID-LC-MS), and isotope dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) procedures performed at NIST, and from results provided by the Centers for Disease Control and Prevention (CDC), Atlanta, GA.

Reference Concentration Values: Reference concentration values for 25(OH)D_2 and 3-epi-25(OH)D_3 are provided in Table 2. Reference values are noncertified values that are the best estimate of the true values based on available data; however, the values do not meet the NIST criteria for certification, and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [1]. The reference values for 3-epi-25(OH)D_3 are based on LC-MS/MS measurements performed at NIST.

Expiration of Certification: The certification of **SRM 972** is valid, within the measurement uncertainty specified, until **30 September 2015**, provided the SRM is handled in accordance with the instructions given in this certificate (see "Instructions for Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certificate: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Support for the development of SRM 972 was provided in part by the National Institutes of Health (NIH) Office of Dietary Supplements (ODS). Technical consultation was provided by J.M. Betz and M.F. Picciano (NIH-ODS).

The overall direction and coordination of the preparation and analytical measurements leading to the certification of this SRM were performed by K.W. Phinney and S.A. Wise of the NIST Analytical Chemistry Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 9 June 2009

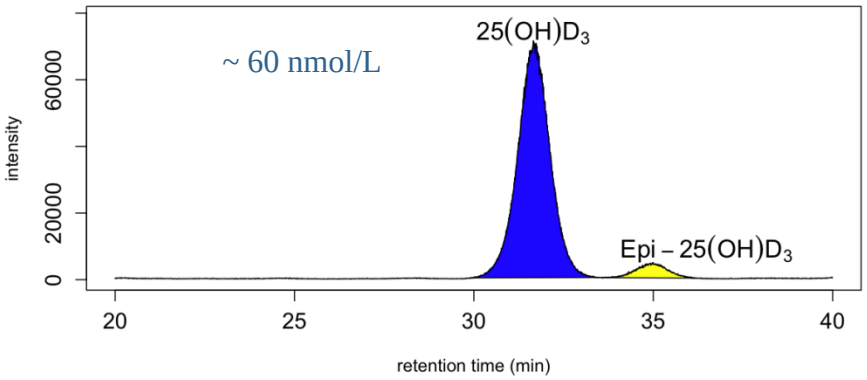
Robert L. Watters, Jr., Chief
Measurement Services Division

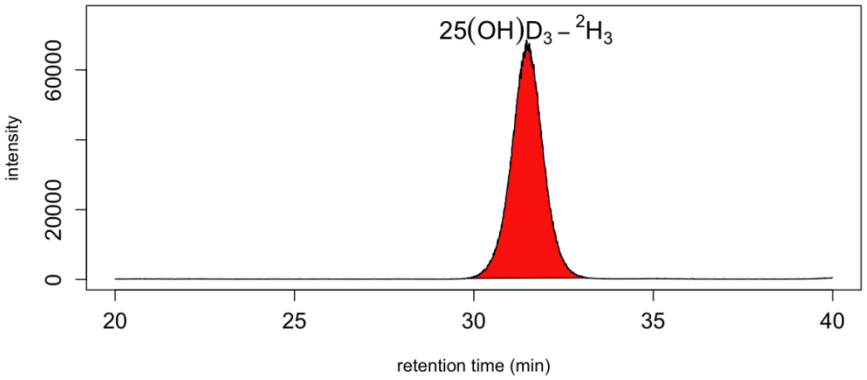
SRM 972 Page 1 of 9

(Courtesy of Karen Phinney, NIST)

