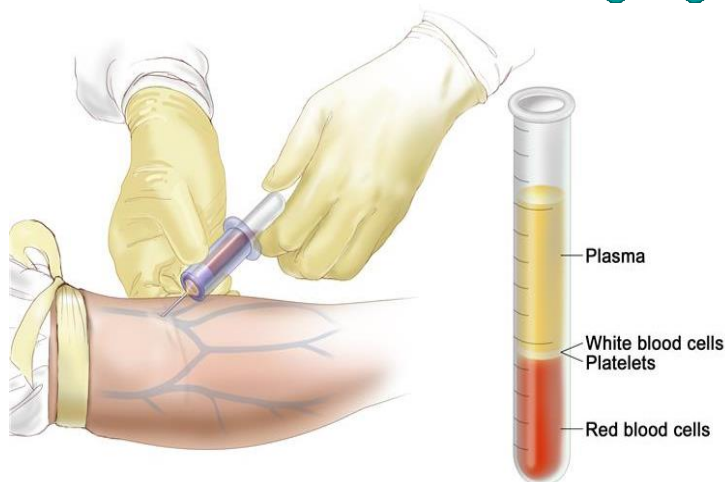
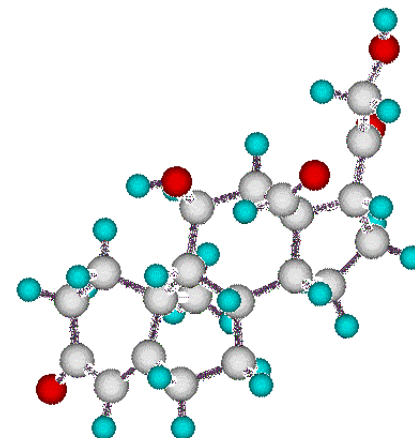


Challenges of implementing JCTLM methods in the routine Clinical Laboratories

R.J. Singh, Ph.D.
Mayo Clinic





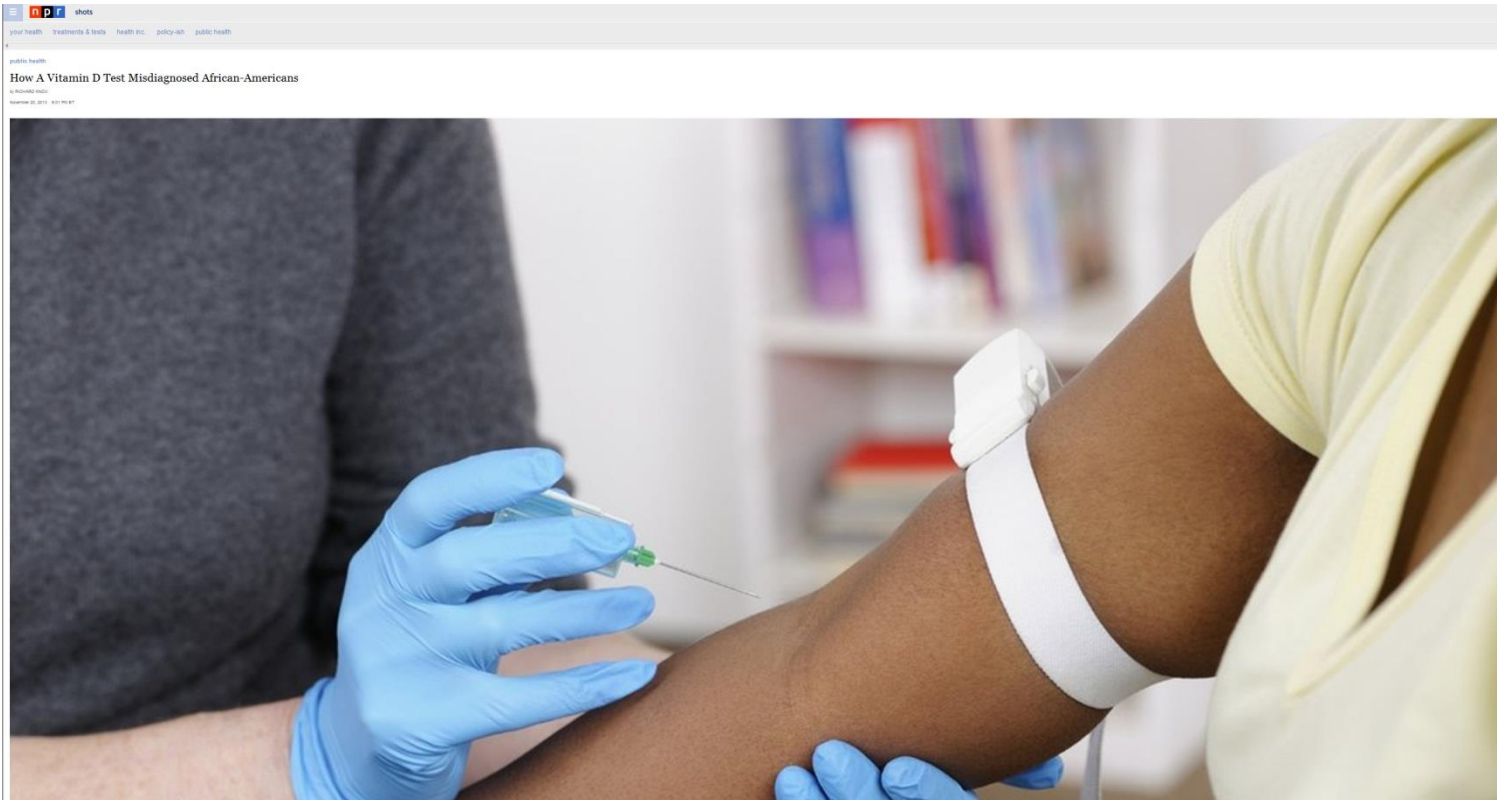
Assessing Vitamin D Status: Time for a Rethink?

Graham D. Carter^{1*} and Karen W. Phinney²

- 100s of Millions of 25-OH Vitamin D test have been run.
- Total 25-OH Vitamin D test is not a useful test in itself.



African Americans were Misdiagnosed-NEJM

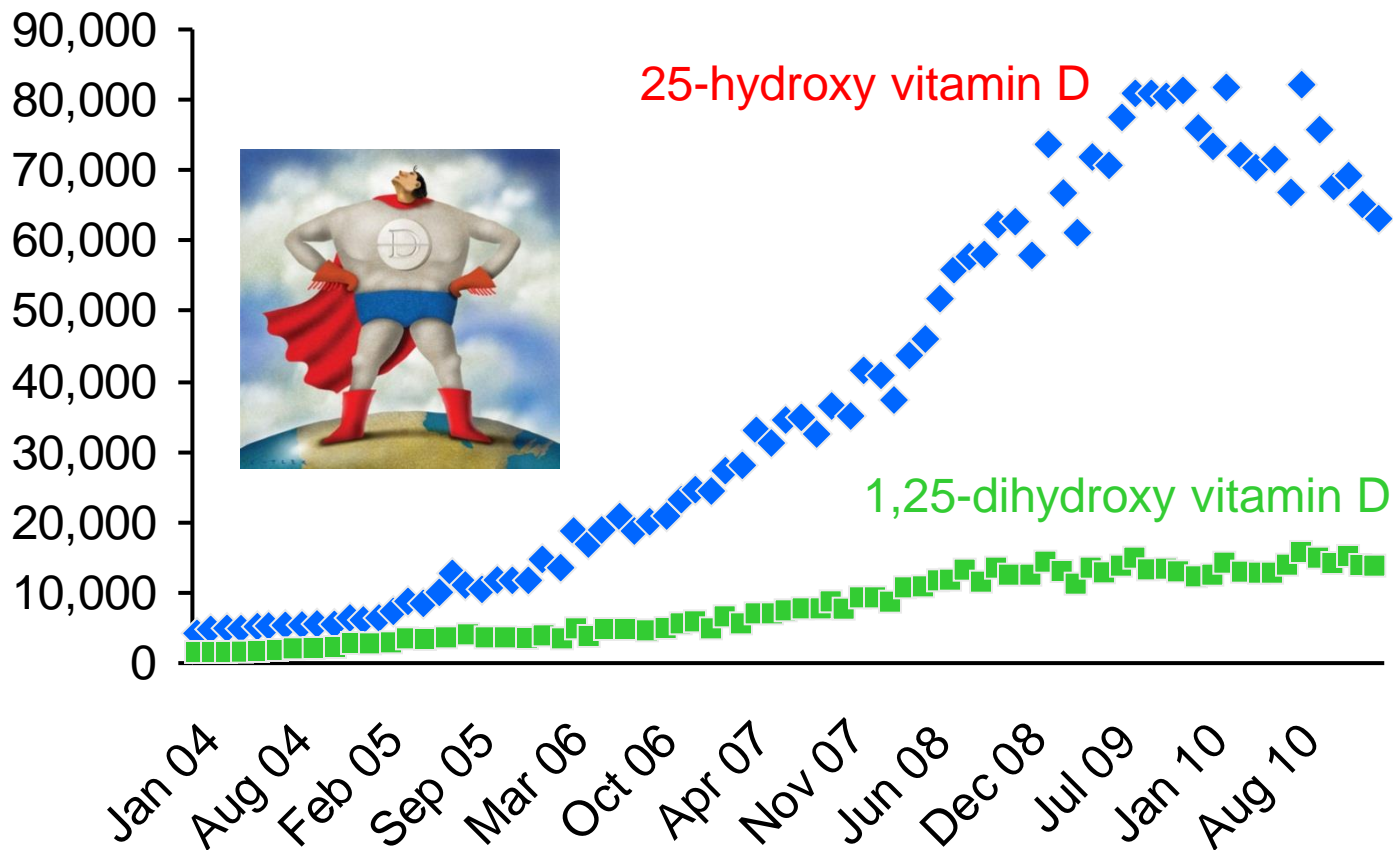


Medicare Payment for 25-OH-D

Top 25 HCPCS Codes by 2016 CLFS Spending

HCPCS Code	HCPCS Code Description	Total 2016 Claim Lines	Total 2016 Payment	% of Total Spending	2017 NLA	Weighted Median	2018 Payment w/Cap	2019 Payment w/Cap	2020 Payment w/Cap
Total		242,885,700	\$4,072,744,372.55	63%					
84443	Assay thyroid stim hormone	21,328,890	\$478,201,900.40	7%	\$23.05	\$14.87	\$20.75	\$18.67	\$16.80
80053	Comprehen metabolic panel	41,058,385	\$464,050,570.56	7%	\$14.49	\$9.08	\$13.04	\$11.74	\$10.56
85025	Complete cbc w/auto diff wbc	41,063,717	\$425,439,439.40	7%	\$10.66	\$6.88	\$9.59	\$8.63	\$7.77
80061	Lipid panel	28,716,199	\$407,636,228.63	6%	\$0.00	\$11.23	\$11.23	\$11.23	\$11.23
82306	Vitamin d 25 hydroxy	8,922,296	\$347,582,955.60	5%	\$40.61	\$26.37	\$36.55	\$32.89	\$29.60
83036	Glycosylated hemoglobin test	19,141,107	\$248,747,306.97	4%	\$13.32	\$8.50	\$11.99	\$10.79	\$9.71
G0483	Drug test def 22+ classes	1,183,239	\$240,503,211.90	4%	\$253.87	\$193.71	\$228.48	\$205.63	\$193.71
80048	Metabolic panel total ca	13,130,016	\$128,574,438.03	2%	\$11.60	\$8.06	\$10.44	\$9.40	\$8.46
G0482	Drug test def 15-21 classes	784,920	\$126,254,803.37	2%	\$204.34	\$132.00	\$183.91	\$165.52	\$148.96
83970	Assay of parathormone	2,176,845	\$119,472,877.14	2%	\$56.62	\$36.76	\$50.96	\$45.86	\$41.28
82607	Vitamin b-12	5,553,620	\$111,752,421.91	2%	\$20.68	\$13.43	\$18.61	\$16.75	\$15.08
85610	Prothrombin time	19,180,431	\$102,955,635.98	2%	\$5.39	\$4.29	\$4.85	\$4.37	\$4.29
84153	Assay of psa total	4,182,938	\$102,655,551.38	2%	\$25.23	\$16.38	\$22.71	\$20.44	\$18.39
84439	Assay of free thyroxine	7,001,009	\$84,030,813.18	1%	\$12.37	\$8.03	\$11.13	\$10.02	\$9.02

Vitamin D Monthly Test Volumes Endocrine Lab Rochester, 2004-2010

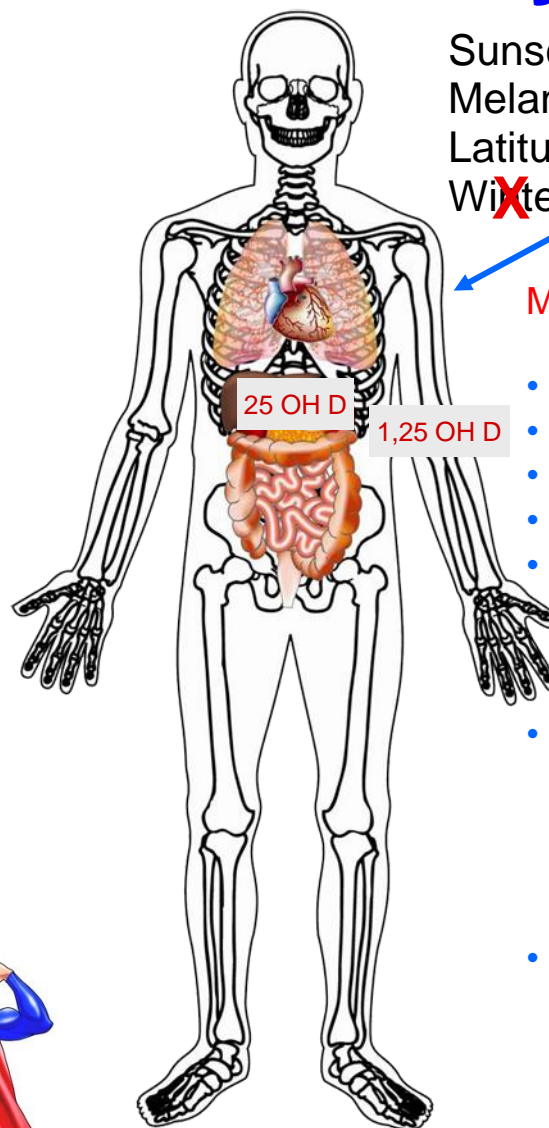




Vitamin D Deficiency

Consequences

- Psychiatric
 - Schizophrenia
 - Depression
- Infections
 - Urinary
 - Tuberculosis
- Circulatory
 - High blood pressure
 - Coronary heart disease
- Cancer
 - Breast
 - Colon
 - Prostate
 - Other
- Other
 - Diabetes
 - Syndrome X
 - Obesity
- Pulmonary
 - Wheezing
 - FEV1
- Bone and muscle
 - Osteoporosis
 - Osteomalacia
 - Osteoarthritis
 - Rickets
 - Muscle aches and weakness



Sunscreen
Melanin
Latitude
Wi~~X~~ter



Medications & supplements

- Antiseizure
- Glucocorticoids
- Rifampin
- HAART
- St John's Wort
- Malabsorption
 - Crohn
 - Whipple
 - Cystic fibrosis
 - Celiac
- Hepatic failure
 - Liver disease
 - Renal failure
 - Nephrotic syndrome

