Vitamin D:
How good are our assays?

Ronda Greaves

Overview

- Background
- Clinical
- Reference intervals
- Measurement systems
- Approaches to quality

The Vitamin Alphabet

- A - Retinol
- B - group of 8
- C - Ascorbic acid
- **D** - **Ergocalciferol**
  - **Cholecalciferol**
- E - Tocopherol
- K - Phylloquinone

B1	Thiamine
B2	Riboflavin
B3	Niacin
(B4	Adenine)
B5	Pantothenic acid
B6	Pyridoxine
B7 (H)	Biotin
(B8	Inositol)
B9	Folate
(B10	PABA)
(B11	Choline)
B12	Cobalamin

Total: 13 = 4 fat soluble + 9 water soluble

( ) indicates B group compound no longer classified as vitamins

Vitamin D: Definitions

VITAMIN

An organic compound required as a nutrient, which cannot be synthesized in adequate amounts, and therefore must be obtained in the diet

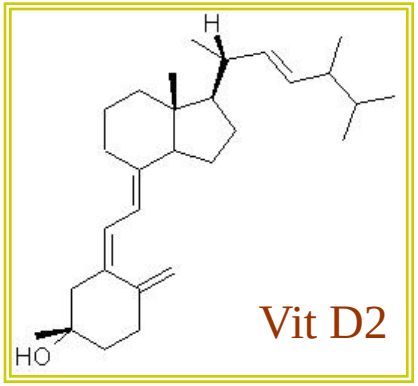
HORMONE

A chemical secreted by a group of cells (gland) into the circulation to affect the function of cells, through interaction with their receptors, in another part of the body

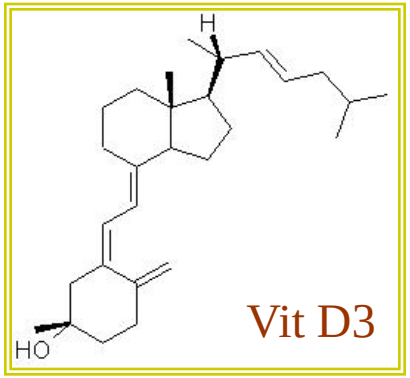
Vitamin D:

- Vit D1 - is a 1:1 mixture of lumisterol and vitamin D2.

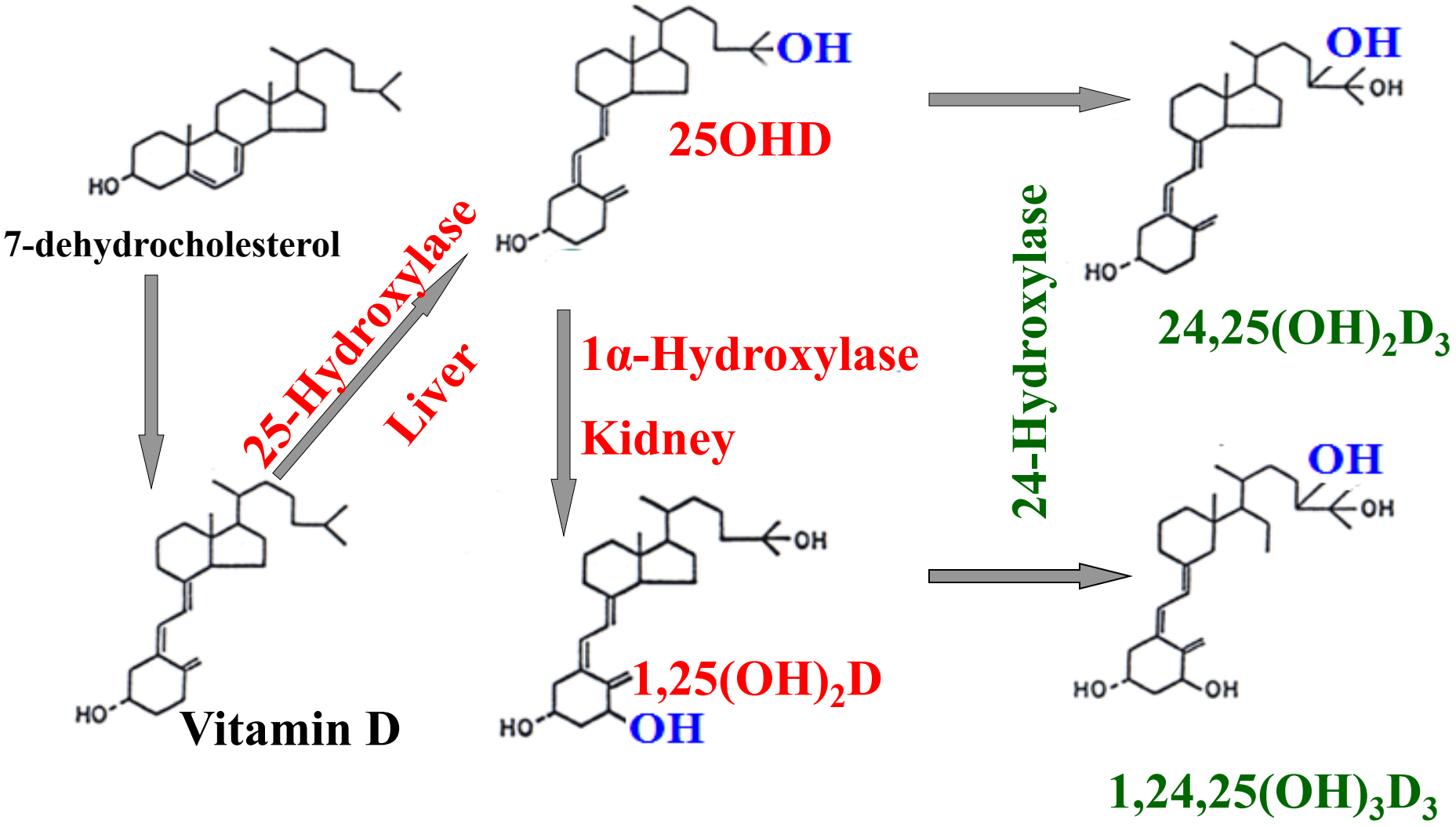
- Vit D2 – **ERGOCALCIFEROL**
  - Plant origin
  - Arises from ultraviolet irradiation of ergosterol
  - Cleaved at the 9,10 bond & develops a double bond b/w C-10 & 19