Development of Methodology

SRM 972 Level 1

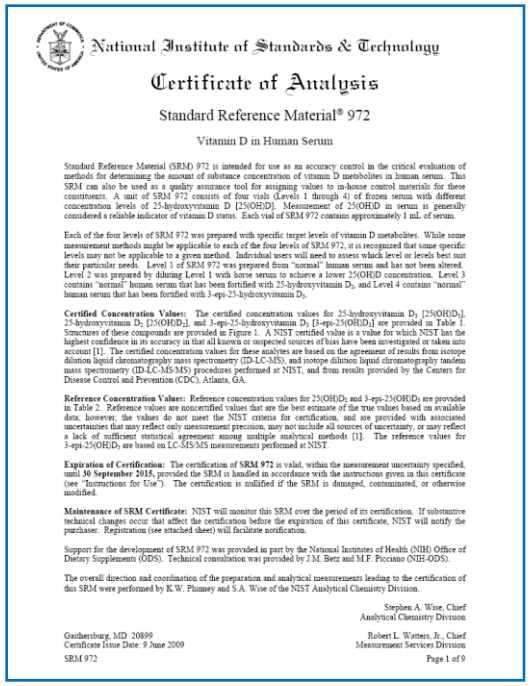




(Courtesy of Karen Phinney, NIST)

- ID-LC/MS/MS and ID-LC/MS methods were developed
- Stable isotope labeled internal standards were utilized for measurements of 25(OH)D_2 and 25(OH)D_3
- ID-LC/MS/MS was validated for submission to JCTLM as a Reference Measurement Procedure
- 3-epi-25(OH)D_3 fully resolved from 25(OH)D_3; (separation based on the work of Lensmeyer et al.)

Impact



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 972

Vitamin D in Human Serum

Standard Reference Material (SRM) 972 is intended for use as an accuracy control in the critical evaluation of methods for determining the amount of substance concentration of vitamin D metabolites in human serum. This SRM can also be used as a quality assurance tool for assigning values to in-house control materials for these constituents. A unit of SRM 972 consists of four vials (Levels 1 through 4) of frozen serum with different concentration levels of 25-hydroxyvitamin D [25(OH)D]. Measurement of 25(OH)D in serum is generally considered a reliable indicator of vitamin D status. Each vial of SRM 972 contains approximately 1 mL of serum.

Each of the four levels of SRM 972 was prepared with specific target levels of vitamin D metabolites. While some measurement methods might be applicable to each of the four levels of SRM 972, it is recognized that some specific levels may not be applicable to a given method. Individual users will need to assess which level or levels best suit their particular needs. Level 1 of SRM 972 was prepared from "normal" human serum and has not been altered. Level 2 was prepared by diluting Level 1 with horse serum to achieve a lower 25(OH)D concentration. Level 3 contains "normal" human serum that has been fortified with 25-hydroxyvitamin D_3 and Level 4 contains "normal" human serum that has been fortified with 3-epi-25-hydroxyvitamin D_3.

Certified Concentration Values: The certified concentration values for 25-hydroxyvitamin D_3 [25(OH)D_3], 25-hydroxyvitamin D_2 [25(OH)D_2], and 3-epi-25-hydroxyvitamin D_3 [3-epi-25(OH)D_3] are provided in Table 1. Structures of these compounds are provided in Figure 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified concentration values for these analytes are based on the agreement of results from isotope dilution liquid chromatography mass spectrometry (ID-LC-MS), and isotope dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) procedures performed at NIST, and from results provided by the Centers for Disease Control and Prevention (CDC), Atlanta, GA.

Reference Concentration Values: Reference concentration values for 25(OH)D_2 and 3-epi-25(OH)D_3 are provided in Table 2. Reference values are noncertified values that are the best estimate of the true values based on available data; however, the values do not meet the NIST criteria for certification, and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [1]. The reference values for 3-epi-25(OH)D_3 are based on LC-MS/MS measurements performed at NIST.

Expiration of Certification: The certification of **SRM 972** is valid, within the measurement uncertainty specified, until **30 September 2015,** provided the SRM is handled in accordance with the instructions given in this certificate (see "Instructions for Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certificate: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Support for the development of SRM 972 was provided in part by the National Institutes of Health (NIH) Office of Dietary Supplements (ODS). Technical consultation was provided by J.M. Betz and M.F. Picciano (NIH-ODS).