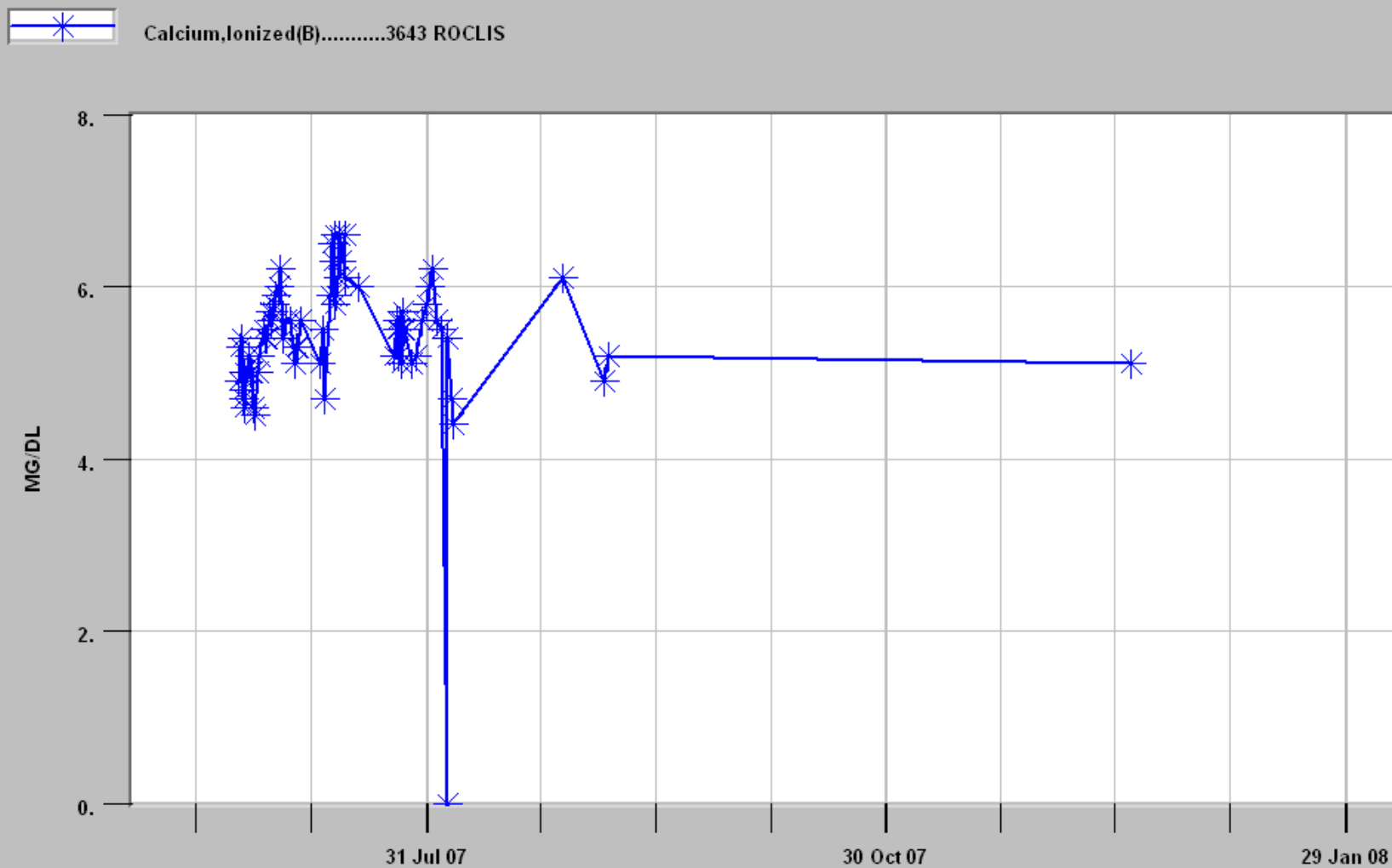


Rickets in a Premature Infant

Birth History

- **Born through C-section**
 - **Intrauterine growth retardation**
 - **Abnormal umbilical blood flow on ultrasound**
- **Apgars of 6 and 7**
- **Infant intubated and given Survanta**
- **Infant quickly transferred to neonatal intensive care unit for medical management**

Ionized Calcium

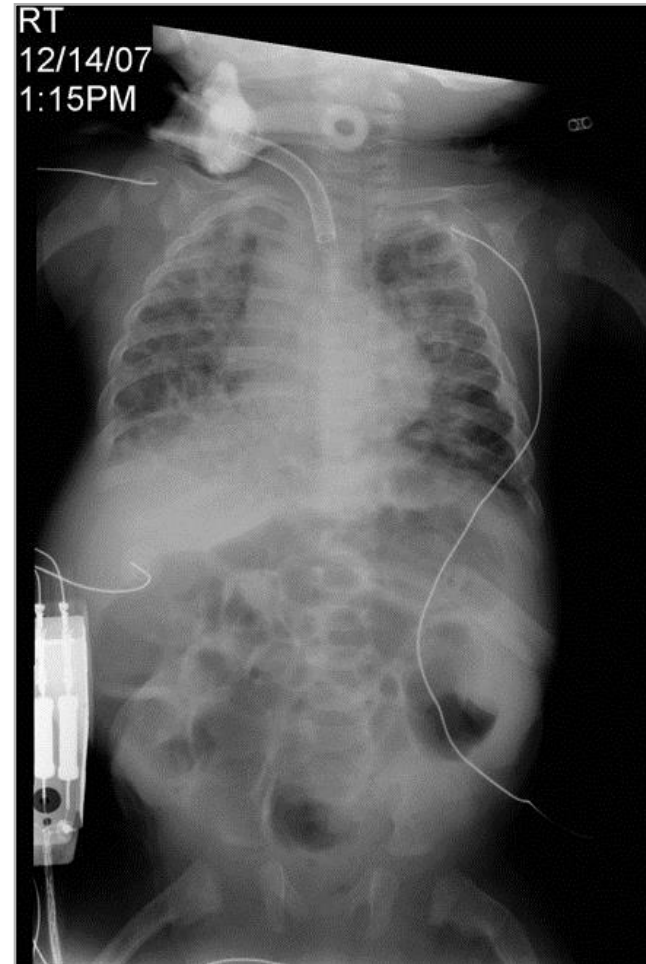
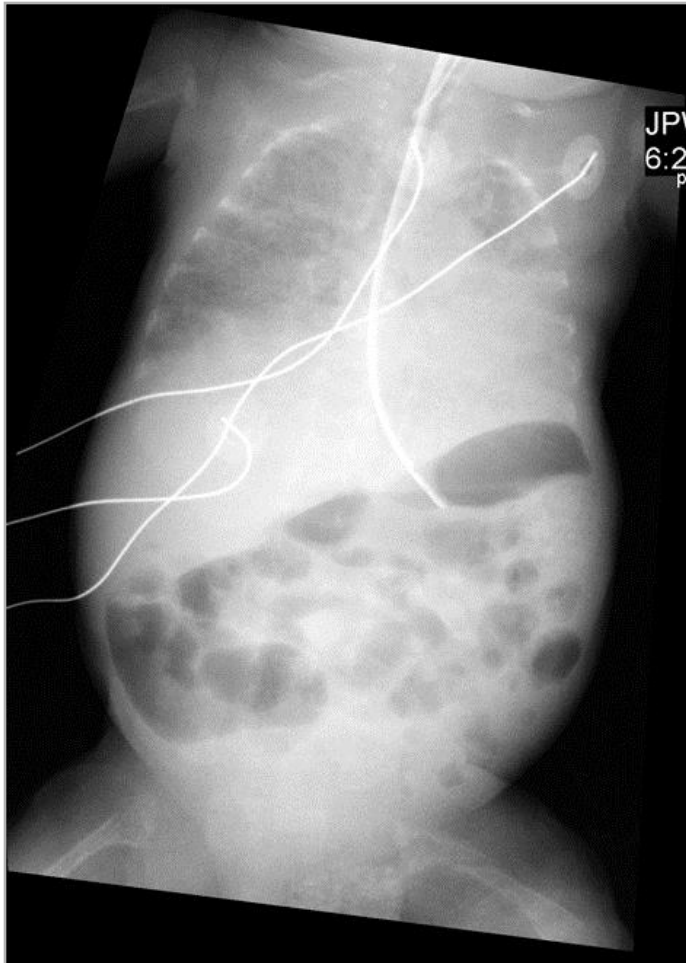


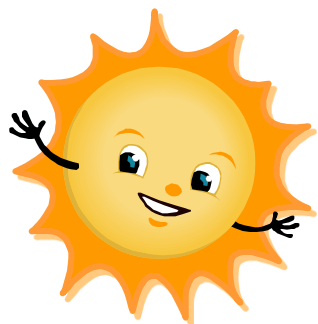
Calcium Testing

- **Calcium Tests are precise and are well standardized**
- **Normal values range from 8.5 to 10.2 mg/dL for Total Calcium**
 - **4.8-5.7 mg/dL for ionized Calcium**
- **Medically Allowable Error – 0.37 mg/dL**
- **CLIA Acceptable Performance 1.0 mg/dL**



Fractures Identified on Routine Radiographic Surveillance for Line Placement

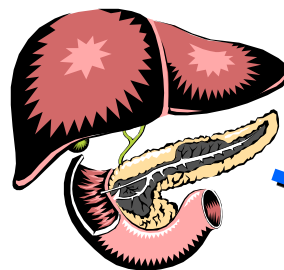




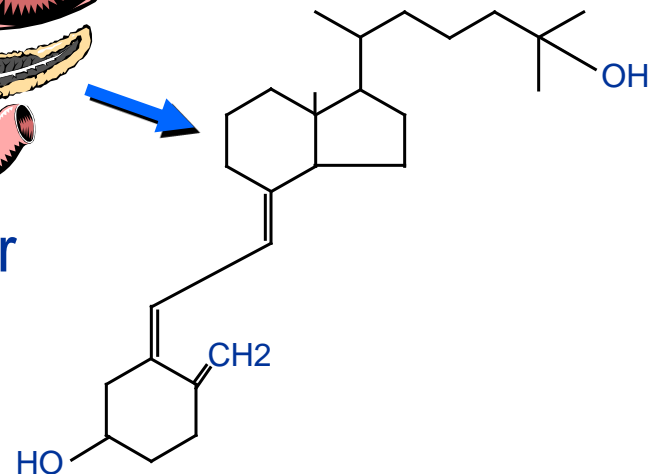
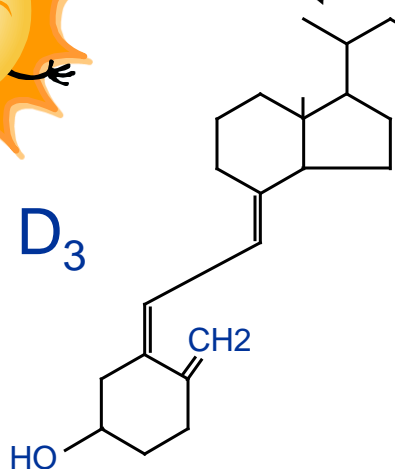
Vitamin D₃

