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Application of LC-MS/MS in Clinical Laboratory for Small Molecule Quantification

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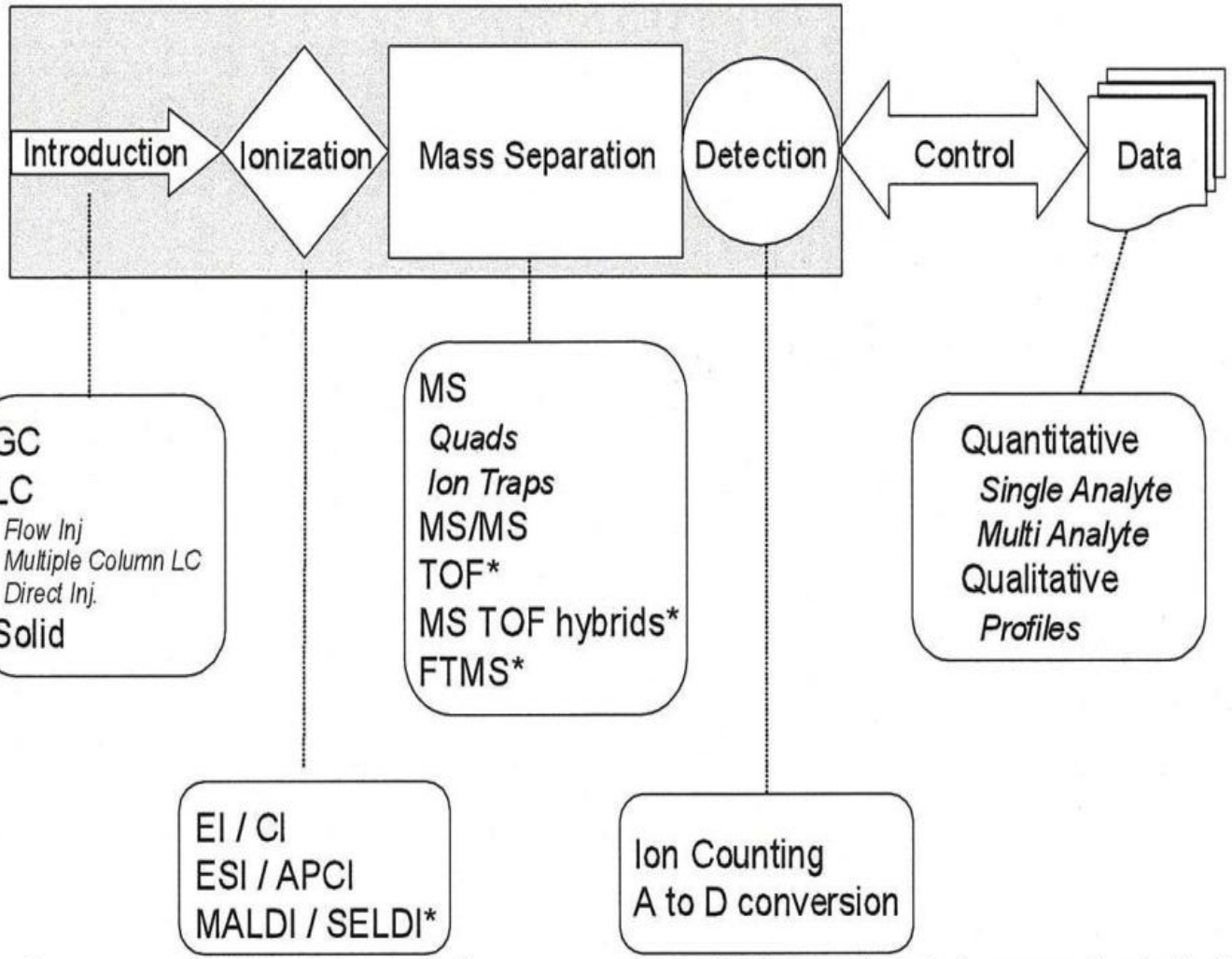
JCST July 23, 2014