The overall direction and coordination of the preparation and analytical measurements leading to the certification of this SRM were performed by K.W. Phinney and S.A. Wise of the NIST Analytical Chemistry Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 9 June 2009

Robert L. Watters, Jr., Chief
Measurement Services Division

SRM 972

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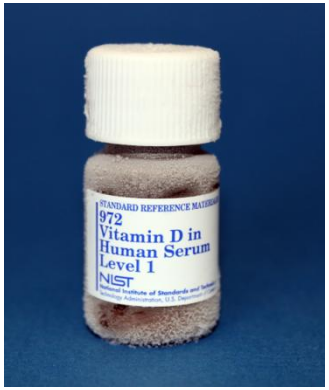


- Sales have greatly exceeded expectations:
  - 250 units sold in first 4 months!
  - Estimated 800 units/year
  - Projected 5 year supply will sell out in FY11
- Objective study of method biases
- Harmonization of measurement results
- Measurement traceability
- Candidate reference measurement procedure*

(Courtesy of Karen Phinney, NIST)

* Manuscript being submitted to Analytical Chemistry

Use of a common standard improves the performance of liquid chromatography-tandem mass spectrometry methods for serum 25-hydroxyvitamin-D

Graham D Carter and Julia C Jones, Clinical Chemistry Department, Imperial College Healthcare NHS Trust, Charing Cross Hospital, London W6 8RF, UK

Background: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is becoming increasingly popular for measuring 25-hydroxyvitamin-D (25-OH-D). Results submitted to the International Quality Assessment Scheme (DEQAS) have shown poor interlaboratory agreement. We investigated whether the use of a common standard would reduce interlaboratory imprecision.

Methods: A commercial standard and two controls were distributed with the DEQAS samples in January 2008. Participants were asked to calculate the results of samples and controls using their usual standard and the commercial standard. A method questionnaire was also distributed.

Results: Use of a common standard reduced the mean interlaboratory imprecision (coefficient of variation [CV]) for total 25-OH-D from 16.4% (in-house standards) to 10.4% (common standard). For 25-OH-D_3 and 25-OH-D_2, the **mean CVs were reduced from 16.7% and 21.1% to 8.5% and 12.6%,** respectively. **Mean values obtained for total 25-OH-D using the common standard were higher by 6.1%.**

Conclusions: Use of a common standard improved agreement among laboratories using LC-MS/MS methods for 25-OH-D. This suggests that problems with assay standardization contribute to interlaboratory imprecision. This may be related to the nature of the matrix used for working standards or errors in the calibration of stock standard solutions of 25-OH-D. Some participants used a gravimetric method, others UV spectrophotometry, to establish the concentration of stock solutions. Among the latter group there was uncertainty over the molar absorption coefficient of 25-OH-D solutions. We conclude that LC-MS/MS is not yet sufficiently robust to become the reference method for 25-OH-D and that gas chromatography-mass spectrometry might be a more suitable candidate.

Current Controversies with 25-OH-D assay

(a) What is the normal range?

(b) Performance Characteristics of Current 25-OH-D Assays

- **i) Vitamin D External Quality Assessment Scheme (DEQAS)**
- **ii) Measurement of Total 25-OH-D in samples**
- **iii) Measurement of 25-OH-D_2 content**
- **iv) Pediatric samples**