Skin
production

Diet: fish, meat

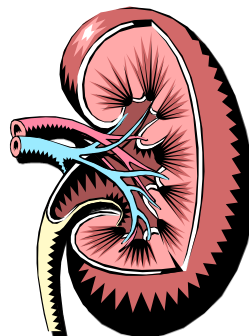


Liver

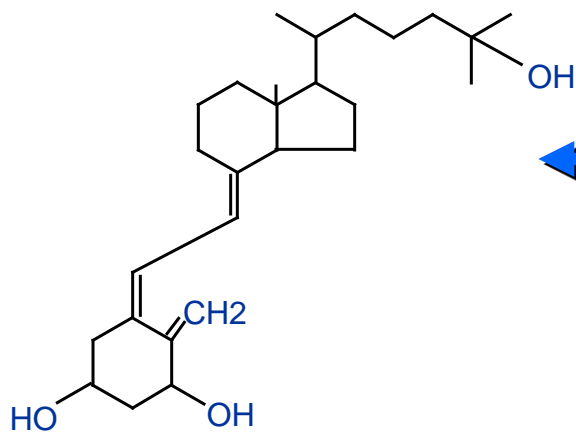


25 OH Vitamin D₃

Kidney



1,25 (OH)₂ Vitamin D₃



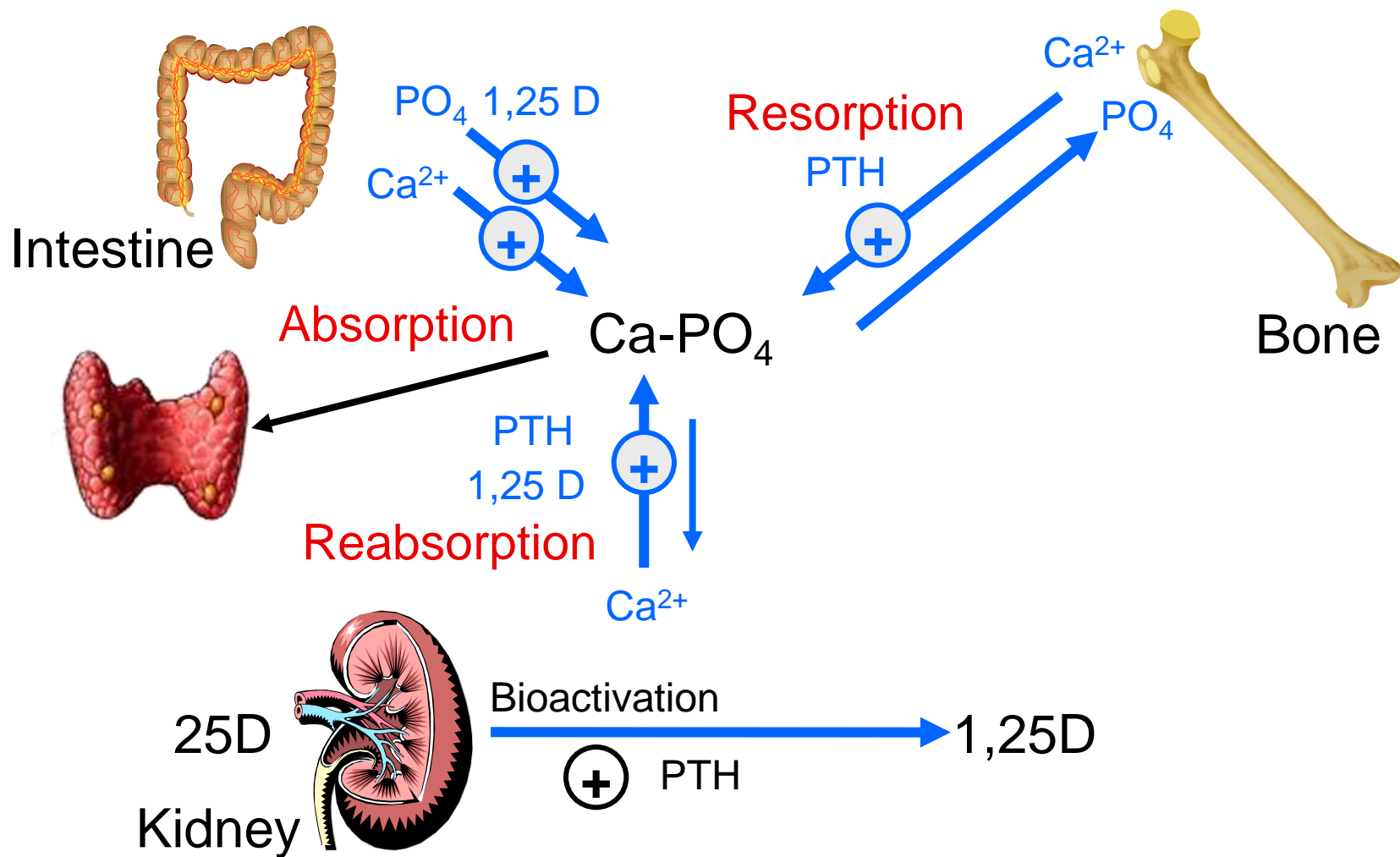


Right side of patient

Left side of patient

On coronals

Vitamin D Endocrine System



Day 1

Dr.'s Questions to the Lab

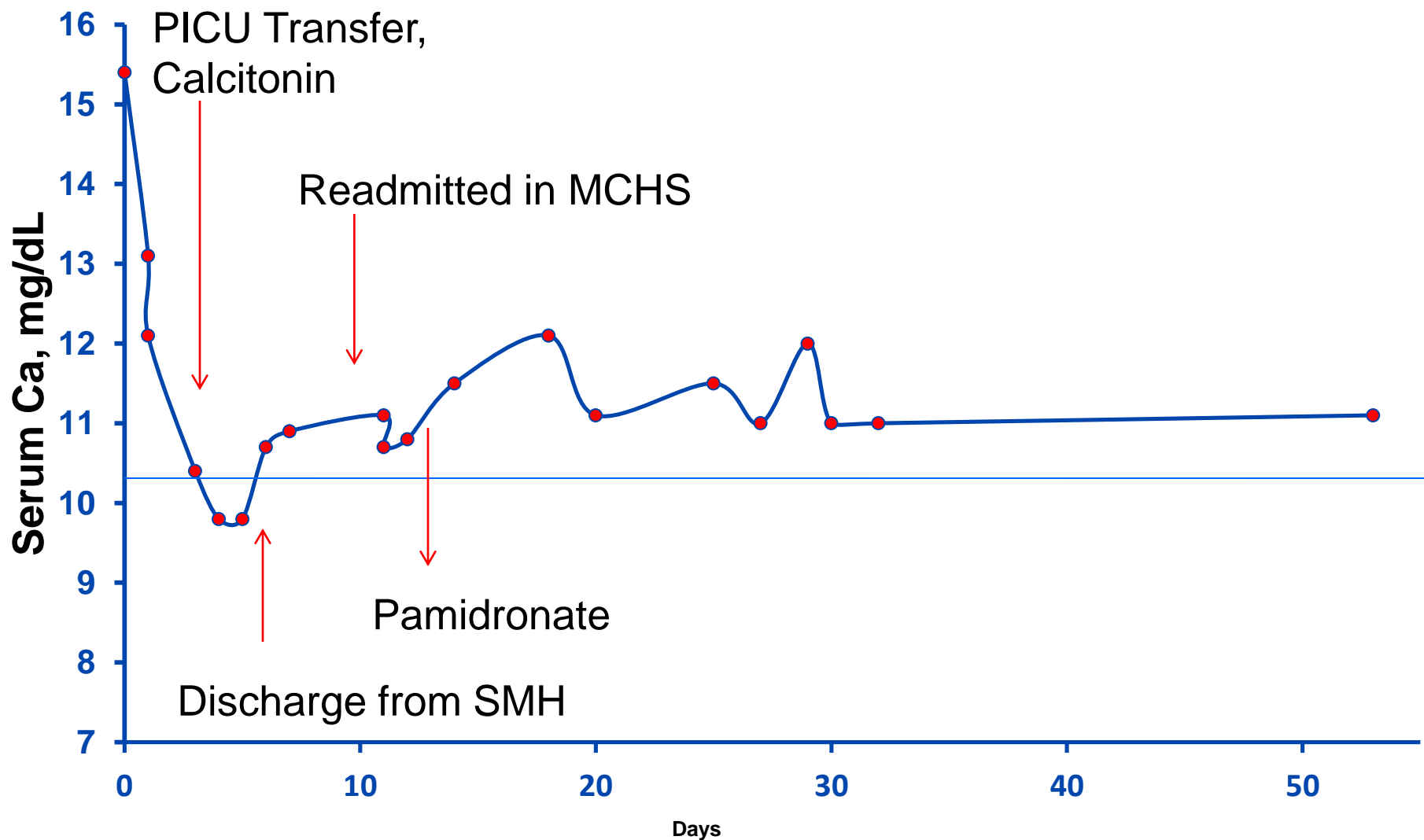
- 4 month old admitted to PICU
- Calcium 18, 15.5, 15.4
- Phosphorus 2 and 1.8
- PTH <6
- PTHrP-Pending
- *What will cause PTH to be low-Any interferences in the PTH assay?*

Day 1

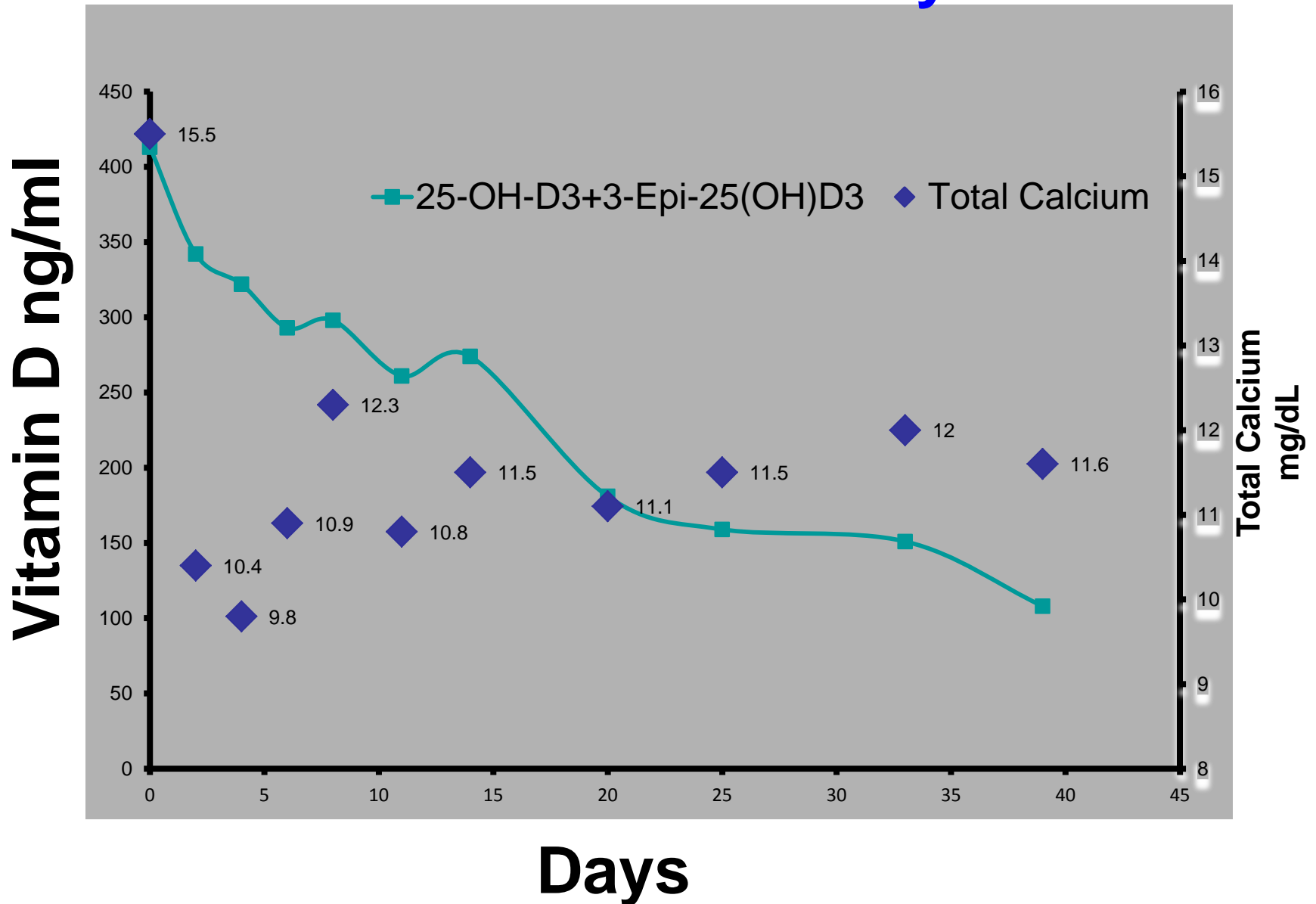
PTH Calcium Question

- Ultrasound of Abdomen and Kidneys Pending
- Breast Fed
- Some teething Medicine-Homeopathic
- Some Natural Vitamin D-Mom doesn't remember the brand
- *But If she is giving excess Vitamin D than P should be high as well*
- skeletal survey as well.
- ?Jensen's chondrodysplasia. Usually though the calcium is not that high

Case: Serum Calcium



Vitamin D Toxicity



Day 2 in PICU

4-month-old female History

- **Since birth, She has been given supplemental vitamin D.**
- **Mom reports that she has given 0.25 mL (likely 100 units) on a daily basis.**
- **Labs and clinical presentation at outside ER were consistent with dehydration. She received a fluid bolus and repeat labs > 12 hours later showed improvement in BUN as well as a decline in her total calcium.**

In US Vitamin D Supplements are not Regulated by FDA



Liquid Vitamin D 3 - 2,000 IU per drop - 900 Servings - High Potency

by [Seeking Health](#)

★★★★★ ▾ [189 customer reviews](#) | [8 answered questions](#)

List Price: \$23.95

Price: **\$19.95** & **FREE Shipping** on orders over \$35. [Details](#)

You Save: **\$4.00 (17%)**

In Stock.

Sold by [Seeking Health](#) and Fulfilled by [Amazon](#). Gift-wrap available.













Want it Thursday, Feb. 13? Order within **2 hrs 50 mins** and choose **Two-Day Shipping** at checkout. [Details](#)

- Physician Formulated Liquid Vitamin D. Hypoallergenic. BPA Free Container. No Plastic.
- Potent Formula at 2,000 IU Vitamin D3 Per Drop. 60,000 IU per Dropperful! 900 Servings Per Container. Only Pennies Per day. Best priced D3 on the market.
- Safe for all ages & easy to take. Kids to Senior Citizens can easily accept a drop of great tasting Liquid Vitamin D3. No swallowing pills needed.
- Pure. No preservatives, additives, colors, yeasts, egg, wheat, soy, fish, salt, sugar, corn or gluten.
- Seeking Health Liquid Vitamin D3 is obtained from the lanolin that comes from the wool of unharmed sheep.

How much vitamin D was the baby getting anyway?

- **The vitamin D content of the supplement was threefold higher (6000 IU of D/drop) than listed on the label (2000IU).**
- **Combined with the gross overdosing, this was estimated to have resulted in a daily vitamin D dose of 50,000 IU for two months**
- **Nephrocalcinosis was noted on ultrasound**



 <p>GNC Vitamin D-3 5000 IU 100 Tablets</p> <p>★★★★★ Read 2 Reviews Write a Review</p> <p>Price: \$19.99</p> <p>Member Price: \$16.99 Become a Member</p> <p>2 for \$30 Sale - Add 2 to Cart for Discount</p>	 <p>GNC Vitamin D-3 2000 IU 100 Tablets</p> <p>★★★★★ Read 1 Review Write a Review</p> <p>Price: \$14.99</p> <p>Member Price: \$12.74 Become a Member</p> <p>\$3.99 Flat Rate Shipping!</p>
<p>NEW!</p>  <p>GNC Vitamin D-3 2000 IU 100 softgels</p> <p>★★★★★ Be the first to Write a Review</p> <p>Price: \$14.99</p> <p>Member Price: \$12.74 Become a Member</p> <p>\$3.99 Flat Rate Shipping!</p>	<p>NEW!</p>  <p>Optimum Nutrition OPTI-MEN® 90 Tablets</p> <p>★★★★★ Be the first to Write a Review</p> <p>Regular Price: \$35.99 Sale Price: \$31.99</p> <p>Member Price: \$25.99 Become a Member</p> <p>\$3.99 Flat Rate Shipping!</p>
<p>NEW!</p>  <p>Optimum Nutrition OPTI-MEN® 150 Tablets</p> <p>★★★★★ Be the first to Write a Review</p> <p>Regular Price: \$51.99 Sale Price: \$45.99</p> <p>Member Price: \$36.99 Become a Member</p> <p>\$3.99 Flat Rate Shipping!</p>	 <p>Now® Vitamin D-3 180 softgels</p> <p>★★★★★ Read 3 Reviews Write a Review</p> <p>Price: \$8.99</p> <p>Member Price: \$7.19 Become a Member</p> <p>\$3.99 Flat Rate Shipping!</p>
<p>NEW!</p>  <p>GNC Vitamin D-3 400 IU 100 Tablets</p> <p>★★★★★ Read 1 Review Write a Review</p> <p>Price: \$4.99</p> <p>Member Price: \$4.24 Become a Member</p> <p>\$3.99 Flat Rate Shipping!</p>	<p>NEW!</p>  <p>GNC Vitamin A & D-3 100 softgels</p> <p>★★★★★ Be the first to Write a Review</p> <p>Price: \$4.49</p> <p>Member Price: \$3.82 Become a Member</p> <p>\$3.99 Flat Rate Shipping!</p>
 <p>Now® Liquid Vitamin D-3 2 fluid ounce(s)</p> <p>★★★★★ Read 3 Reviews Write a Review</p> <p>Price: \$9.99</p> <p>Member Price: \$7.99 Become a Member</p> <p>\$3.99 Flat Rate Shipping!</p>	 <p>Nature's Plus® Adult Chewable Vitamin D3 90 chewable tablets</p> <p>★★★★★ Read 1 Review Write a Review</p> <p>Price: \$13.35</p> <p>Member Price: \$10.68 Become a Member</p> <p>\$3.99 Flat Rate Shipping!</p>
 <p>Jarrow Formulas® Vitamin D3 100 softgels</p> <p>★★★★★ Be the first to Write a Review</p> <p>Price: \$7.99</p> <p>Member Price: \$6.39 Become a Member</p> <p>\$3.99 Flat Rate Shipping!</p>	 <p>Natural Factors® Vitamin D-3 120 softgels</p> <p>★★★★★ Be the first to Write a Review</p> <p>Price: \$14.99</p> <p>Member Price: \$11.99 Become a Member</p> <p>\$3.99 Flat Rate Shipping!</p>



History of 25-OH Vitamin D Testing

- **HPLC assay in 1981**
 - **50 samples/day**
- **RIA in March 2000**
 - **150 samples/day**
- **LC-MS/MS in November 2004**
 - **3500 samples/day**

Deciding on an Assay Platform

“if an RIA or ELISA better suits the needs of the laboratory, the results of these assays should correlate closely with result from the physical chemical ‘gold standard’ methods of HPLC or mass spectrometry.”



MAYO CLINIC

Laboratory Automation





MAYO CLINIC

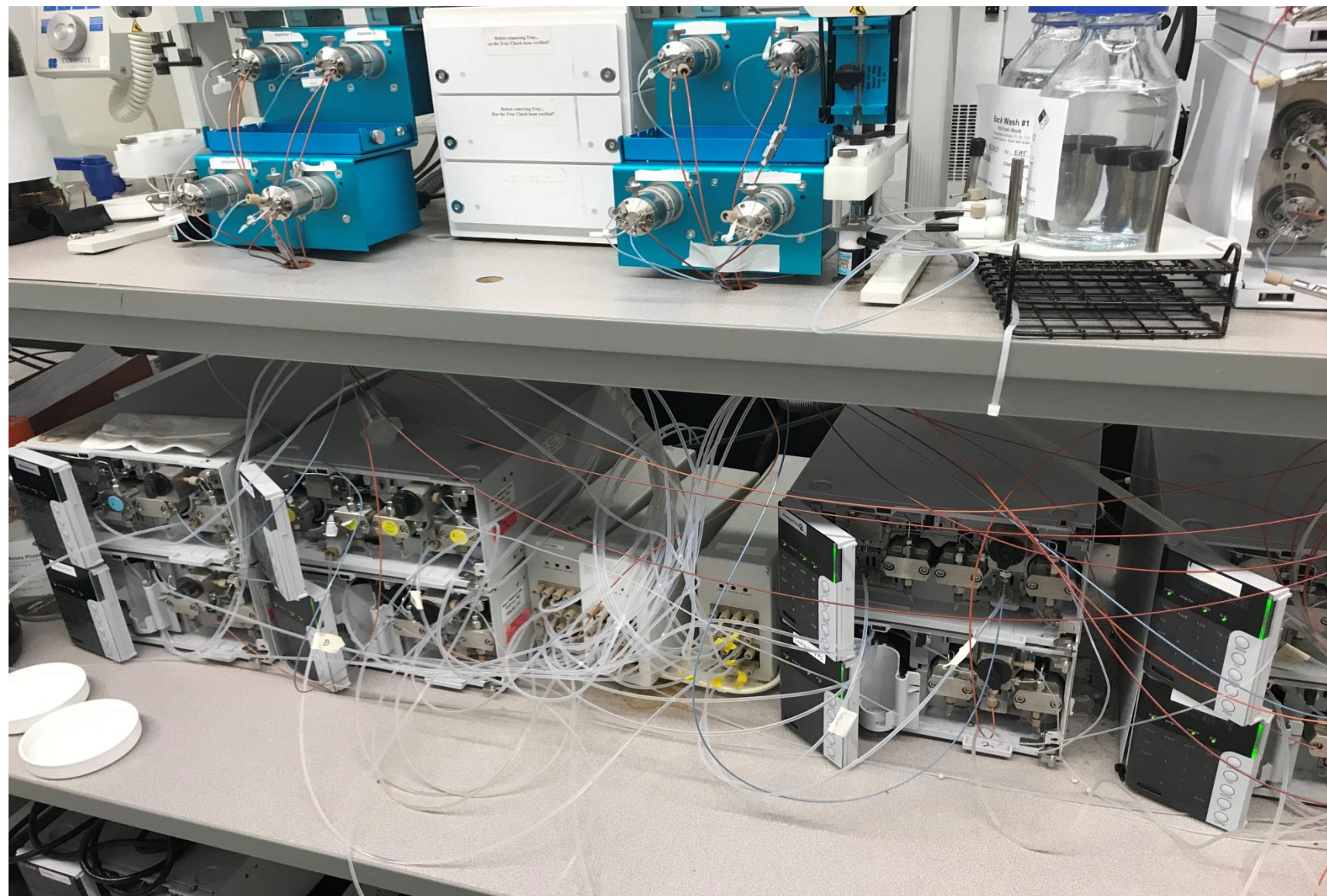
Workstation and Modular Pre-Analytical Automation