Objectives

- To explain the advantages and challenges of LC-MS/MS in quantifying small molecules in patient specimens
- To illustrate unique contributions that LCMS/MS may bring for patient care
  - 25-hydroxyvitamin D
  - 1,25-dihydroxyvitamin D
- To demonstrate how to validate a quantitative assay by LC-MS/MS

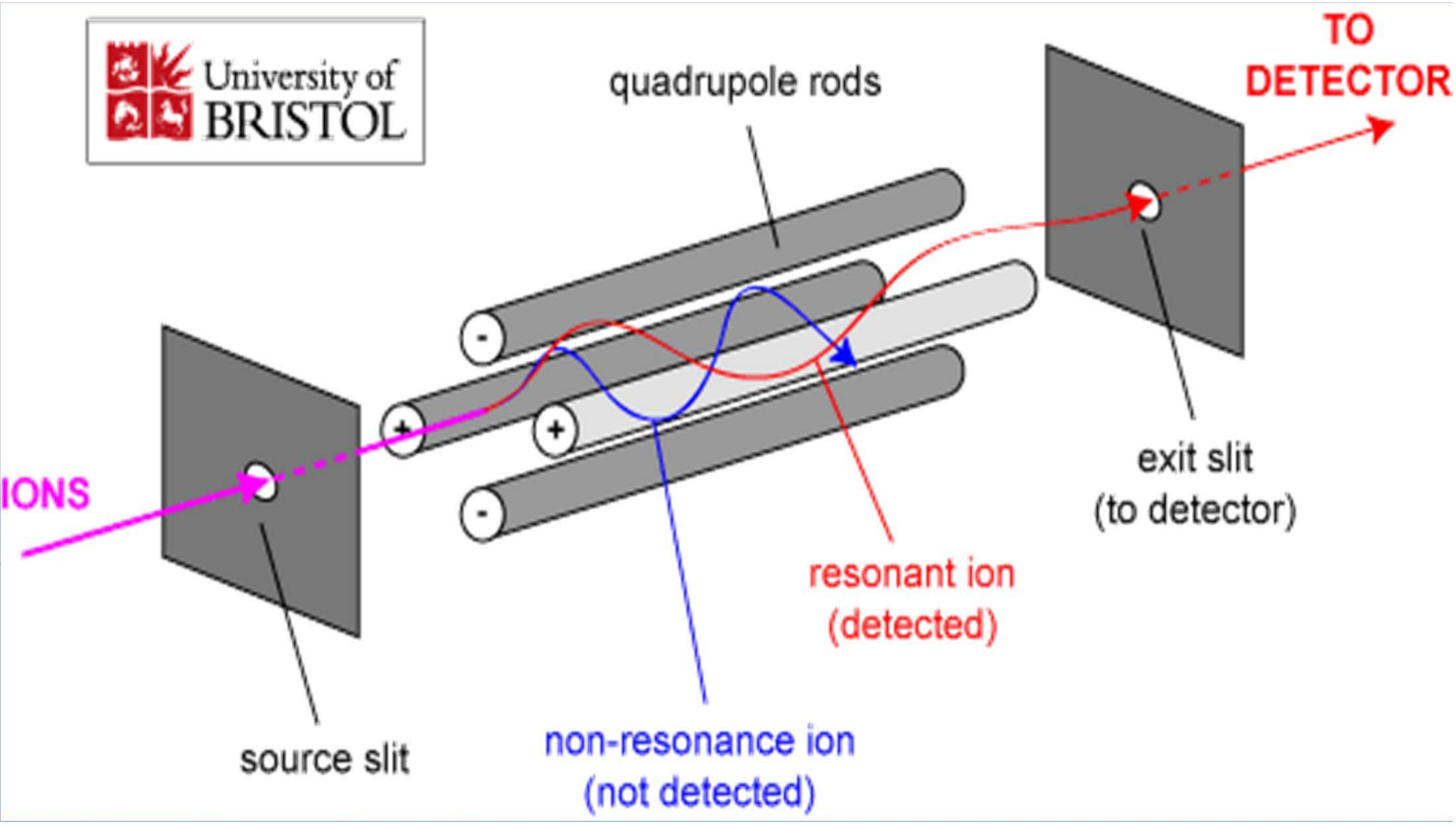


GC	gas chromatography
LC	liquid chromatography
TOF	time of flight
FTMS	Fourier transform mass spectrometry

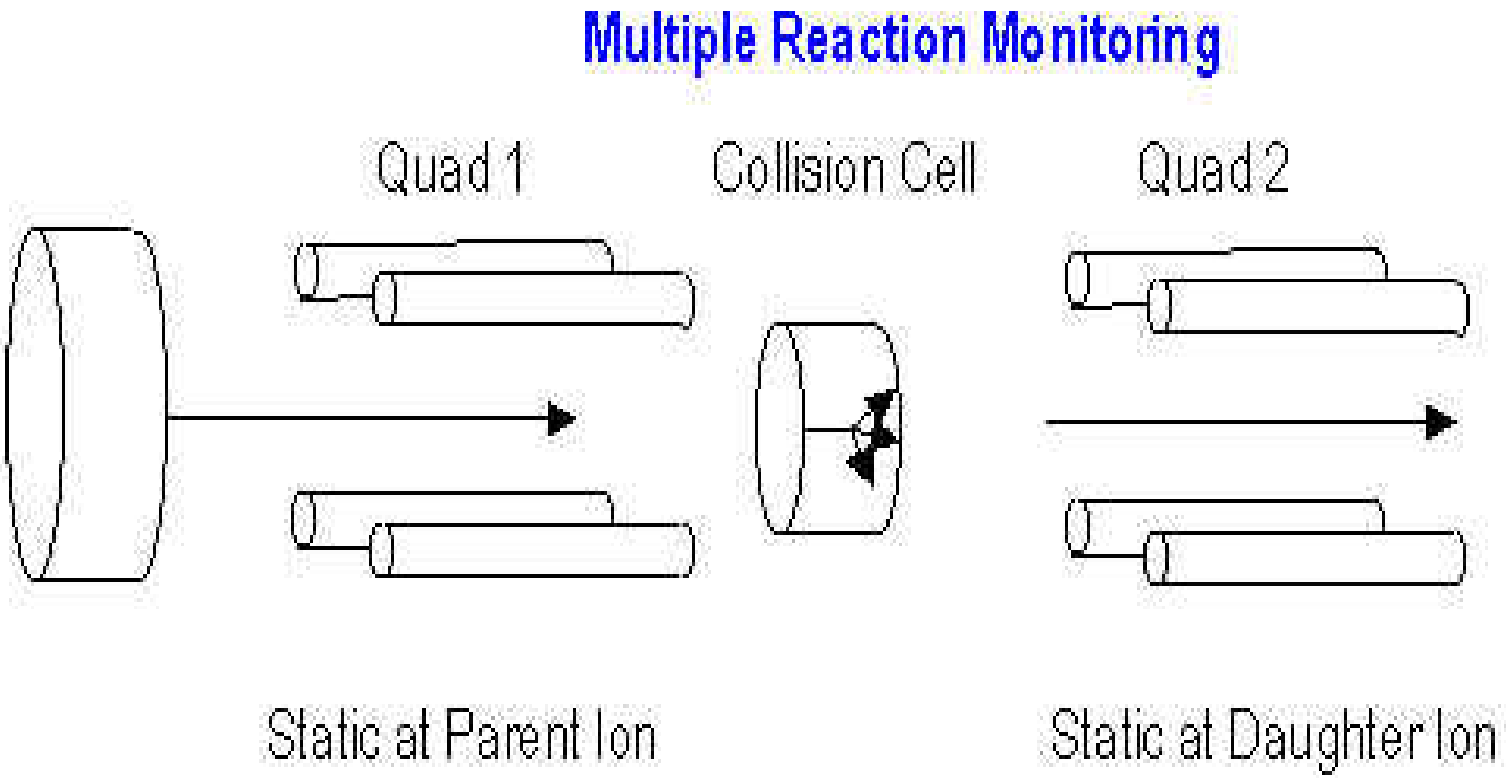
EI	electron ionization
CI	chemical ionization
ESI	electrospray ionization