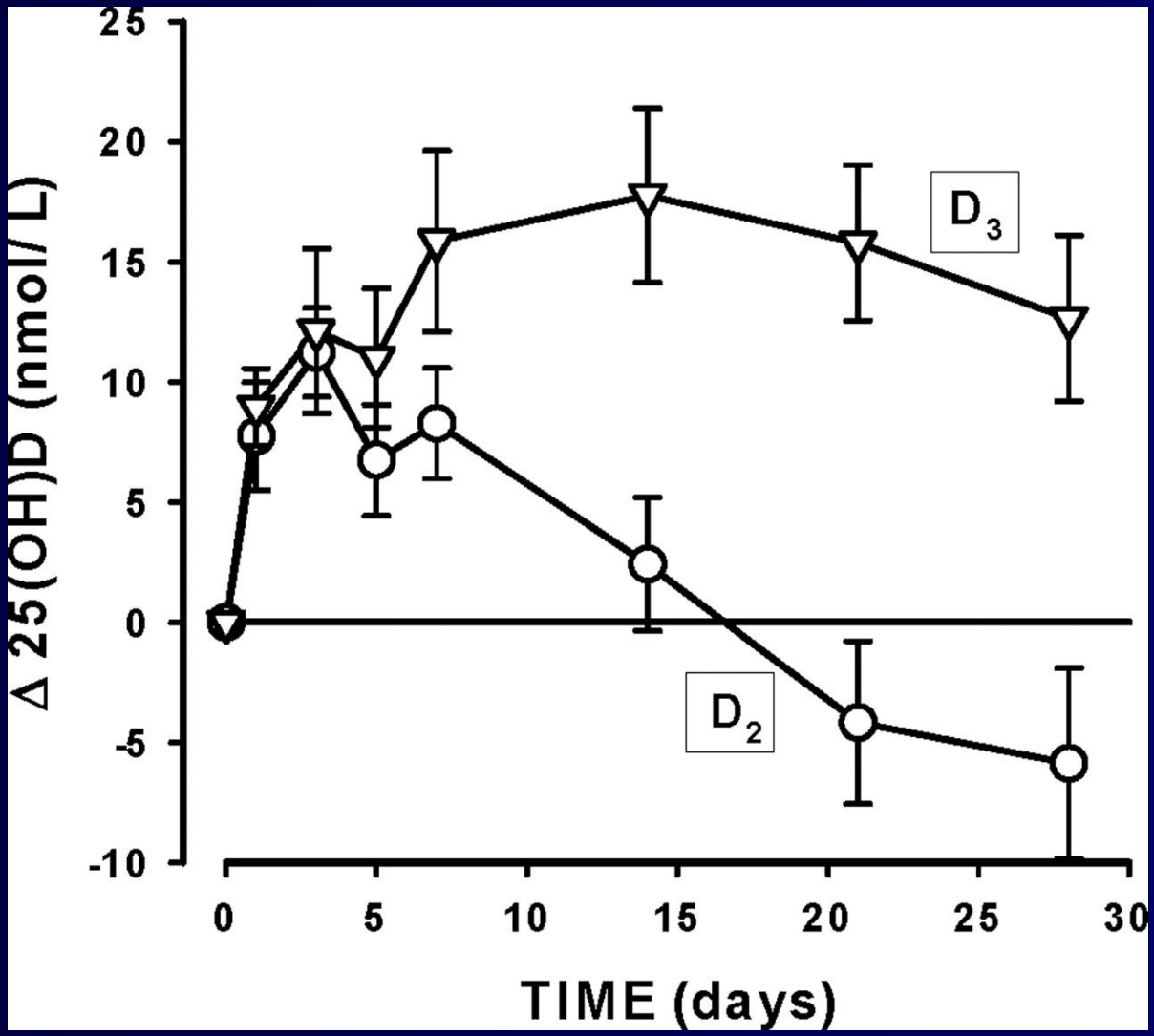
(c) Are Vitamin D_2 and Vitamin D_3 biologically equivalent ?
Are separate assays of 25-OH-D_2 and 25-OH-D_3 clinically useful?

(d) Can we avoid use of 25-OH-D assay?
Suggested frequency of 25-OH-D Testing

Are 25-OH-D_2 and 25-OH-D_3 bio-equivalent?

- Most *in vitro* findings suggest 25-OH-D_2 & 25-OH-D_3 and their active forms are biologically equivalent
- Data supporting bioequivalence of D_2/D_3 at curing rickets
- Recent results suggest that smaller doses (1000-1500 IU/d) are bio-equivalent at raising 25-OH-D levels
- Vitamin D_2 is less toxic than vitamin D_3
- Some evidence that 50,000 IU doses of vitamin D_2 are less effective than vitamin D_3 for raising 25-OH-D level