- Vit D3 – **CHOLECALCIFEROL**
  - Animal origin
  - Formed by breakage of the 9,10 bond in 7-dehydrocholesterol by ultraviolet irradiation, yielding a double bond b/w C-10 and C-19
  - Found in the skin, fur, and feathers of animals and birds exposed to sunlight, and also in butter, brain, fish oils, and egg yolk



Vitamin D


HO
7-dehydrocholesterol
25-Hydroxylase
Liver
Vitamin D
OH
HO
25OHD
1α-Hydroxylase
Kidney
OH
HO
OH
1,25(OH)2D
24-Hydroxylase
OH
OH
HO
24,25(OH)2D3
OH
OH
HO
OH
1,24,25(OH)3D3

Clinical Utility

- Classically
  - Rickets
  - Osteomalacia
- Modern era
  - Bone health
  - Diabetes
  - Autoimmune diseases
  - Immune regulation
  - Infections
  - Cancer
  - Cardiovascular disease

Before vitamin D treatment

After 14 months of vitamin D treatment

(b)

Photos from Lehninger, Principles of Biochemistry

Increasing testing numbers

“In 2009, US laboratories were reporting surges in the number of vitamin D tests being ordered - increases of 50% to even 100%. But beyond the growth in testing and usage, what's the quality required by this type of testing?”

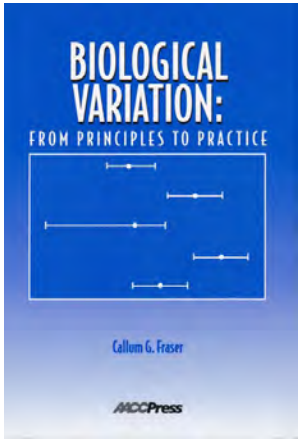
www.Westgard.com

Vitamin D: What level is appropriate?

- RCH
  - 1990's: Reference range quoted 23 to 90 nmol/L
  - 2000's: Change to recommended range of 50 to 150 nmol/L
- Other ranges
  - >60 nmol/L proposed based on rise in PTH
  - >75 nmol/L proposed for health
  - >100 nmol/L for cancer prevention
- On going debate of what range is needed for health
- BUT – we don't have harmonisation of methods!!!!

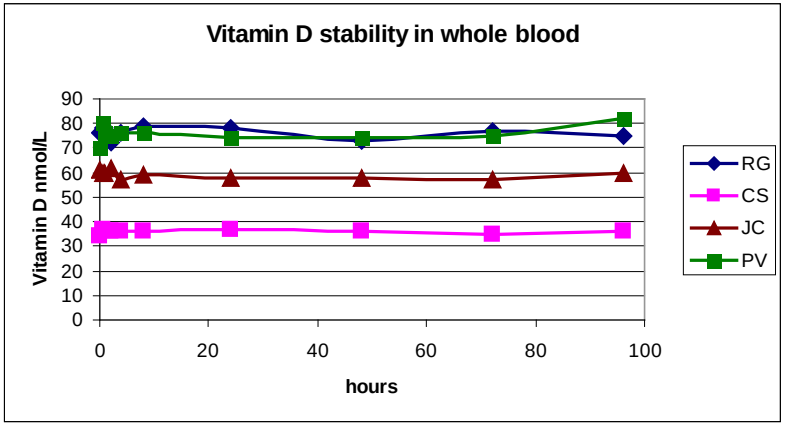
Pre analytical factors

- Biological Variation
- Seasonal variation
- Skin pigmentation
- Racial differences



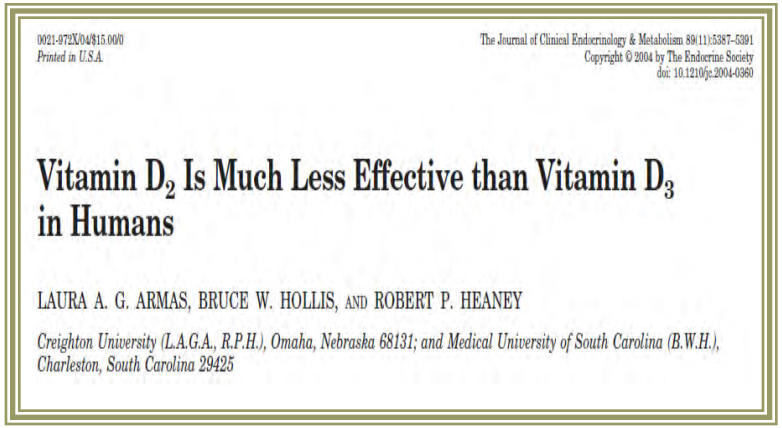
- Vitamin D is stable in whole blood stored at room temperature in sunlight for up to 96 hours.