APCI	atmospheric pressure chemical ionization
APPI	atmospheric pressure photo ionization
MALDI	matrix-assisted laser-desorption ionization
SELDI	surface laser desorption ionization

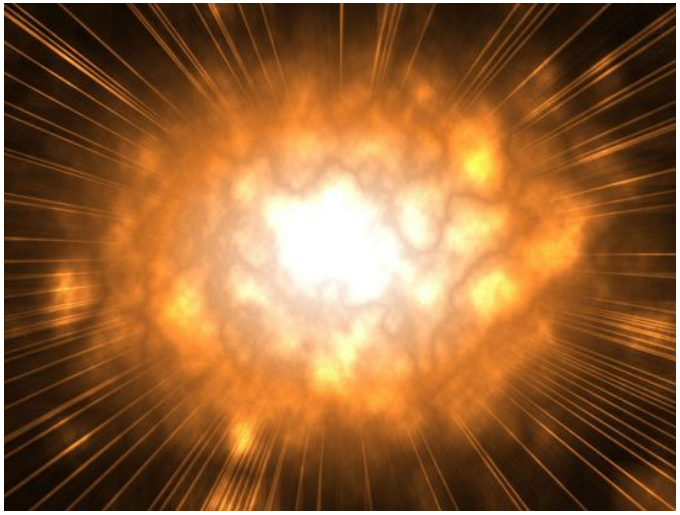
The Quadrupole Mass Analyzer



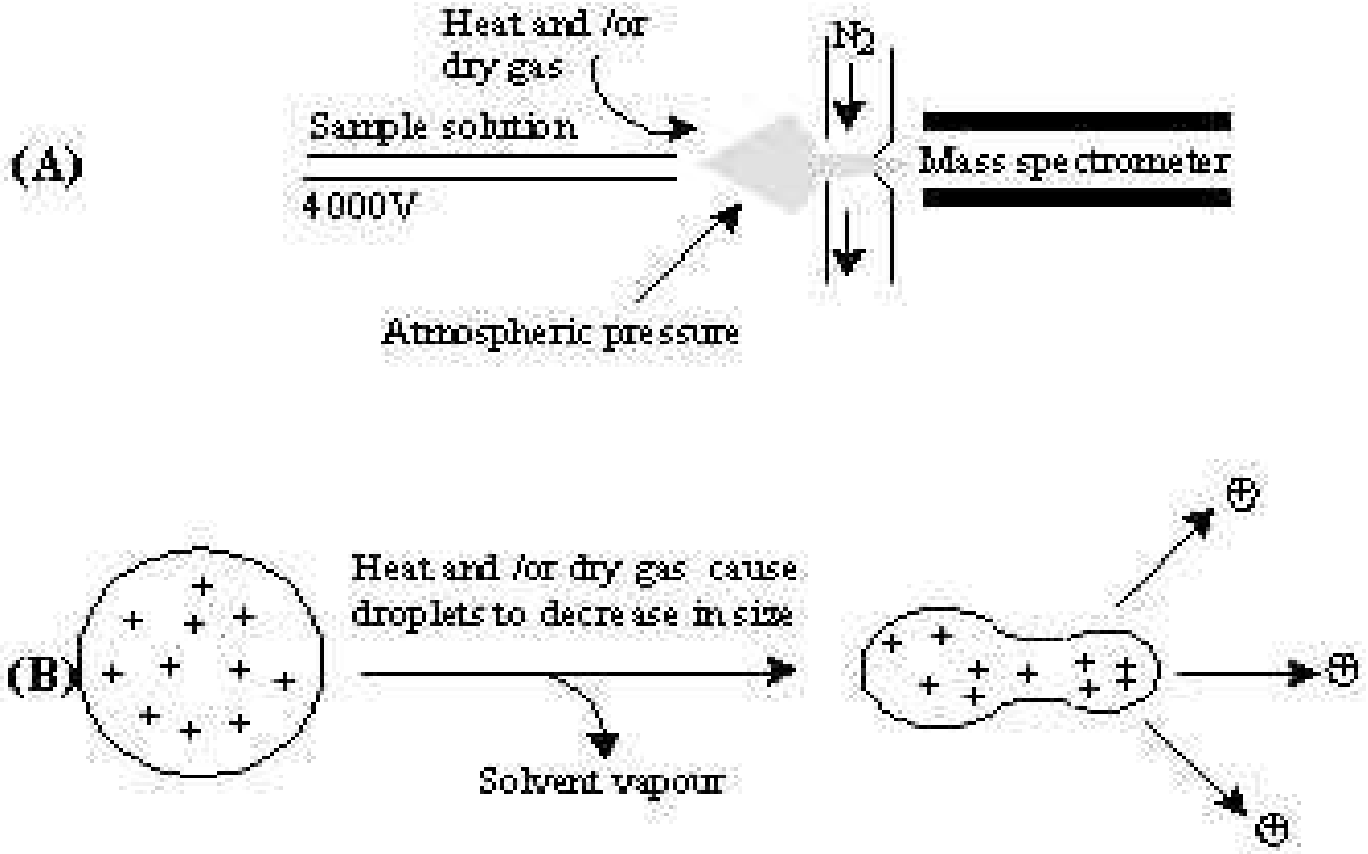
Tandem Mass Spectrometry (MS/MS)