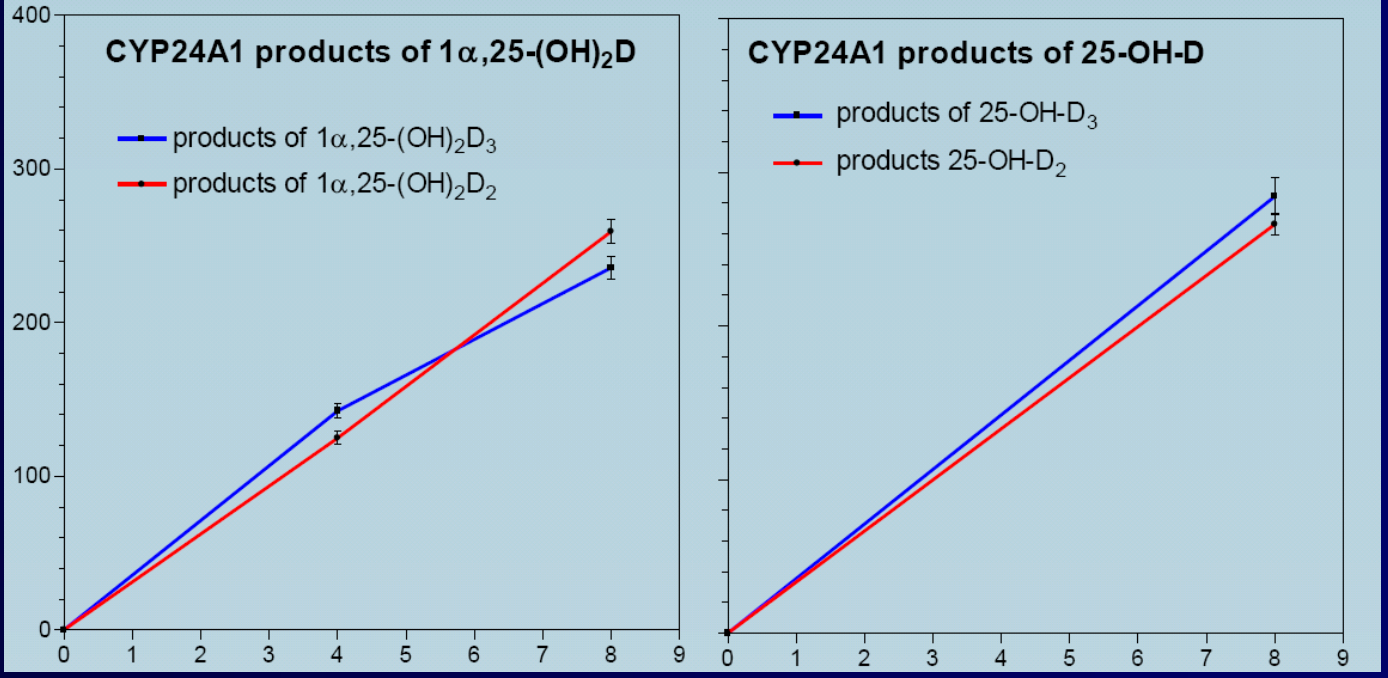
Armas, L. A. G. et al. J Clin Endocrinol Metab 2004;89:5387-5391