Mass Spec Environment









History of Vitamin D Testing

Tribute to Dr. Deluca

Plasma (3-5 ml) + ^3H Metabolites



Diethylether (2X)



Methanol: Methylene Chloride (1:3)



Backwash w/ 0.1m NaPO_4 (pH 10.5)



Sephadex LH-20

(1:1:9 Chloroform : Methanol : Hexane)



Vitamin D



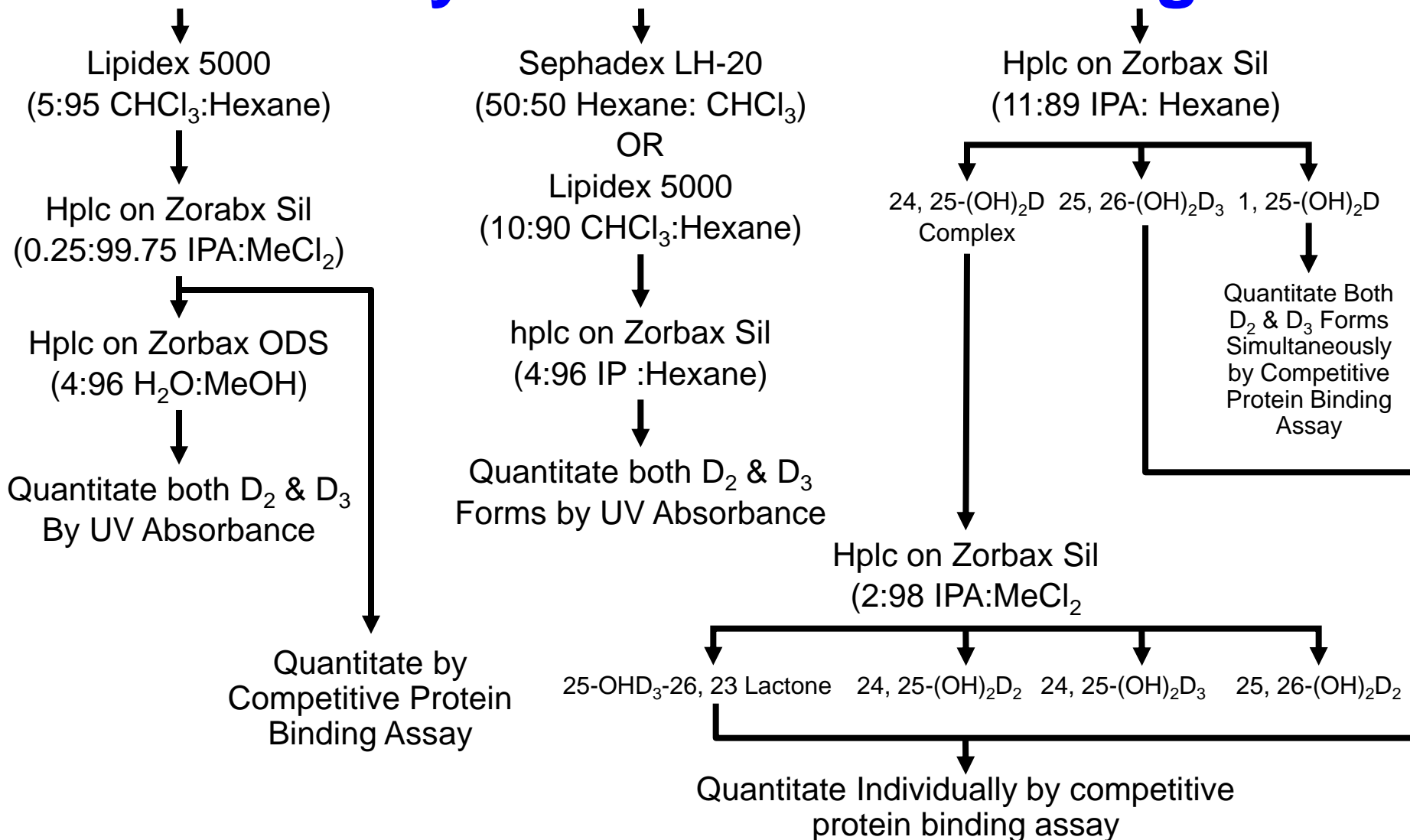
25(OH)D



Dihydroxyvitamin D



History of Vitamin D Testing





25-OH-Vitamin D is Sticky

TABLE 1. Comparison of DBP with other hormone-carrier proteins in human plasma

	DBP	TBG	CBG	SHBG
Mol. wt.	58,000	54,000	52,000	95,000
Ligand/carrier	mol/mol	mol/mol	mol/mol	mol/mol
Plasma concentration (mM)	6	0.3	0.8	0.08 (♀) 0.04 (♂)
Carrier binding sites normally occupied	2%	50%	50%	50% (T)
Fraction of total ligand concentration on carrier	88% (25-OHD)	77% (T ₄)	70% (F)	45% (T)
% of total ligand concentration in free form	0.04% (25-OHD) 0.4% [1,25(OH) ₂ D]	0.03% (T ₄) 0.3% (T ₃)	8% (F)	2% (T)
Influence of estrogen on carrier concentration	↑	↑	↑	↑

TBG, binding globulin; CBG, cortisol binding globulin; SHBG, sex hormone binding globulin; F, cortisol; T, testosterone.

LC-MS/MS Method

- **Pipet 100 μ L serum, add internal standard and incubate 15 minutes**
- **Precipitate proteins with 250 μ L Acetonitrile**
- **Vortex and centrifuge 15 minutes at 3000 rpm**
- **Pipet 200 μ L of supernatant into autosampler vials**
- **Initiate analysis on LC-MS/MS**
- **Results are produced every 3 minutes 15 seconds**

Result of the search: list of reference measurement methods/procedures

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➔ **Your search criteria:** Reference measurement methods/procedures; Analyte: 25-hydroxyvitamin D3; Analyte category: -; Matrix category: -

➤ [Save as PDF file](#)

➤ [Modify your selection](#)

Results of the search

Isotope dilution mass spectrometry methods for 25-hydroxyvitamin D3 in blood serum

► CDC reference measurement procedure for serum 25-hydroxyvitamin D metabolites

Applicable matrice(s)	fresh, lyophilized, or frozen human serum
Full description of technique(s)	Isotope dilution liquid chromatography mass spectrometry (ID-LC-MS/MS)
Quantity	Amount-of-substance concentration
Applicable range	10 nmol/L to 100 nmol/L
Expected uncertainty (level of confidence 95%)	2.7 % to 3.9 %
Reference(s)	A candidate reference measurement procedure for quantifying serum concentrations of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 using isotope-dilution liquid chromatography-tandem mass spectrometry, Mineva E M et al., <i>Anal. Bioanal. Chem.</i> , 2015, 407 (19), 5615-5624
JCTLM DB identification number	C12RMP2

► NIST methods for 25-hydroxyvitamin D

Applicable matrice(s)	serum, plasma
Full description of technique(s)	ID-LC-MS/MS
Quantity	Mass fraction
Applicable range	6.311 ng/g to 26.972 ng/g
Expected uncertainty (level of confidence 95%)	2 % to 2.1 %
Reference(s)	<i>Anal. Chem.</i> , 2010, 82 , 1942
JCTLM DB identification number	C7RMP17

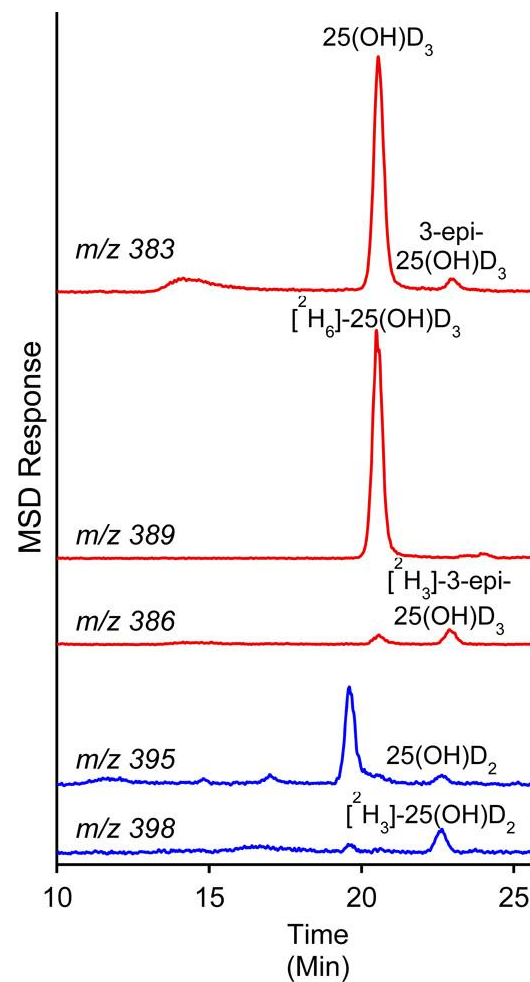
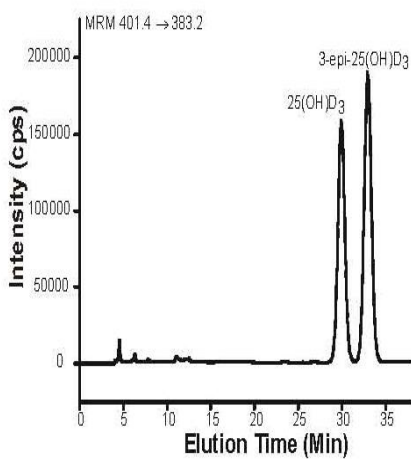
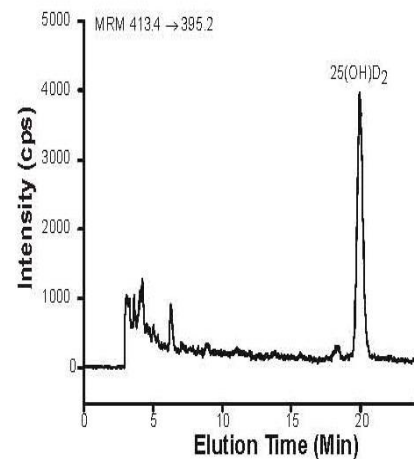
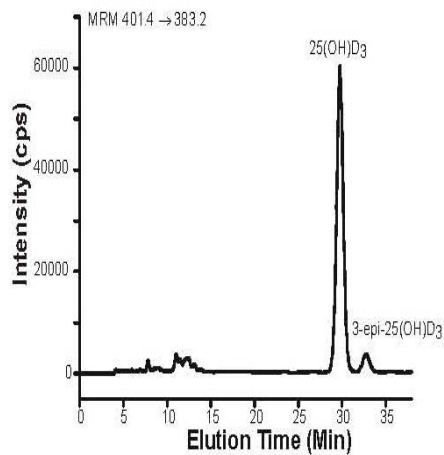
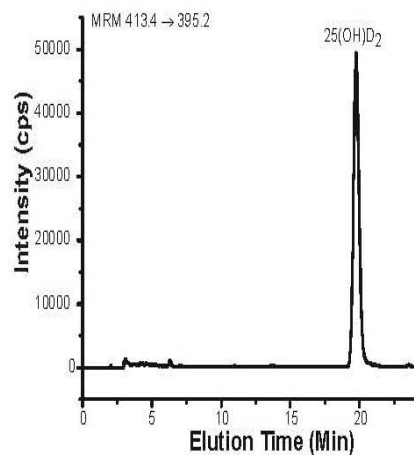
► University of Ghent reference measurement procedure for 25-hydroxyvitamin D3

Applicable matrice(s)	lyophilized, fresh, or frozen serum
Full description of technique(s)	ID/LC/MS
Quantity	Amount-of-substance concentration
Applicable range	1.1 nmol/L to 600 nmol/L
Expected uncertainty (level of confidence 95%)	3.4 %
Reference(s)	<i>Clin. Chem.</i> , 2011, 57 (3), 441-448
JCTLM DB identification number	C8RMP3

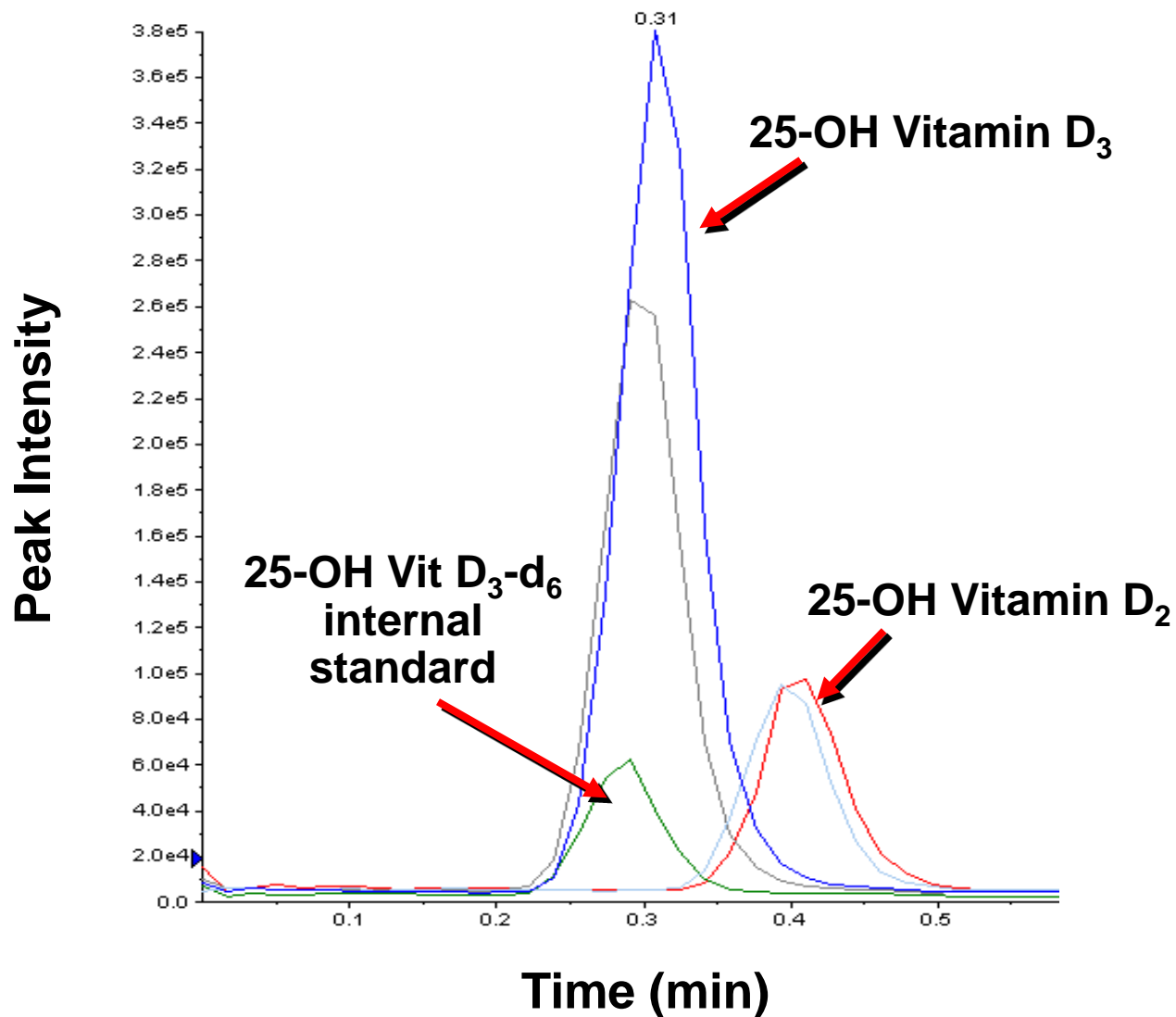
Sample Preparation for LC-MS/MS

- Serum samples (1 to 2 g) were weighed into a 50 mL glass centrifuge tube.
- At least one level of SRM 972 was used as a quality control material with each set of samples. Each sample was spiked (exact mass known) with an appropriate amount of the internal standard solution (see Supporting Information) to achieve an approximately 1:1 ratio of analyte to internal standard.
- After equilibration for 1 h at room temperature, the pH of each sample was adjusted to $\text{pH } 9.8 \pm 0.2$ with carbonate buffer (0.1 g/mL, pH 9.8).
- Analytes were isolated from the serum matrix by liquid–liquid extraction with 8 mL of hexane:ethyl acetate (50:50, volume fractions).
- Samples were extracted twice, and the extracts were combined and dried under nitrogen at 45 °C.
- The residue was reconstituted with methanol (200 to 250 μL).