(Poster: AACB ASM in 2005)



Vitamin D

- Choice for routine assessment of vitamin D status
- Need to extract to remove Vit D from DBP (Vit D binding protein)
- Standards calibrated against D3
- Some immunoassays cross react with D2
  - Traditionally considered an advantage
- Supplementation in Australia originally D2 now mainly D3



0021-972X/04/$15.00/0
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The Journal of Clinical Endocrinology & Metabolism 89(11):5387–5391

doi: 10.1210/jc.2004-0360

Vitamin D_2 Is Much Less Effective than Vitamin D_3 in Humans

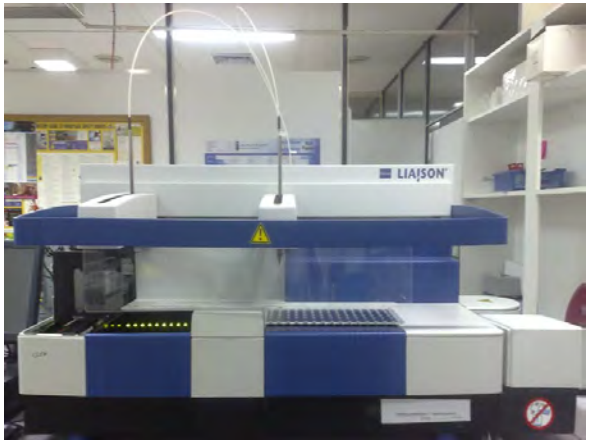
LAURA A. G. ARMAS, BRUCE W. HOLLIS, AND ROBERT P. HEANEY

Creighton University (L.A.G.A., R.P.H.), Omaha, Nebraska 68131; and Medical University of South Carolina (B.W.H.), Charleston, South Carolina 29425

Vitamin D: Automated analysis

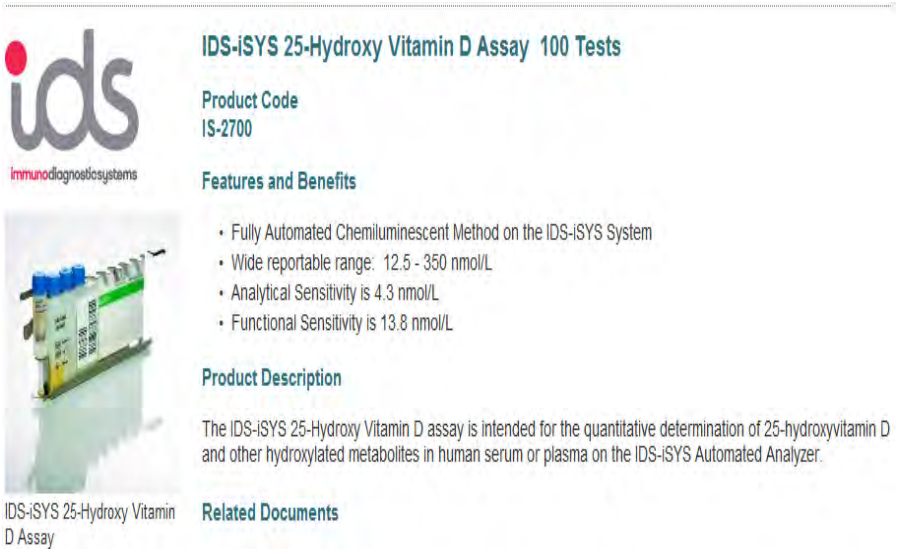
- Roche Cobas e601
  - 25 OH Vit D3 only
  - 0% cross reactivity with 25 OH Vit D2

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



Vitamin D: Other Immunoassays

- Enzyme Linked Immunosorbent Assay
- Radio-immunoassay
  - Diasorin (Sorin)
  - IDS



IDS-iSYS 25-Hydroxy Vitamin D Assay

- NEW AUTOMATED
  - IDS – ISYS platform
  - Abbott – recent lab trials
  - Siemens – under development

ISYS: http://www.intermedico.com/productpage.php?id=IS-2700

Chromatography + MS (+MS)

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

Live rates at 2010.05.23 11:09:07 UTC

500,000.00 AUD = 7,896,262,319.09 VND

Australia Dollars	Vietnam Dong
1 AUD = 15,792.52 VND	1 VND = 0.0000633211 AUD

THE ANALYSIS OF 25-HYDROXYVITAMIN D IN SERUM USING UPLC/MS/MS

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

LJ Calton[1], SD Gillingwater[1], GW Hammond[1], DP Cooper[1] & S Wilson[2]
[1]Waters Corporation, Manchester, UK [2]Waters, Australia

INTRODUCTION

Several recent studies have shown that vitamin D deficiency is common in adults and children around the world. In addition to the well known effects of vitamin D deficiency, such as calcium malabsorption, there is growing evidence that the risk for other conditions (e.g. cancers[1,2]) may be increased. The measurement of 25-hydroxyvitamin D [25(OH)D] is accepted as the clinical indicator of vitamin D status[3] and is important in the diagnosis and treatment of vitamin D deficiency. The major issue with immunoassays is that they cannot differentiate between the two forms of 25(OH)D; 25(OH)D2 & 25(OH)D3, and instead, rely on the cross-reactivity of the antibody to measure a total 25(OH)D concentration. If that cross-reactivity is less than 100% then vitamin D2 therapy may not be monitored effectively.[4] The aim of this study was to develop a quantitative method for 25(OH)D2 and 25(OH)D3 in serum to prevent the misdiagnosis of vitamin D deficiency in patients.

Figure 1. System configuration of Waters ACQUITY UPLC / TQD

METHODS

A Waters® ACQUITY® Tandem Quadrupole Detector (TQD) coupled to an ACQUITY UPLC® (Waters Corporation, Manchester, UK) was used for all analyses (Figure 1). The 25(OH)D compounds were separated from endogenous interferences using an ACQUITY UPLC BEH C8 Column 2.1 x 50 mm, 1.7 µm employing a gradient elution profile, 73-98%B in 1.5min following a 2min initial hold at a flow rate of 0.4ml/min, where mobile phase A and B are 2mM ammonium acetate+0.1% formic acid in water and methanol respectively.