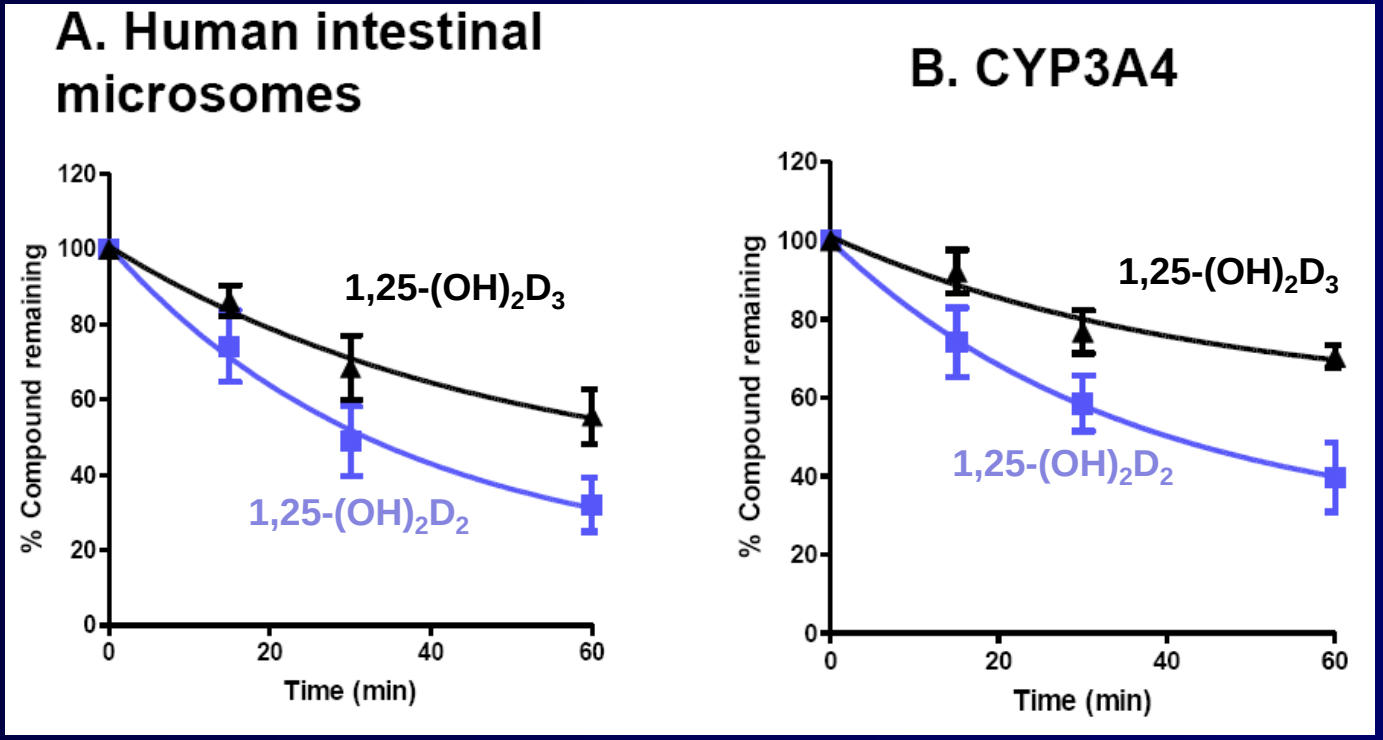
THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

FIG. 2. Time course of the rise in serum 25OHD after a single oral dose of 50,000 IU of either cholecalciferol (vitamin D3) or ergocalciferol (vitamin D2) to two groups of 10 normal men each

CYP24A1



CYP3A4



Jones et al
Vitamin D Workshop
Brugge , Oct 2009

ARE VITAMIN D_2 AND D_3 EQUIPOTENT?

- BOTH CURE RICKETS EQUALLY WELL
- AT PHYSIOLOGICAL LEVELS METABOLISED AT A SIMILAR RATE
- AT PHARMACOLOGICAL LEVELS D_2 COMPOUNDS METABOLISED FASTER PROBABLY AS THE RESULT OF CYP3A4 ACTION
- MAY EXPLAIN THE OBSERVED LOWER TOXICITY OF VITAMIN D_2

SO WHAT SHOULD WE MEASURE?

Total 25-OH-D or separate [25-OH-D_2] & [25-OH-D_3]?

- **Total 25-OH-D is the clinically-important parameter**
- **Total 25-OH-D will generally suffice to assess health**
- **Plasma 25-OH-D_2 may be useful as a marker of dietary D or to assess the effectiveness of supplemental vitamin D_2 (the only source of prescription vitamin D in US)**

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COST AND FREQUENCY OF 25-OH-D TESTING?