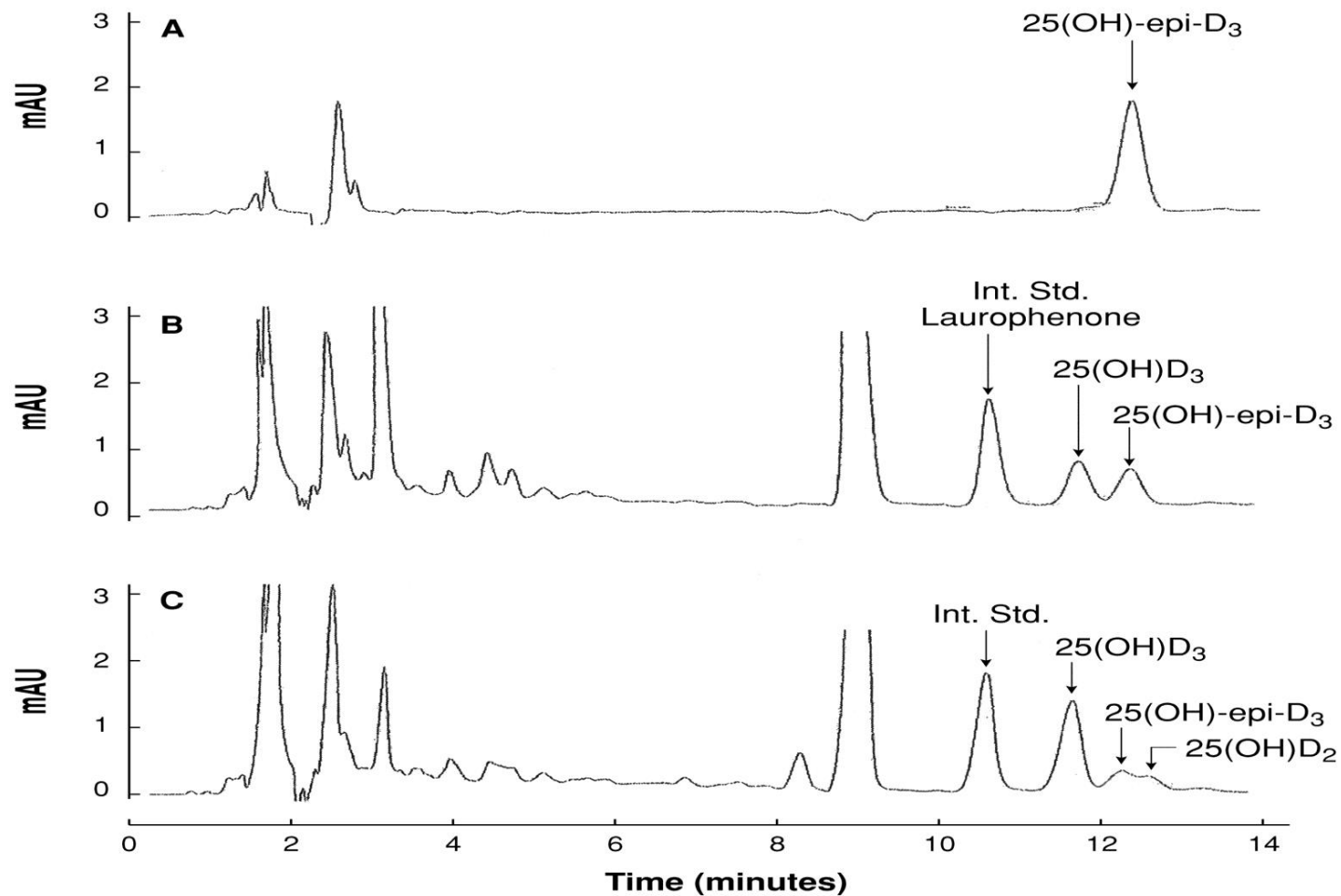
Challenge Throughput



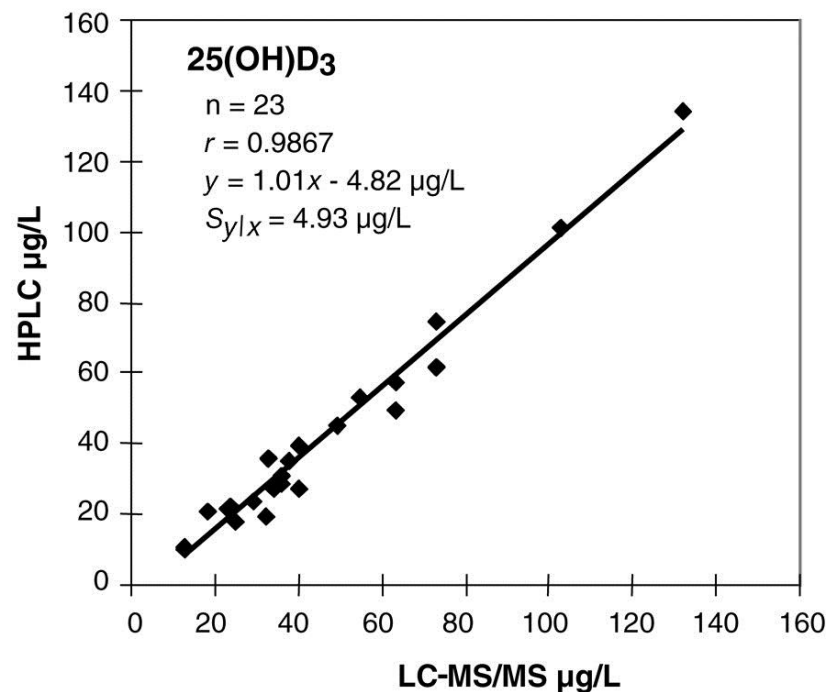
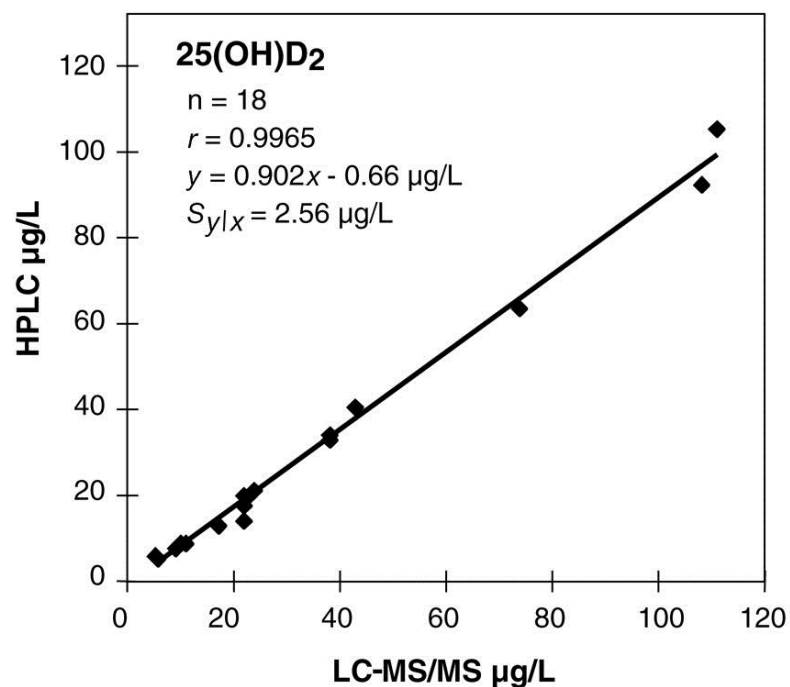
LC-MS/MS VITAMIN D



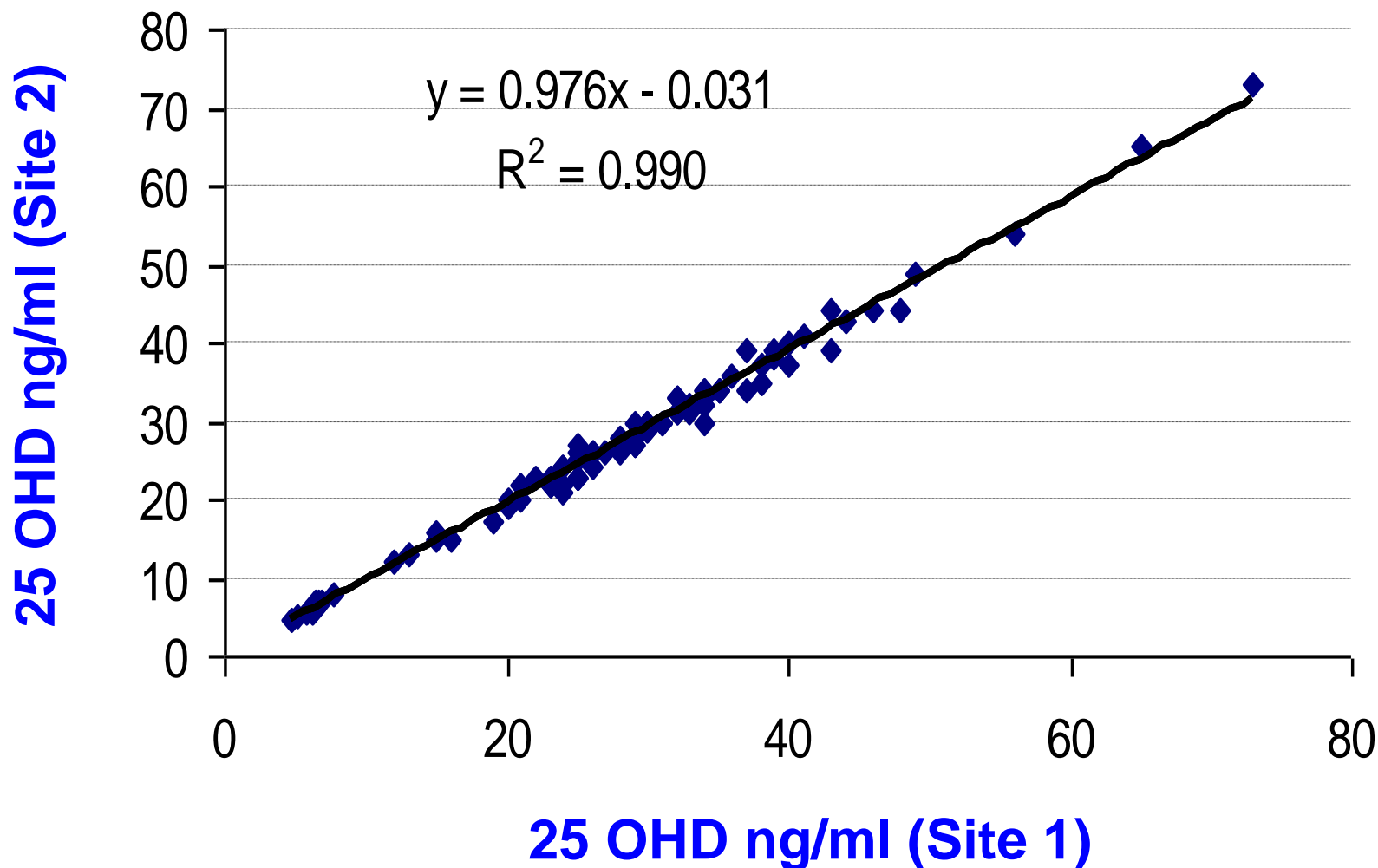
Chromatograms



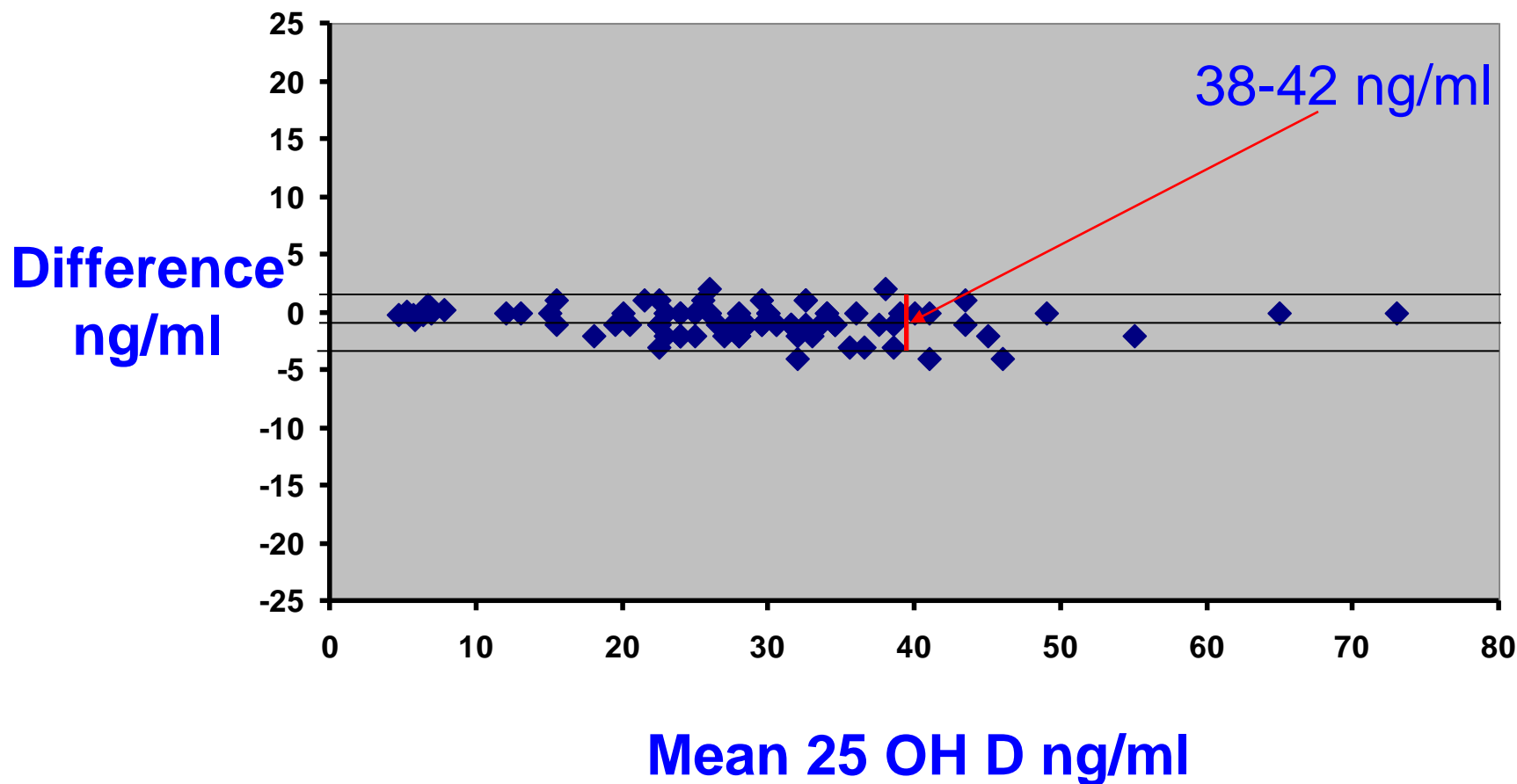
Comparison of the Proposed HPLC Method with LC-MS/MS



Performance of LC-MS/MS Method at Two Different Sites



LC-MS/MS-LC/MS/MS



Methods for Testing of 25-OH-Vitamin D

- 1. Competitive protein-binding assay (CPB)**
- 2. High-performance liquid chromatography (HPLC)**
- 3. Radioimmunoassay RIA and chemiluminescence immunoassay CLIA**
- 4. Liquid chromatography mass spectrometry (LC-MS, LC-MS/MS)**