The instrument was operated in positive electrospray ionisation mode using MassLynx™ 4.1 software with auto data processing by the QuanLynx™ Application Manager. Specific Multiple Reaction Monitoring (MRM) experiments for each compound were created as shown in Table 1.

A single calibrator and bi-level QC's (Chromsystems, Munich, Germany) were prepared as per the manufacture's instructions. A low QC was prepared by pooling human serum and adding a known concentration of 25(OH)D2 and 25(OH)D3. The final concentrations of the low, medium and high QC samples were 19, 27 and 84ng/mL for 25(OH)D2 and 13, 29 and 89ng/mL for 25(OH)D3 respectively.

Compound	MRM	Dwell (secs)	Cone Voltage (V)	Collision Energy (eV)
25(OH)D3	401.35 > 159.1	0.05	24	28
25(OH)D3*	401.35 > 380.3	0.05	24	10
d_6-25(OH)D3	407.35 > 159.1	0.05	24	28
25(OH)D2	413.35 > 83.1	0.05	24	22
25(OH)D2*	413.35 > 395.3	0.05	24	10

*Table 1. The tuning parameters used when monitoring for 25(OH)D2 and 25(OH)D3 and the internal standard. *denotes optional qualifier ion*

To assess linearity, calibrators were prepared in mammalian serum over the concentration range 2.5-100ng/mL for 25(OH)D2 and 25(OH)D3.

The samples were prepared using a liquid-liquid-extraction protocol that involves the addition of internal standard (250ng/mL hexa-deuterated 25(OH)D3, Synthetica AS, in 80% MeOH/20% IPA), $ZnSO_4$, MeOH and Hexane to 150µL of serum. Following centrifugation for 5 mins at 13,000rpm, the hexane layer was removed and placed into Waters maximum recovery vials and evaporated to dryness under nitrogen at 50°C. The samples were reconstituted in 75µL of 70% methanol in water and 20µL was injected.

RESULTS

Accuracy

The accuracy of the assay was determined by the analysis of external quality control samples from DEQAS (www.deqas.org). The Chromsystems single point calibrator was used and a calibration line constructed through zero to calculate the DEQAS sample concentrations. Passing-Bablok linear regression was used to compare the Waters 25(OH)D3 results with the DEQAS LC/MS method mean. All results were within ±11.5% deviation of the expected value (Figure 2).

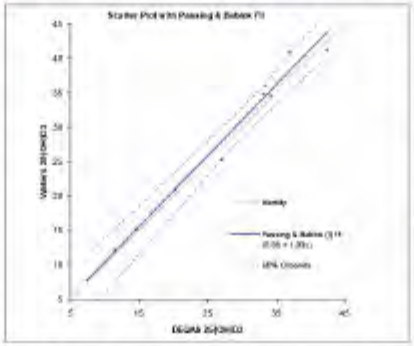


Figure 2. Passing-Bablok linear regression analysis comparing the Waters 25(OH)D3 results to DEQAS LC/MS method mean

Linearity

The coefficient of determination (R^2) for 25(OH)D3 was >0.999 and the calculated concentrations for the calibrators were all within ±4% of the assigned values. The coefficient of determination (R^2) for 25(OH)D2 was >0.997 and the calculated concentrations for the calibrators were all within ±10% of the assigned values.

Precision

The intra-assay precision was determined by extracting and analysing five replicates of the low , medium and high QC samples. The coefficient of variation (CV) for 25(OH)D over the three levels were calculated. The inter-assay precision was determined over five consecutive days using the low, medium and high QC samples. The results are shown in Table 2.

	Low QC		Medium QC		High QC	
	25(OH) D2	25(OH) D3	25(OH) D2	25(OH) D3	25(OH) D2	25(OH) D3
Intra-assay %CV	5.6	7.5	3.0	3.9	5.1	6.2
Inter-assay %CV	8.9	6.1	9.2	5.5	7.2	5.9

Table 2: Summary of the intra and inter-assay precision of the assay

DISCUSSION

A method for the UPLC/MS/MS analysis of 25(OH)D2 and 25(OH)D3 in serum has been developed. The methodology involves a simple liquid-liquid extraction of the analytes from serum and the MRM detection of each analyte using two transitions. Quantifier and qualifier ion ratios were monitored to ensure lack of interference[5]. The assay demonstrates good sensitivity with acceptable intra and inter-day precision. Using this methodology it is feasible to manually process and analyse up to 100 samples per day.

CONCLUSION

- **A method for the independent quantification of 25(OH)D2 and 25(OH)D3 in serum has been developed with good linearity, sensitivity and precision.**
- **UPLC/MS/MS offers significant advantages over the traditional HPLC/UV methodology through reduced sample volume, increased sensitivity, specificity and speed.**
- **UPLC/MS/MS allows for the accurate and reliable measurement of 25(OH)D2 and 25(OH)D3 in serum to prevent the misdiagnosis of vitamin D deficiency in patients who are receiving vitamin D2 supplementation.**