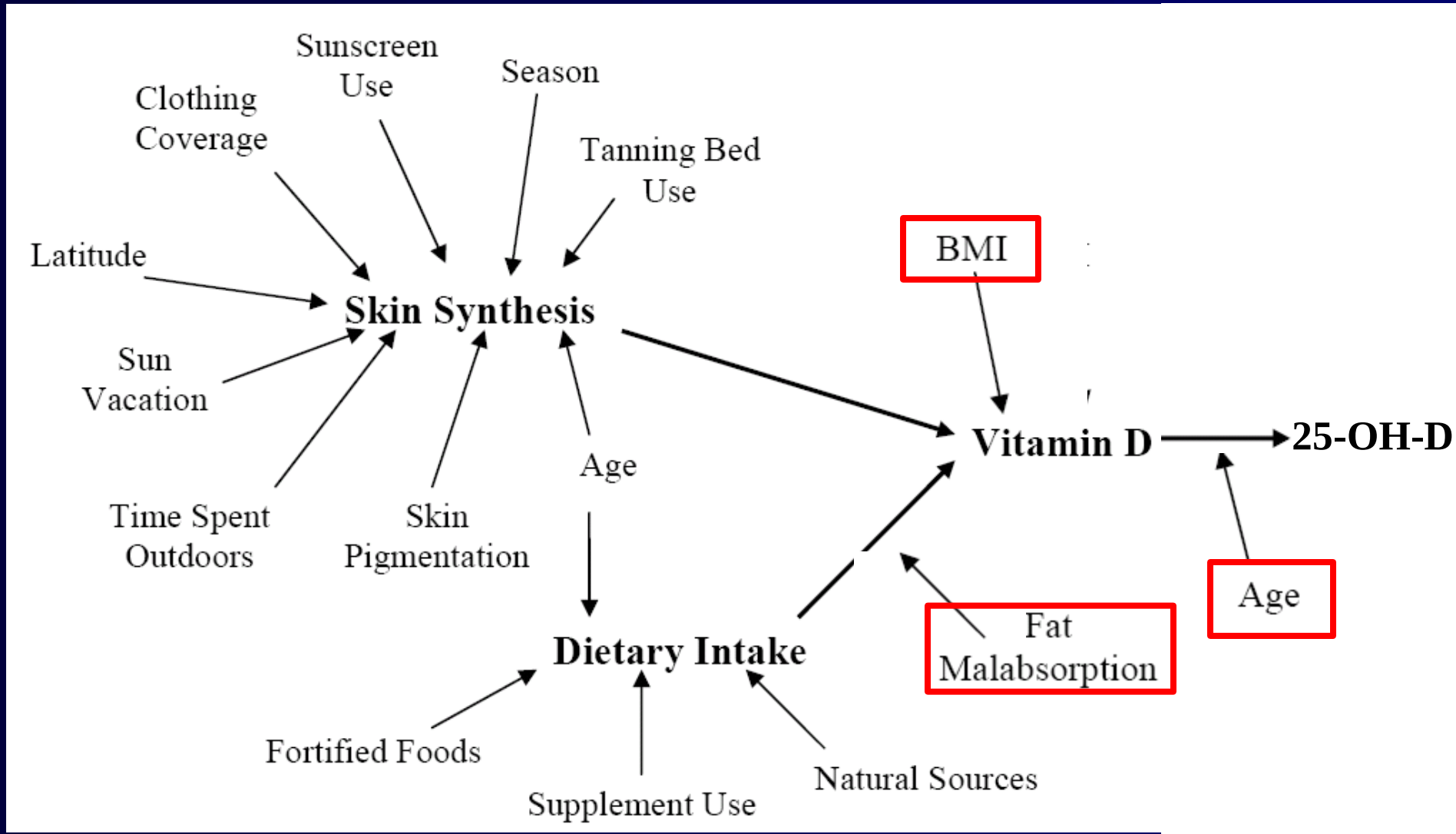
- COST OF 25-OH-D TESTING IS A HEALTH CARE-BURDEN
  - 25-OH-D TESTING & REPLETION MAY SAVE $$$$

- WHAT IS THE IDEAL FREQUENCY OF 25-OH-D TESTING?
  - t½ IS 15-20 DAYS

- GIVEN CURRENT D REPLETION TOOLS :
  -TEST AT BASELINE AND ABOUT 4 MONTHS
  - IF REPLETION HAS OCCURRED EVERY 6 MONTHS

- CAN ALL TESTING BE AVOIDED?
  - POOR RESPONDERS eg high BMI

FACTORS AFFECTING VITAMIN D INTAKE



Summary

- Emergence of extra-renal 1α-hydroxylase emphasizes the value of serum 25-OH-D assay as a tool to monitor vitamin D status

- Performance of 25-OH-D assays has gradually improved but still has a long way to go. Introduction of NIST standards may improve.

- Research suggests that vitamin D_2 and D_3 have different rates of metabolism especially at pharmacological concentrations

- Repletion of 25-OH-D levels complicated by factors such as BMI, age and GI problems making monitoring of 25-OH-D important