Immunoassays in Clinical Laboratories and the way of the Dodo

**Tandem Mass-spectrometry - the quiet
revolution in endocrine-, drug- and
metabolic laboratory testing**



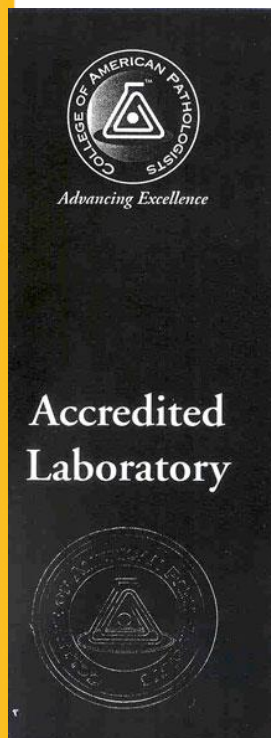
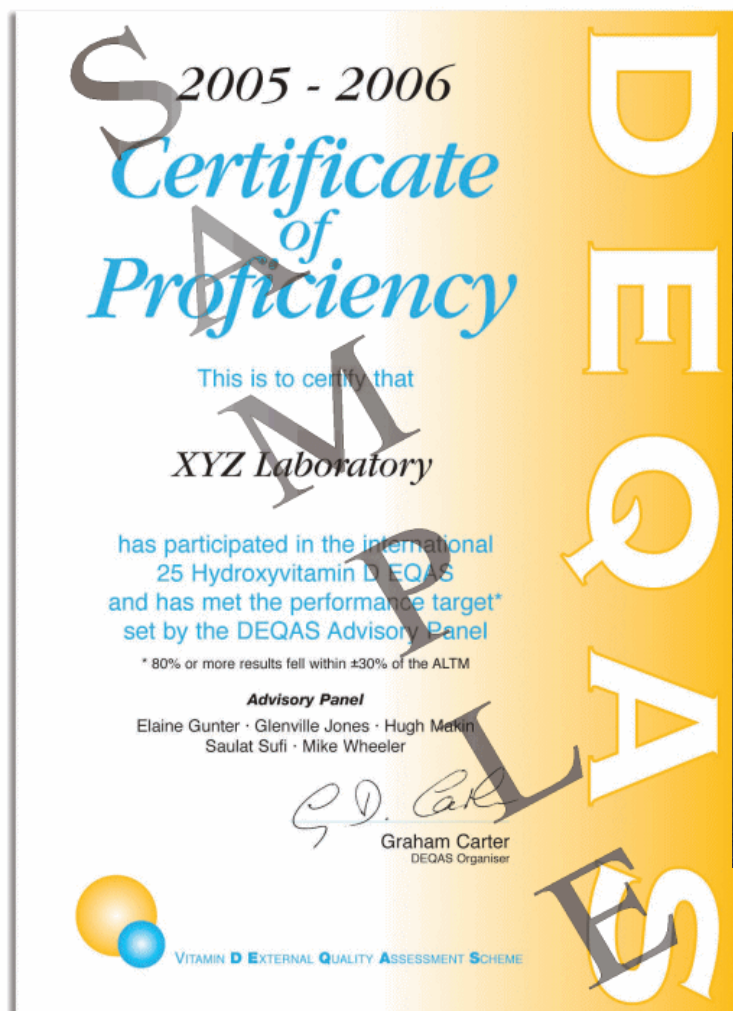
Immunoassays – central to current lab testing

- **Immunoassays allow sensitive detection of a wide range of analytes**
- **Immunoassays can be very specific**
- **Immunoassays come in a wide range of formats and can be automated**
- **Today's reference ranges for a wide range of analytes have been established with Immunoassays**
- **Immunoassay problems and limitations are well understood**

Methods Participating in DEQAS

From	Method	Returns	
		July 2017	April 2016
Oct 1989	Chromatographic competitive protein binding assay		
April 1991	HPLC	18	24
April 1993	IncStar RIA (until January 1999)		
July 1999	DiaSorin RIA (formerly IncStar)		5
	IDS RIA	4	6
July 2001	Nichols Advantage (discontinued in April 2006)		
Oct 2002	IDS EIA (OCTEIA)	12	17
April 2004	DiaSorin Liaison		
Oct 2005	LC-MS/MS	159	159
Jan 2006	IDS Automated EIA	2	5
April 2007	DiaSorin Liaison Total	214	243
Oct 2007	Roche 25-OHD3		
July 2008	DIASource 25-OHD3 RIA (formerly BioSource)		
Jan 2009	IDS iSYS automated chemiluminescence immunoassay	63	88
Jan 2011	Abbott Architect	9	38
April 2011	Siemens ADVIA Centaur	60	65
	Roche Total 25-OHD	165	161
Jan 2012	Diazyme 25-OHD EIA		2
	DiaSource Total 25-OHD RIA	1	2
	DiaSource Total 25-OHD ELISA	1	1
July 2012	Euroimmun ELISA	10	11
Oct 2012	DRG ELISA	1	1
	Tosoh AIA	3	5
Jan 2013	Ortho Total 25-OHD	4	5
	Immunodiagnostik ELISA		
April 2013	Quidel Microvue 25-OH Vit D		
July 2013	SNIBE Maglumi 25-OH Vit D	2	1
Jan 2014	Beckman Access 2 Total 25-OHD	4	4
	Beckman Unicel Dxi Total 25-OHD	32	31
April 2014	Diazyme 25-OH VitD Chemistry Analysers	3	2
	Fujirebio Lumipulse G 25-OH Vit D	3	3
April 2015	bioMerieux 25OH Vitamin D Total	4	2
	Bio-Rad BioPlex 2200	1	1
	Qualigen Fstpak IP Vitamin D	1	1
Oct 2015	DRG Hybrid XL	1	1
Jan 16	Abbott Architect New Kit	71	42
July 2016	Human 25-OH Vitamin D ELISA	1	
	Organtec Alegria 25-OH Vitamin D	2	
Jan 17	Roche Vitamin D total II (cobas e801)	8	
April 17	IDS iSYS New	6	

Accreditation Agencies look for only Precision among Peers and not Necessarily Accuracy



The College of American Pathologists
certifies that the laboratory named below

Mayo Clinic
Department of Laboratory Medicine & Pathology

LAP Number: 1808201
AU-ID: 1183832

has met all applicable standards for accreditation and is hereby fully accredited by the College of American Pathologists' Laboratory Accreditation Program. Reinspection should occur within 30 days prior to September 15, 2006 to maintain accreditation.

Accreditation does not automatically survive a change in director, ownership, or location and assumes that all interim requirements are met.

Ronald S. Heyman
Chair, Commission on Laboratory Accreditation

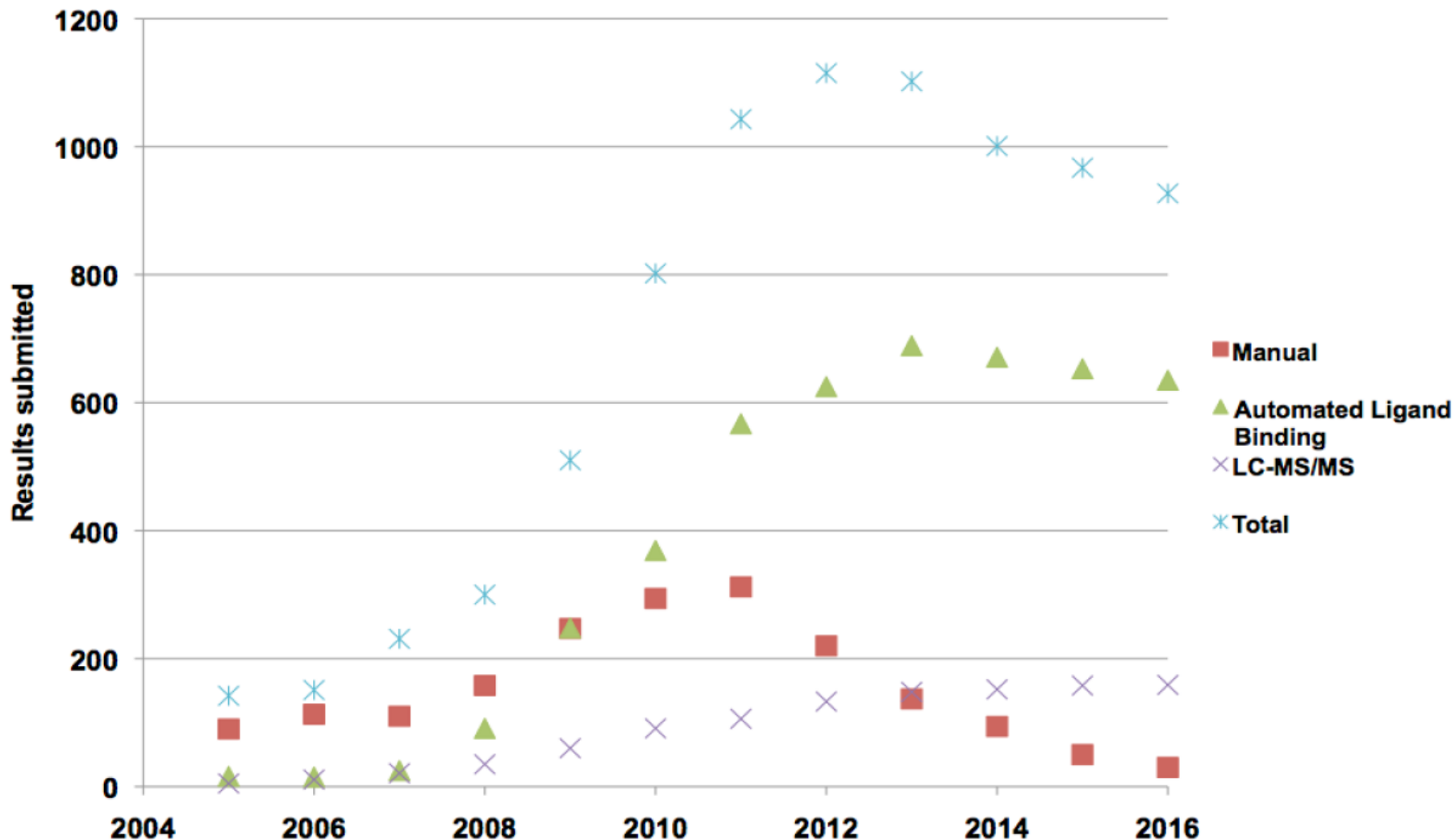
Mary E. Kass, M.D.
President, College of American Pathologists



An Endocrine Society Position Statement: Precision and Accuracy Matters

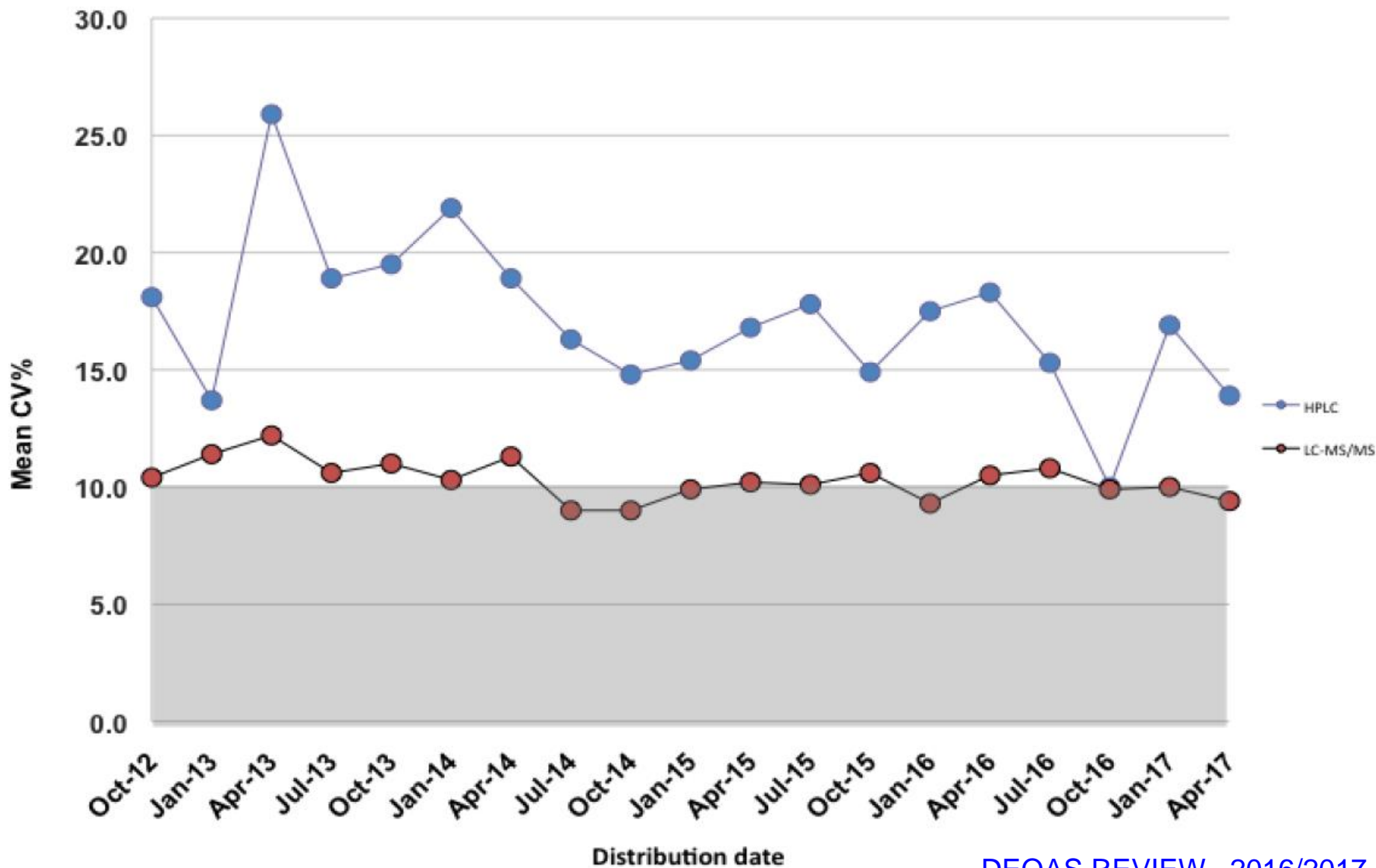
Laboratory proficiency testing should be based on the ability to measure accurately and precisely samples containing known concentrations of testosterone, not only on agreement with others using the same method