References

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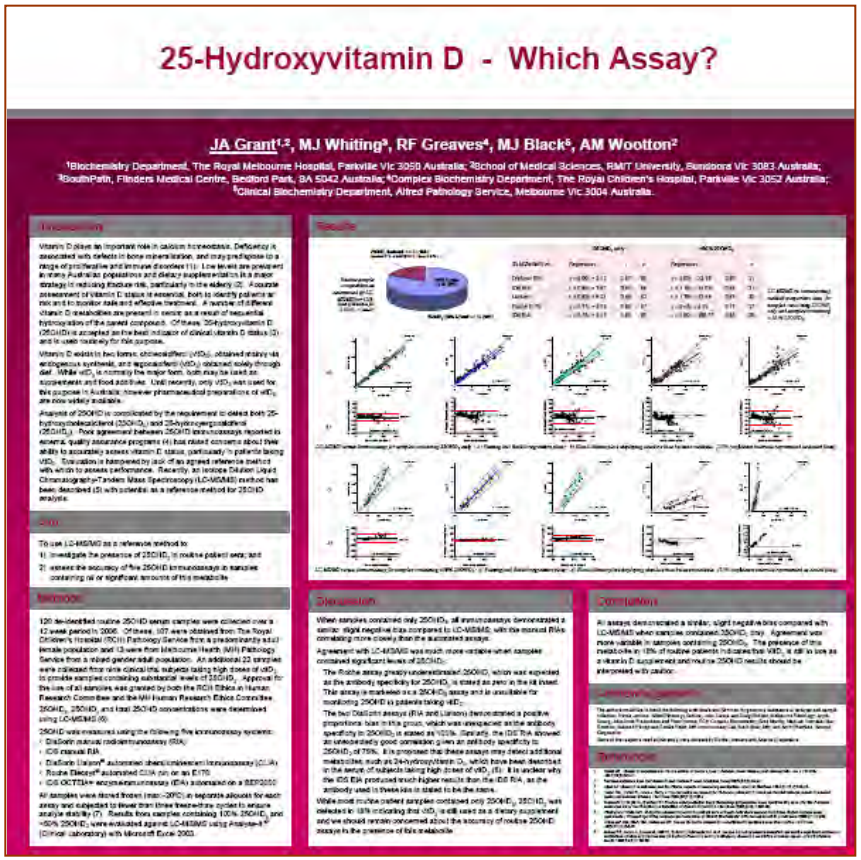
5. CLSI. Mass Spectrometry in the Clinical laboratory:general principles and guidance. C50-A. 2007.

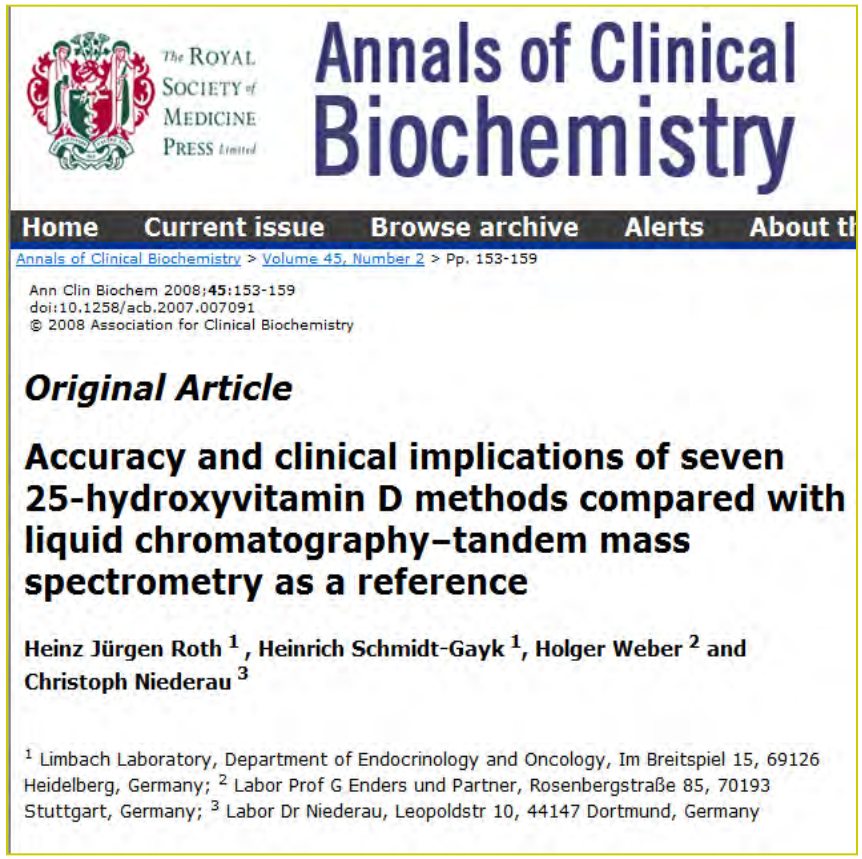
Method comparisons



25-Hydroxyvitamin D - Which Assay?

JA Grant[1,2], MJ Whiting[3], RF Greaves[4], MJ Black[5], AM Wootton[2]

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



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Original Article

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]

[1] Limbach Laboratory, Department of Endocrinology and Oncology, Im Breitspiel 15, 69126 Heidelberg, Germany; [2] Labor Prof G Enders und Partner, Rosenbergstraße 85, 70193 Stuttgart, Germany; [3] Labor Dr Niederau, Leopoldstr 10, 44147 Dortmund, Germany

2009 QAP end of cycle 32 report

Welcome | Program Selection | End Of Cycle Report: Vitamin D3 (25-hydroxycholecalciferol) Cycle: 32 | Summary Data

Vitamin D3 (25-hydroxycholecalciferol) (nmol/L) - Summary Data
Endocrine Program Cycle 32

Analytical Principle	No. Labs	S.D.	CV	Low 6.0	High 254.0
Isotope Diln Tandem Mass Spec (IDMSMS)	4	5.4	4.45	7.0	242.0
Radioimmunoassay	5	8.9	9.0	12.0	214.0
Electrochemiluminescence	26	12.5	11.75	44.0	160.0
HPLC	1	15.5	12.5	3.0	245.0
Chemiluminescence	24	15.0	15.3	36.0	170.0
ELISA	4	20.3	19.25	44.0	186.0