No of Labs Participating in DEQAS



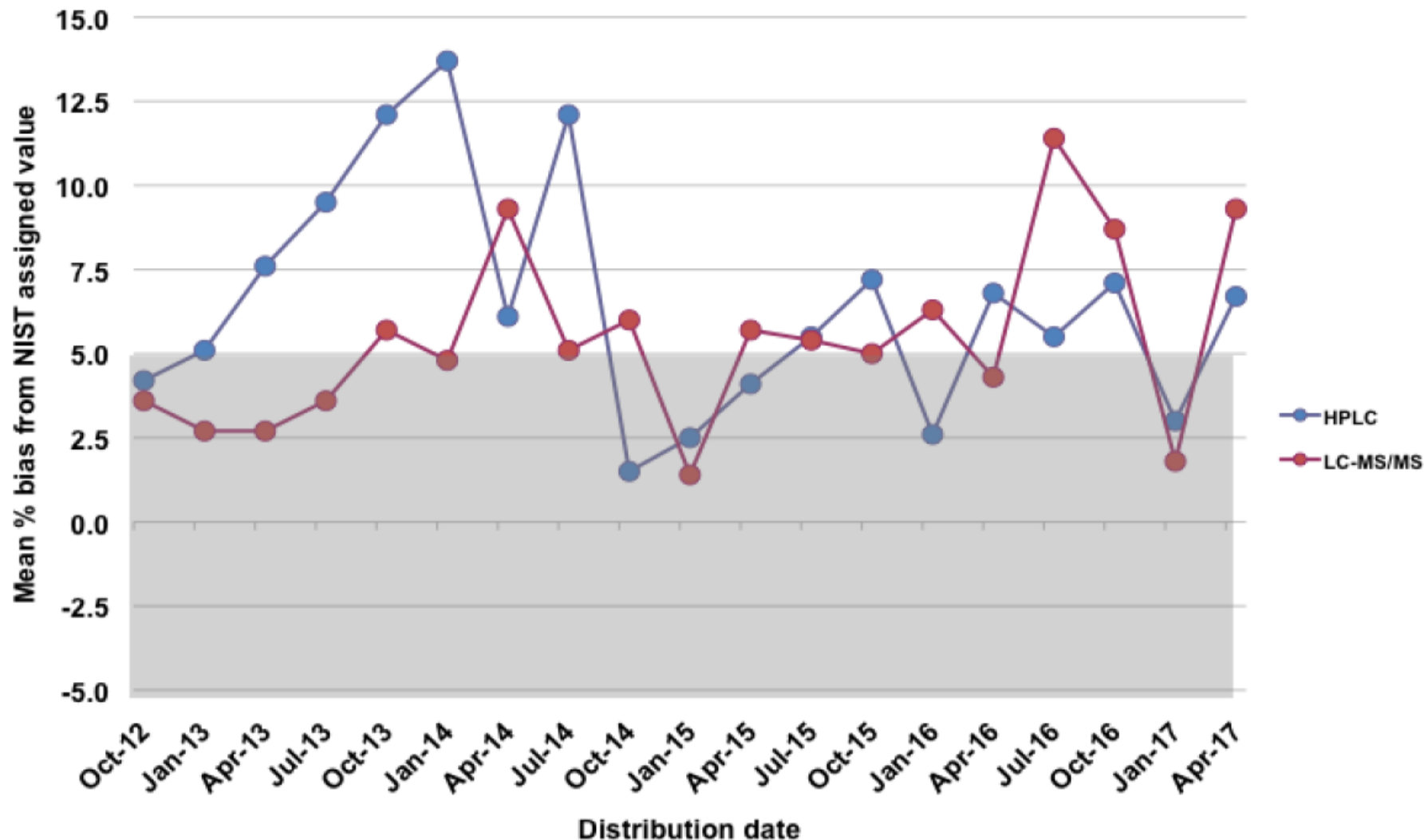


Mean %CV HPLC and LC-MS/MS

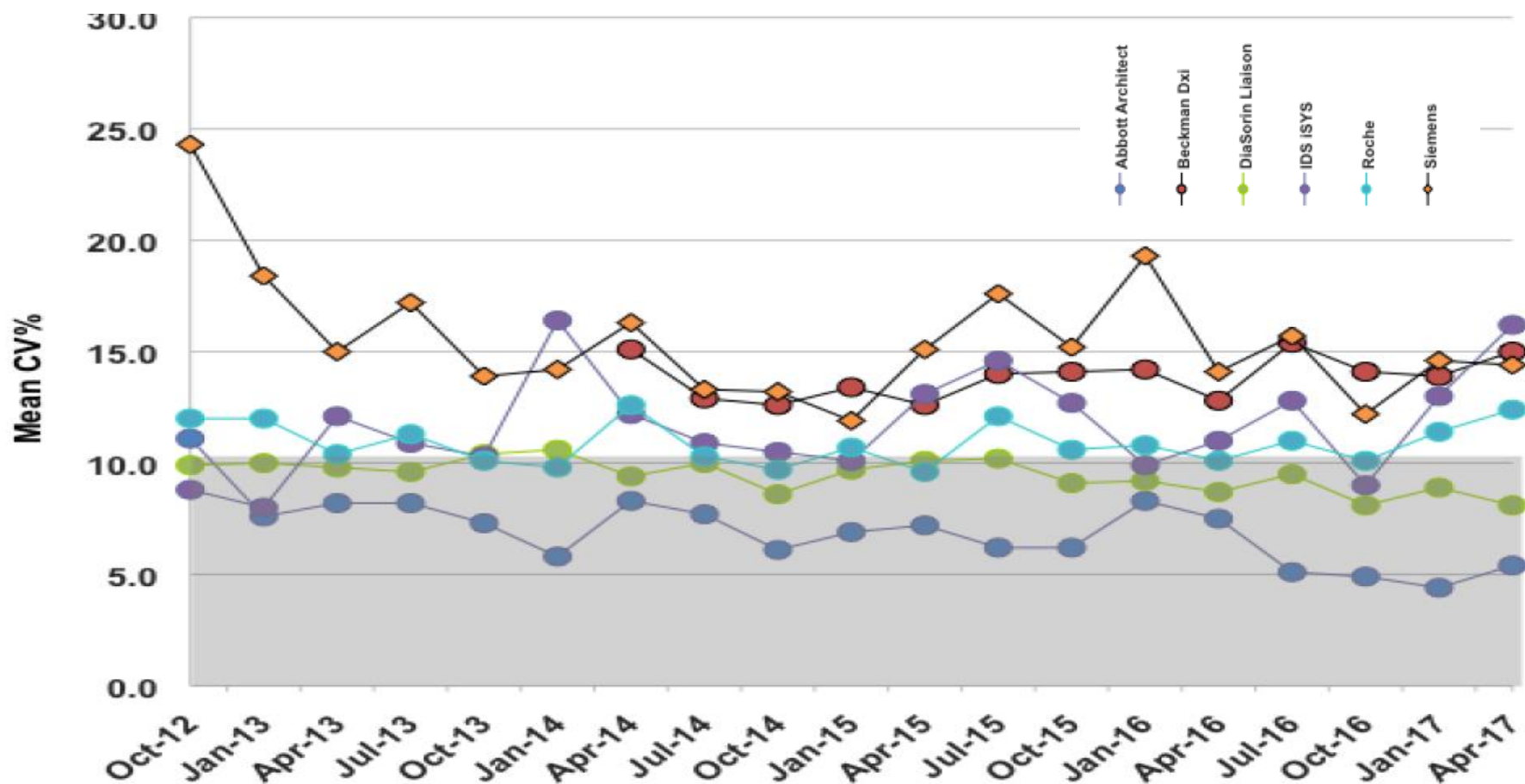




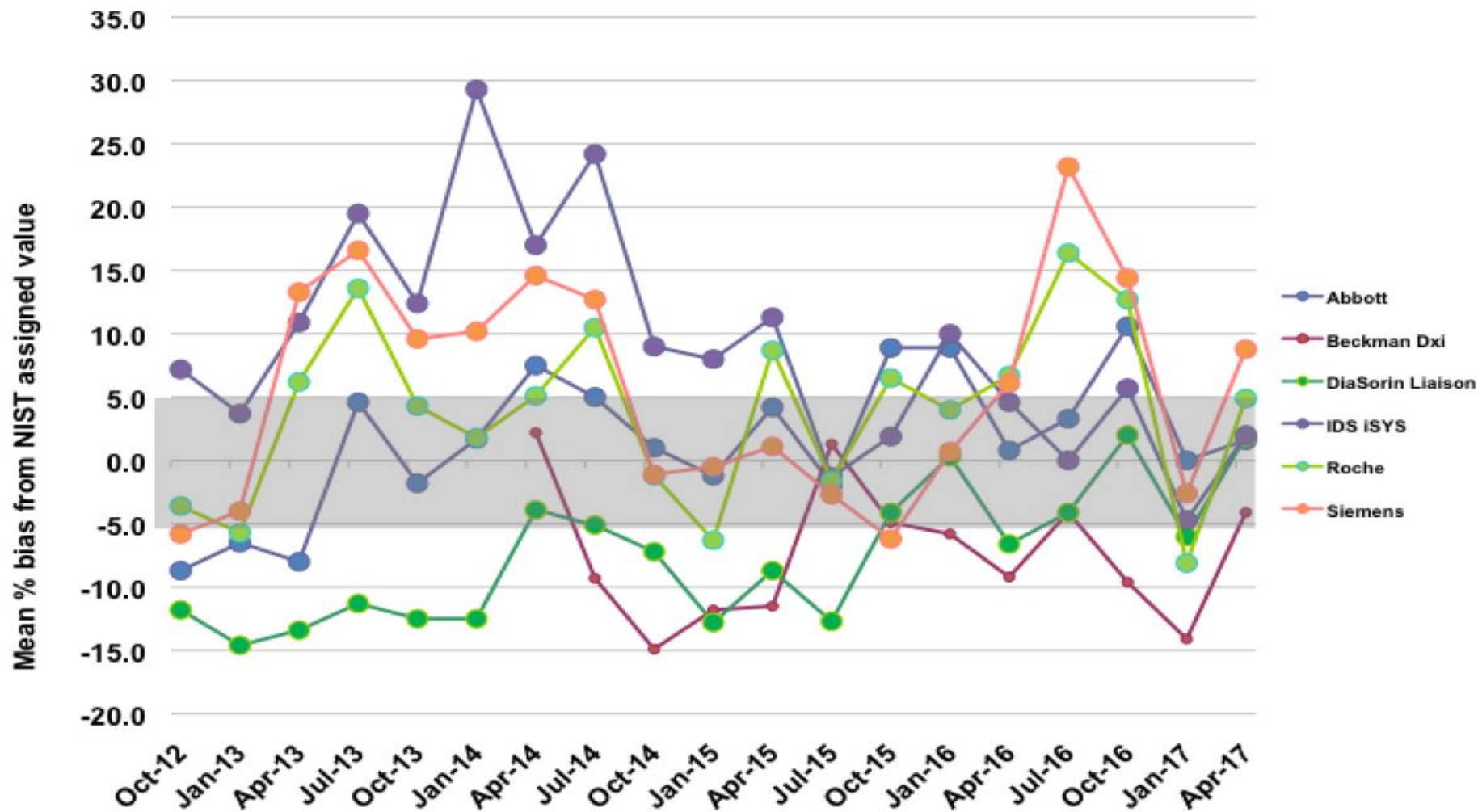
Mean %CV HPLC and LC-MS/MS



Mean CV% for Major Automated Assays

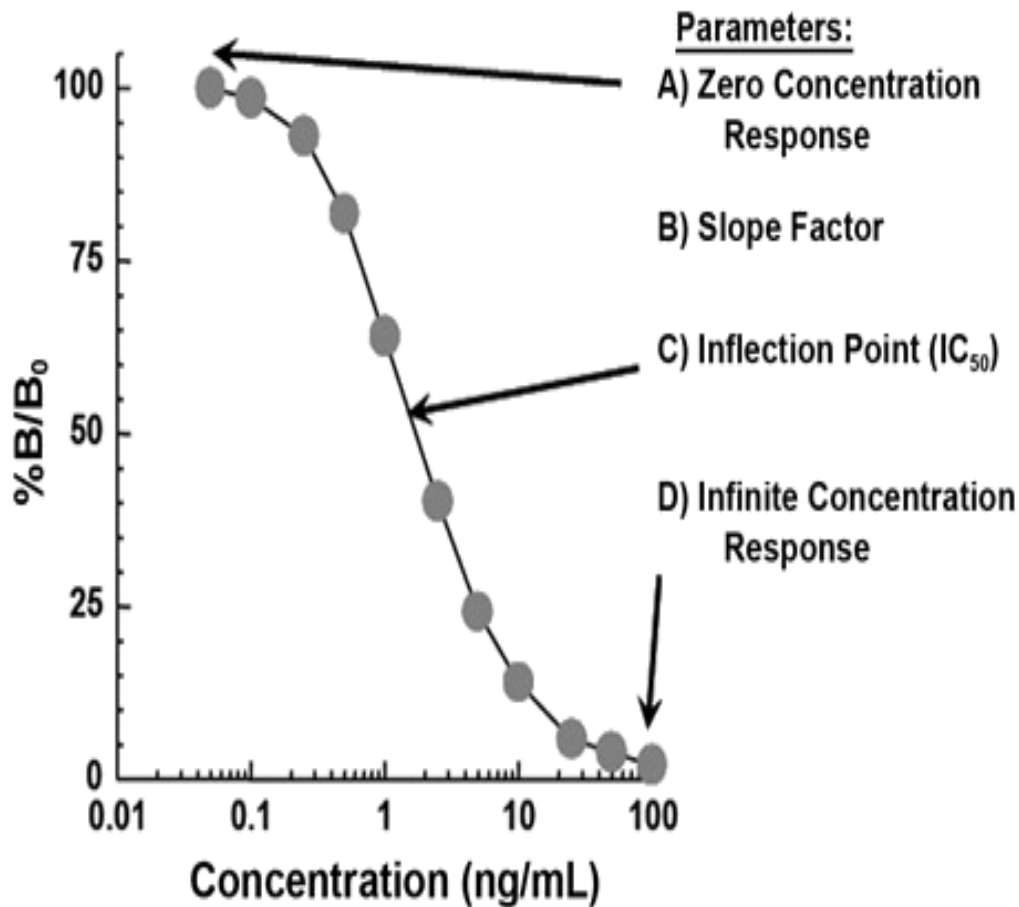
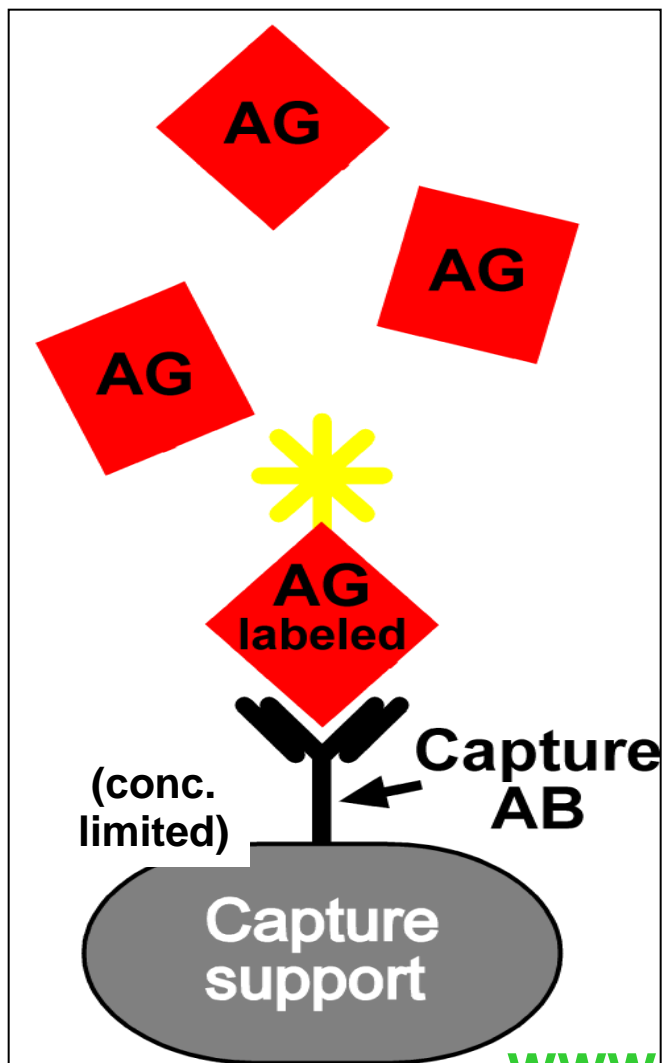


Mean % Bias from NIST Major Automated Assays





Competitive Immunoassays



Characteristics of 25-OH-D Methods

Principle	Instrument	Specificity D ₂ & D ₃ (% Recovery)	Antibody used
CMIA	Abbott	D ₃ – 100% D ₂ – 52%	sheep polyclonal / mouse <u>nonoclonal</u>
CLIA	ISYS	D ₃ – 100% D ₂ – 100%	sheep polyclonal
CLIA	LIAISON	D ₃ – 100% D ₂ – 104%	goat <u>polyclona</u>
RIA	Manual / γ -counter	D ₃ – 100% D ₂ – 100%	goat polyclonal
ECLIA	<u>Elecsys/</u> Modular/ <u>Cobas</u>	D ₃ – 100% D ₂ – <10%	monoclonal
CLIA	Centaur / Centaur XP	D ₃ – 106% D ₂ – 97%	mouse monoclonal
LCMS	Waters ACQUITY UPLC	D ₃ – 100% D ₂ – 100%	n/a
LCMS	Waters XE	D ₃ – 100% D ₂ – 100%	n/a



25-OH-Vitamin D is Sticky

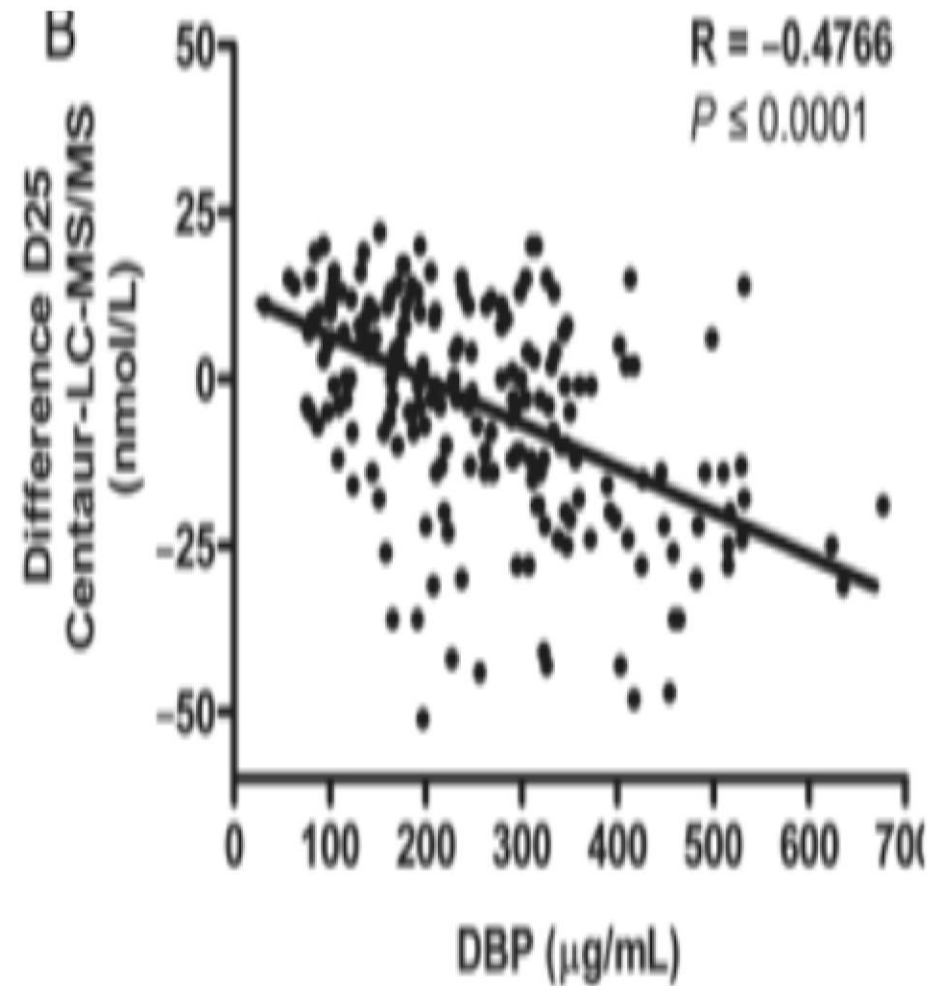
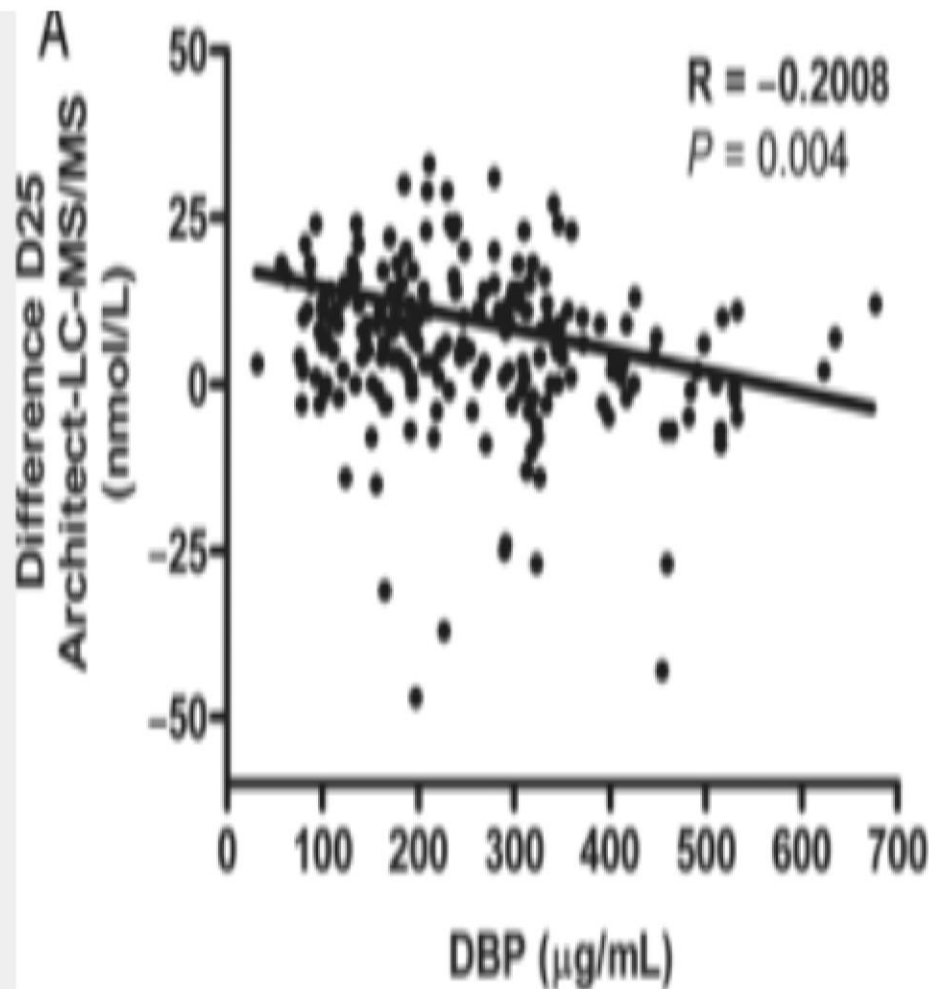
TABLE 1. Comparison of DBP with other hormone-carrier proteins in human plasma

	DBP	TBG	CBG	SHBG
Mol. wt.	58,000	54,000	52,000	95,000
Ligand/carrier	mol/mol	mol/mol	mol/mol	mol/mol
Plasma concentration (mM)	6	0.3	0.8	0.08 (♀) 0.04 (♂)
Carrier binding sites normally occupied	2%	50%	50%	50% (T)
Fraction of total ligand concentration on carrier	88% (25-OHD)	77% (T ₄)	70% (F)	45% (T)
% of total ligand concentration in free form	0.04% (25-OHD) 0.4% [1,25(OH) ₂ D]	0.03% (T ₄) 0.3% (T ₃)	8% (F)	2% (T)
Influence of estrogen on carrier concentration	↑	↑	↑	↑

TBG, binding globulin; CBG, cortisol binding globulin; SHBG, sex hormone binding globulin; F, cortisol; T, testosterone.

Clinical Chemistry Accuracy of 6 Routine 25-OH-vitamin D assays:

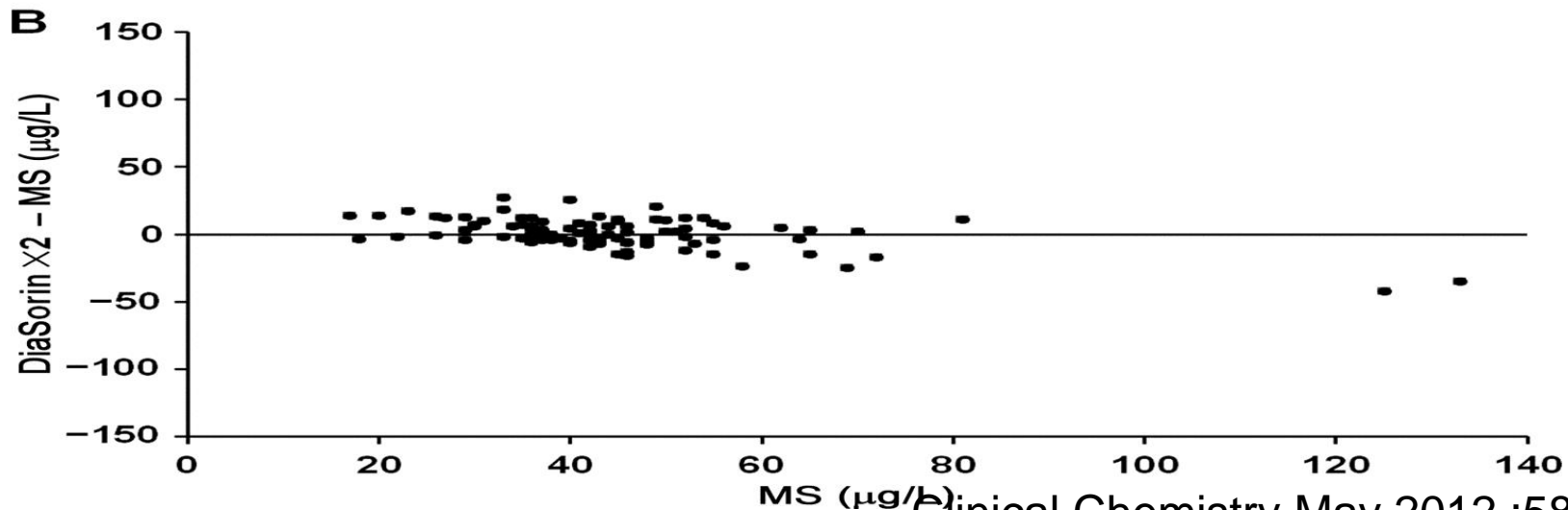
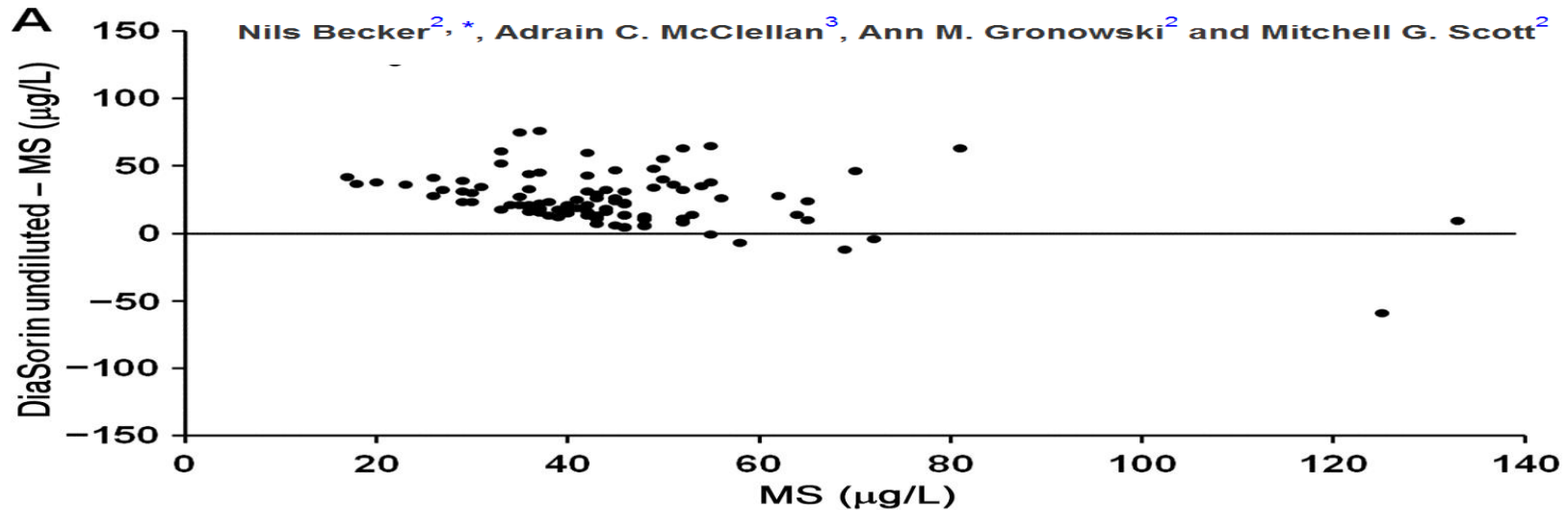
Influence of Vitamin D Binding Protein Concentration





Inaccurate 25-Hydroxyvitamin D Results from a Common Immunoassay

Nils Becker^{2, *}, Adrain C. McClellan³, Ann M. Gronowski² and Mitchell G. Scott²



Impact of MS/MS in Laboratory Medicine (Mayo)

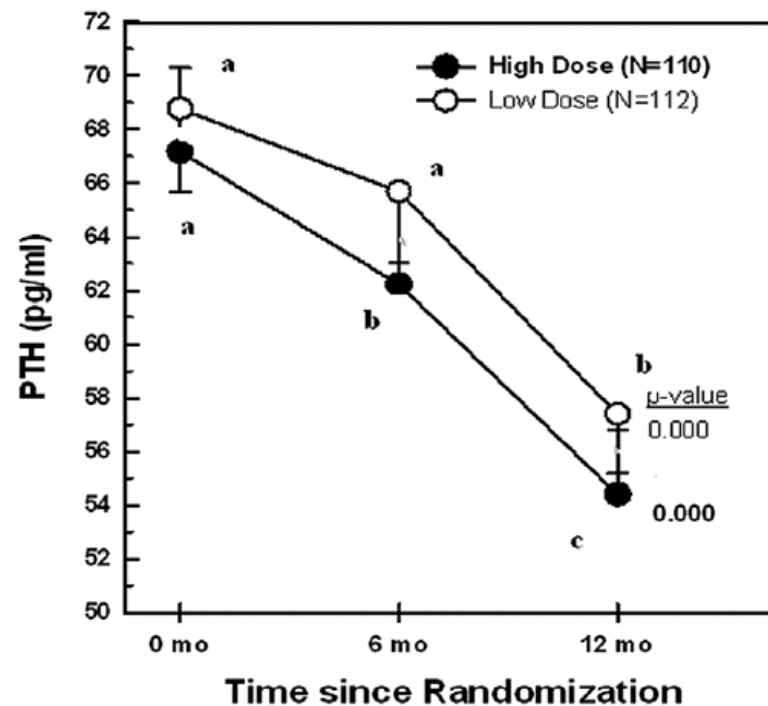
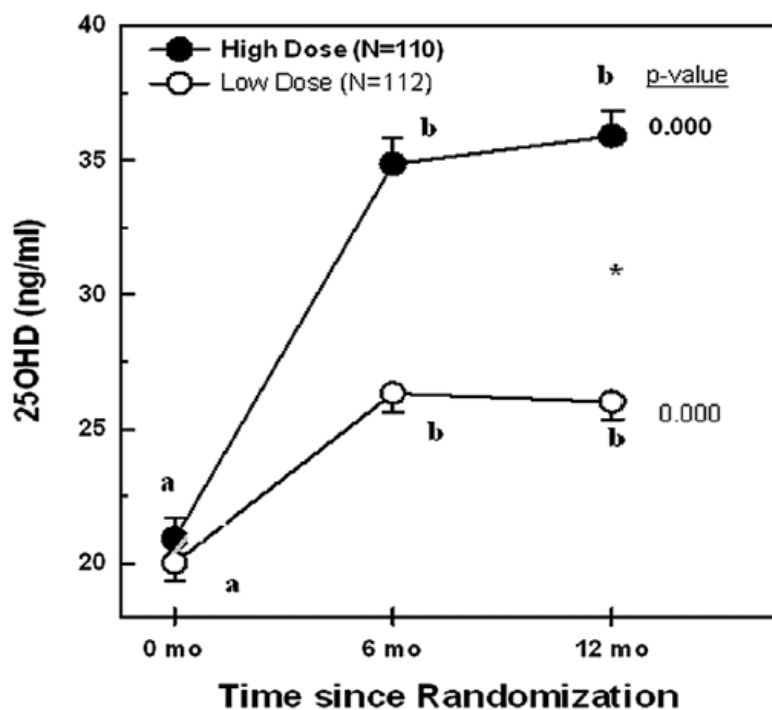
Platform	1998	2009
HPLC	>400	<100
GC/MS	>50	<30
MS/MS	0	58
Competitive Immunoassays	Many	Few

MS/MS Presence in Mayo-DLMP

DLMP laboratory	2008 Test Volume	MS/MS Test Volume	MS/MS Test %	No. of tests	No. of units
Anatomic Pathology	785,000	456	0.1%	1	1
Biochemical Genetics	563,268	229,721	41%	28	15
Cardiovascular (CVLM)	157,805	34,001	22%	3	2
Dev & Validation Center	n/a	n/a	n/a	n/a	4
Endocrinology	1,544,654	1,058,768	69%	28	16
Mayo Jacksonville	184,000	3,526	2%	3	3
MML New England	697,000	133,307	19%	3	4
Toxicology	461,985	155,829	34%	15	13
DLMP (total)	22,000,000	1,615,608	7%	81	58

(not sum of above)

Precision Required to See Change in a Single Patient



The way out of the chaos

Vitamin D measurement standardization:

- The lack of standardized laboratory measurement of serum 25-hydroxyvitamin D [25-OHD] in vitamin D impedes development of consensus to define stages of vitamin D status.
- 60,000 vitamin D papers have been published with nearly all the values from non-standardized assays. We cannot just ignore this old data and focus on standardizing 25-OHD in current and future research.
- Retrospective standardization results can vary widely depending on if initial 25-OHD measurements were determined using an assay that was positively or negatively biased and the level of bias in the old assay.

The way out of the chaos Vitamin D measurement standardization

- An International effort is needed to identify key prior studies with stored samples for re-analysis and standardization, initially, to define the 25-OHD level associated with D deficiency (rickets/osteomalacia).
- Subsequent work could focus on defining inadequacy.
- Suspending publication of meta-analyses based on unstandardized 25(OH)D results.



MAYO CLINIC

Vitamin D Test Expert Discusses Mass Spectrometry

**LC-MS/MS Vitamin 25(OH) D
test results can be**

**“analytically accurate” but
not**

“clinically relevant”

Thanks
singh.ravinder@mayo.edu