Instrument	No. Labs	S.D.	CV	Low 6.0	High 254.0
Applied Biosystems API 3200 Q-TRAP	2	4.3	3.4	7.0	246.0
HPLC Waters	1	5.3	4.4	6.0	238.0
Applied Biosystems API 4000 Q-TRAP	1	6.4	5.0	8.0	246.0
Nichols Institute Diagnostics Advantage	1	5.2	5.2	38.0	162.0
Roche Diagnostics Hitachi Modular	1	8.1	8.0	46.0	156.0
Scintillation Counter - Gamma	5	8.9	9.0	12.0	214.0
Roche Diagnostics Elecsys 1010/2010/cobas e 411	4	11.3	10.9	46.0	159.0
Roche Diagnostics E170/ e 601 (cobas 6000- IA)	21	12.6	12.9	43.0	161.0
DiaSorin Liaison	23	15.4	15.4	35.0	170.0
Spectrophotometer/Plate Reader Spectrophotometer/Plate Reader	3	19.5	20.9	42.0	183.0

Reagent	No. Labs	S.D.	CV	Low 6.0	High 254.0
Own Preparation	3	5.5	4.5	8.0	246.0
Roche Diagnostics (Integra)	1	5.4	4.7	44.0	183.0
Chromsystems	2	10.4	8.45	5.0	242.0
Roche Diagnostics (Hitachi)	25	12.6	12.9	44.0	159.0
DiaSorin	28	14.3	14.75	32.0	172.0
IDS Ltd	5	19.5	17.6	42.0	183.0

Calibrator	No. Labs	S.D.	CV	Low 6.0	High 254.0
Chromsystems	1	6.4	5.0	8.0	246.0
Inhouse Calibrator	5	9.9	9.5	42.0	165.0
LC-MS-MS	21	13.6	14.1	42.0	159.0
UV Quantification	4	17.5	14.7	29.0	202.0

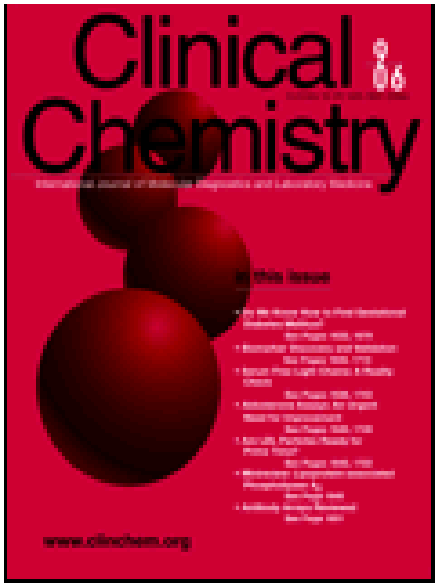
Your Method Code: I: Electrochemiluminescence 11L : Roche Diagnostics E170/ e 601 (cobas 6000- IA) 21: Roche Diagnostics (Hitachi) C: LC-MS-MS

Jan 2010 DEQAS: Vitamin D

25-HYDROXYVITAMIN D3							
		346	347	348	349	350	
HPLC	Mean	29.9	93.4	24.6	47.2	48.8	nmol/L
	SD	8.3	21.6	6.0	9.0	9.3	nmol/L
	n	19	19	19	19	19	
	cv	28	23	25	19	19	%
LCMS	Mean	31.3	94.2	25.1	48.0	50.5	nmol/L
	SD	5.4	14.0	5.1	7.1	11.7	nmol/L
	n	43	43	43	43	43	
	cv	17.2	14.9	20.3	14.8	23.1	%

LC-MS/MS Reference methods

- Various LC-MS/MS methods available
- Variation between these methods
- Roche assay based on the method below
- This was developed in cooperation with Dr. Vogeser Klinikum Grosshadern)
- Then further optimized at Roche

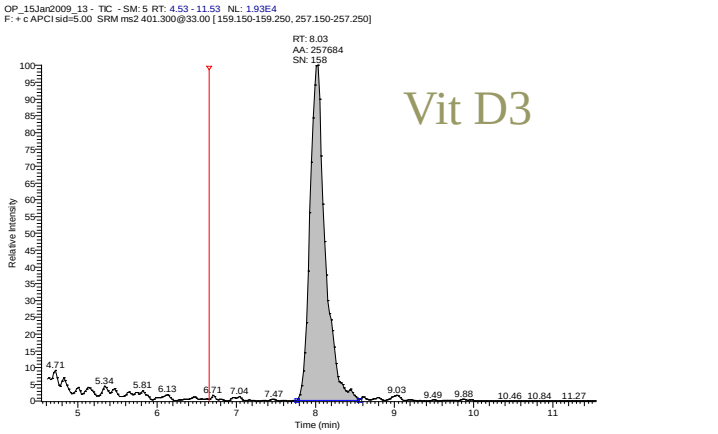


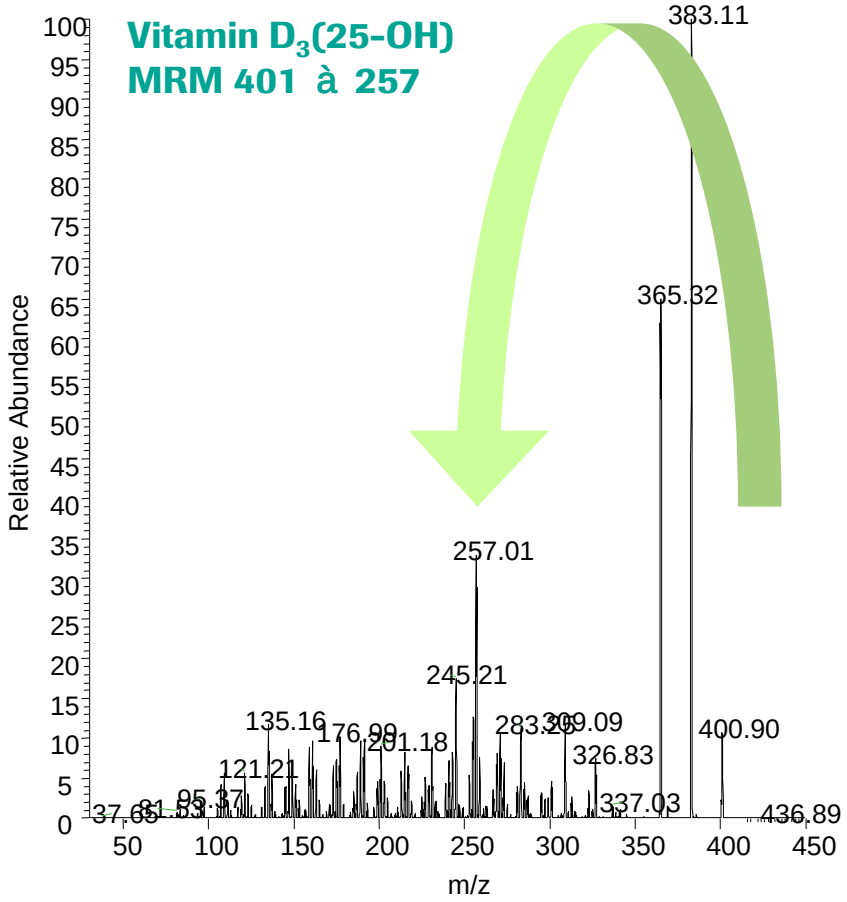
Clinical Chemistry 50, No. 8, 2004

Candidate Reference Method for the Quantification of Circulating 25-Hydroxyvitamin D_3 by Liquid Chromatography–Tandem Mass Spectrometry, *Michael Vogeser,*[1*] *Apostolos Kyriatsoulis,*[2] *Erasmus Huber,*[2] *and Iwe Kobold*[2] ([1] Institute of Clinical Chemistry, Hospital of the University of Munich, Munich, Germany; [2] Roche Diagnostics GmbH, Penzberg, Germany; * address correspondence to this author at: Institute of Clinical Chemistry, Hospital of the University of Munich, D-81366 Munich, Germany; fax 49-89-7095-3240, e-mail Michael.Vogeser@med.uni-muenchen.de)

Information provided courtesy of Roche Australia

Vit D3 chromatography + MS/MS





Vitamin D3 (25-OH) elutes at approx. 8 min.
total gradient time: 20 min. incl. extensive column cleaning

Information provided courtesy of Roche Australia

Current challenges in vitamin D standardization

- *There is considerable variability in reference methods* Lack of a „real“ vitamin D standard reference material which can be used for immunoassays

- Variability in methods for reference standardization (methodological risks, influence of chromatographic resolution)

- No “real“ reference values existing

NIST human serum SRM

SRM	Description	Certified Constituents	Reference	Form	No. of Levels
909b	Human Serum	Calcium, Chloride, Cholesterol, Creatinine, Lithium, Magnesium, Potassium, Sodium,Total Glycerides, Triglycerides, Urea, and Uric Acid	Bilirubin	Lyophilized	2
1951b	Lipids in Frozen Human Serum	Total Cholesterol, Total Glycerides, Triglycerides		Frozen	2
956b	Electrolytes in Frozen Human Serum	Total Ca, Li, Mg, K, Na	Ionized Ca	Frozen	3
965a	Glucose in Frozen Human Serum	Glucose		Frozen	3
967	Creatinine in Frozen Serum	Creatinine		Frozen	2
970	Ascorbic Acid in Frozen Human Serum	Total Ascorbic Acid		Frozen	2
1952a	Cholesterol in Human Serum (Freeze-dried)	Cholesterol		Lyophilized	3
968c	Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum	Vitamins (4), Cholesterol, Carotenoids (4)	Carotenoids (8), Vitamin D	Lyophilized	2
1589a	PCBs, Pesticides, and Dioxins/Furans in Human Serum	PCB Congeners (16), Chlorinated Pesticides (5), Total Cholesterol	PCB Congeners (9), Chlorinated Pesticides (5), Total Cholesterol, Triglycerides, "Free" Cholesterol, Phospholipids	Lyophilized	1
1599	Anticonvulsant Drug Level Assay (valproic acid and carbamazepine)	valproic acid carbamazepine		Lyophilized	1
900	Antiepilepsy Drug Level Assay	Antiepileptics (4)		Lyophilized	3
1955	Homocysteine and Folate in Human Serum	Homocysteine 5-Methyltetrahydrofolic acid	Total Folate, Folic Acid	Frozen	3

Now 968d

Vit D
Released 2009
SRM 972

Allows for a common primary calibrator

Current challenges in vitamin D standardization

- *Limitations of NIST controls* SRM 972:
  - Level 1: native human serum
  - Level 2: level 1 diluted with horse serum
  - Level 3: human serum spiked with vitamin D2 (25-OH)
  - Level 4: human serum spiked with vitamin D3 (25-OH) and 3-epi 25(OH)

- ***In vitro anomaly affecting immunoassays***
  - Exogeneously added vitamin D does not distribute to the vitamin D binding protein (VDBP) as it occurs as in vivo
  - Exogeneously added material binds to other moieties than the VDBP

- à failure of quantitative recovery in immunoassays

- ***Is there a way out of this dilemma ?***

Information provided courtesy of Roche Australia

NIST SRM 972 standard is detectable by the Roche LC-MS/MS

					Conc. found with Roche LC-MS/MS		
NIST Level	Target conc. Vit. D3 (25-OH)	Target conc. 3-epi-Vit.D3 (25-OH)	Target conc. Vit.D2 (25-OH)	NIST total Vitamin D	Vit. D3 (25-OH)	Vit. D2(25-OH)	Total Vitamin D Roche LC-MS/MS
Level 1	23.9 +/- 0.8	1.39 +/- 0.04	0.60 +/- 0.20	**25.9**	24.8	1.5	**26.3**
Level 2	12.3 +/- 0.8	0.76 +/- 0.02	1.71 +/- 0.08	**14.8**	14.6	1.2	**15.8**
Level 3	18.5 +/- 1.1	1.06 +/- 0.03	26.4 +/- 2.0	**46.0**	20.5	25.2	**45.7**
Level 4	33.0 +/- 0.8	37.7 +/- 1.2	2.4 +/- 0.21	**73.1**	72.1	3.1	**75.2**

Information provided courtesy of Roche Australia

How safe are LC-MS/MS data ?
The Quest Story

08. Jan 2009 - New York Times:

- **“Quest acknowledges errors in vitamin D tests”**

- The nation's largest medical laboratory company provided possibly **erroneous results to thousands of people** who had their vitamin D levels tested in the last two years, the company has acknowledged.

- Quest's problems with the vitamin D analysis arose after it shifted in 2006 and 2007 to a new test of its own design, replacing an older F.D.A.-approved test.

- The new test promised to be more accurate and offer more detailed information, Quest executives said. But the test relied on a **sophisticated instrument called a mass spectrometer, which can be tricky to use, especially for high-volume testing.**

Information provided courtesy of Roche Australia

Specifications for trueness and precision of a reference measurement system for serum/plasma 25-hydroxy vitamin D analysis

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Dietmar Stöckl, Patrick M. Sluss and Linda M. Thienpont

Abstract

Background

The divergence in analytical quality of serum/plasma 25-hydroxy-vitamin D analysis calls for defining specifications for a reference measurement system.

Methods

Fundamentally, in a reference measurement system, there should be a relationship between the analytical specifications for higher- (reference) and lower-order (routine) measurements. Therefore, when setting specifications, we started with limits for routine imprecision (CV_{rou}) and bias (B_{rou}) using 4 models: (1) the misclassifications in diagnosis, (2) biological variation data (reference interval (RI) and monitoring), (3) expert recommendations, and (4) state-of-the-art performance. Then, we used the derived goals to tailor those for reference measurements and certified reference materials (CRMs) for calibration by setting the limits for CV_{ref} at 0.5 CV_{rou}, B_{ref} at 0.33 $B_{rou,}$ max. uncertainty (U_{max}) at 0.33 B_{ref}.

Results

The established specifications ranged between $CV_{rou} \leq 22\%$, $B_{rou} \leq 10\%$, $CV_{ref} \leq 11\%$, $B_{ref} \leq 3.3\%$, U_{max} 1.1% (model 3) and $CV_{rou} \leq 4\%$, $B_{rou} \leq 2.6\%$, $CV_{ref} \leq 2\%$, $B_{ref} \leq 0.9\%$, U_{max} 0.3% (model 2, monitoring).

Conclusions

Model 2 (monitoring) gave the most stringent goals, model 3, the most liberal ones. Accounting for state-of-the-art performance and certification capabilities, we used model 2 (RI) to recommend achievable goals: for routine testing, $CV_{rou} \leq 10\%$, $B_{rou} \leq 5\%$, for reference measurements, $CV_{ref} \leq 5\%$, $B_{ref} \leq 1.7\%$, and for CRMs, U_{max} 0.6%.

Keywords: Serum/plasma 25-hydroxyvitamin D2; Serum/plasma 25-hydroxyvitamin D3; Quality goals; Bias; Imprecision; Uncertainty

Stockl *et al: Approaches for quality*

- Four different approaches
- Followed the 1999 Stockholm consensus conference guidelines on quality specifications

1. Clinical Interpretation: Analyses the impact of bias and analytical imprecision on interpretation of results based on clinical decision limits
2. Biological Variation: Relates analytical performance to the intra- and inter-individual biological variation of vitamin D
3. Expert Opinion: Considered performance goals set by expert opinion from external quality assurance programs
4. State of the art: Evaluated the literature on currently used measurement procedures for vitamin D analysis with stated recovery and imprecision data.

Stockl *et al:* What they found

- Routine measurement systems Expert opinion gave the most liberal goals i.e. 5x the CV's for the biological variation goals

- Expert opinion CV 22% Bias 10%
- Biological Variation CV 4% Bias 2.6%

- Survey of 14 "state of the art" studies of methods
  - **only one method** was close to achieving the performance required by the Biological Variation model i.e. the most stringent model.

Stockl *et al:* Recommendations

- Using the biologic variation approach i.e. Gowan model

- Routine Testing
  - CV $\leq$ 10%
  - Bias $\leq$ 5%
- Reference Measurements
  - CV $\leq$ 5%
  - Bias $\leq$ 1.7%

- Westgard: "The requirements are challenging, but Stock *et al* believe the laboratory community is up to the challenge"

Moves for Harmonisation

- LC-MS/MS for standardization of Vitamin D assays needs
  - high analytical investment
  - critical interpretation of data (especially in HPLC method validation)
  - accurate and reliable methods

- LC-MS/MS methods for standardization can only be compared if specific mass transitions are used

Summary

- Vitamin D: How good are our assays?
  - Clinical understanding of the importance of vitamin D has increased in recent years
  - Both automated immunoassays and chromatography MS methods have developed
  - Discussion surrounds acceptable performance of vitamin D assays
  - Harmonisation is the next objective