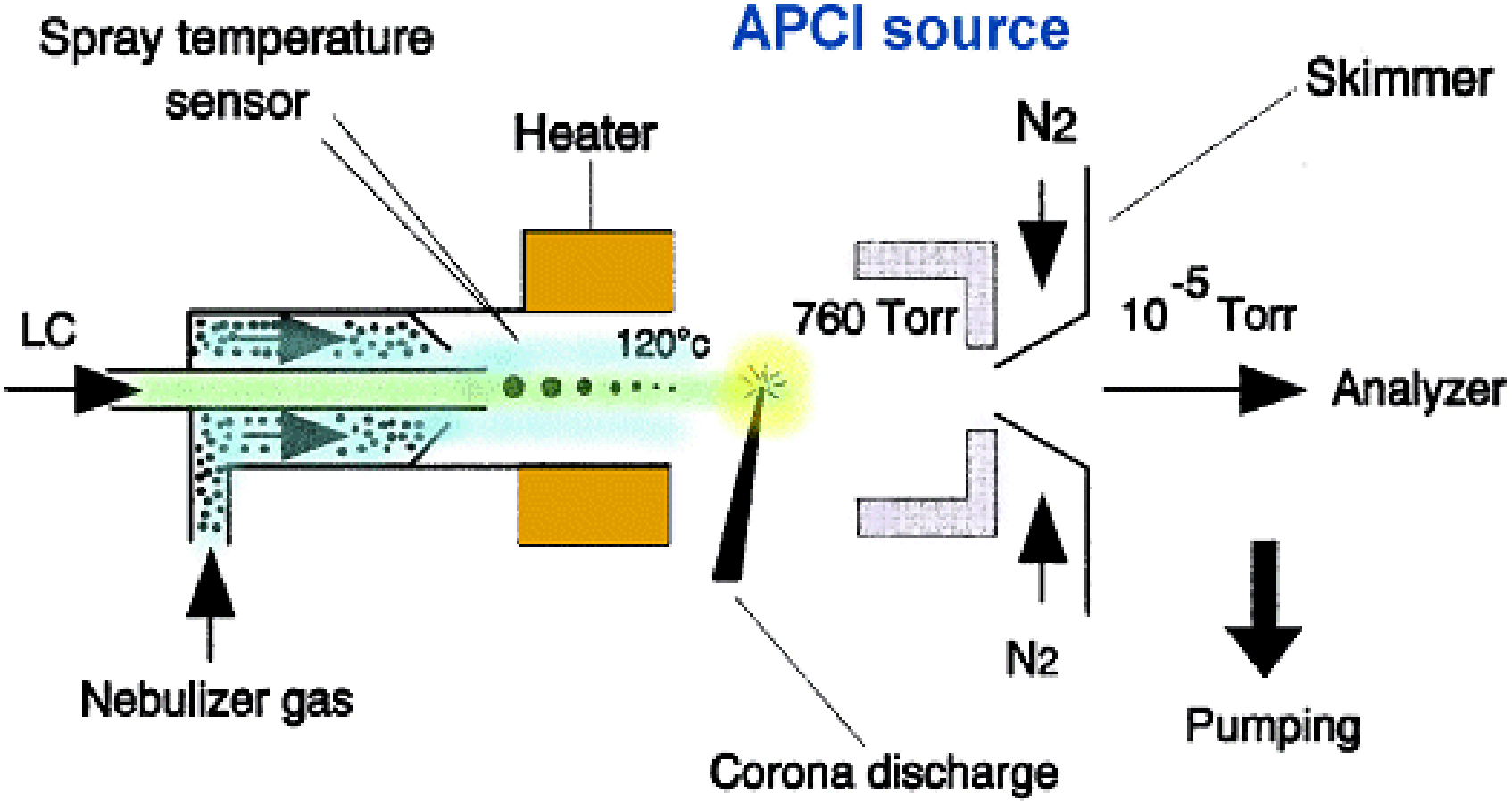
Specificity through MS/MS



ElectrosprayIonization (ESI)



Atmospheric Pressure Chemical Ionization (APCI)



Clinical Applications of LC-MS/MS

- Neonatal screening
- Inborn errors of metabolism
- Toxicology and Drugs of abuse
- Pain management drug testing
- Therapeutic drug monitoring
- Endocrine
- Protein identification and quantitation

Vogeser, Clin Chem Lab Med 2003;41:117-126. Dooley, Clin Biochem 2003;36:471-481.
Vogeser, Clin Biochem 2008;41:649-662. Wu, Clin Chimica Acta 2013.

Advantages of Immunoassays

- Easy to operate
- Low maintenance by laboratory personnel
- Pre-defined performance by manufactures
- Approved by regulatory agencies
- High throughput through automation and parallel reactions

Limitations of Immunoassay Tests for Small Molecule Quantification

- Sensitivity—10-100 pg/mL
- Specificity
  - Structurally similar molecules
  - Human anti-mouse antibodies
  - Heterophilic antibodies
- Hard to develop
- Variation between assays using different antibodies
- Epitopes recognized by different antibodies

Advantages of LC-MS/MS Methods

- High specificity
- High sensitivity (< 1pg/mL)
- Wide range of applicability
  - ➢ Volatility
  - ➢ Polarity
- Assay flexibility
  - ➢ Relatively easy to establish and change
- Information rich detection
  - ➢ Multiple analytes in one run
  - ➢ Structural information

Challenges of Using LC-MS/MS

- Instrument complexity
  - ➢ Hard to learn
- Home brewed assays
  - ➢ Lack of regulatory agency cleared assays
- Throughput is limited by
  - ➢ Analytical cycle time
  - ➢ Sample preparation
  - ➢ Interface with lab information system

Inaccurate Results by LC-MS/MS

- Poor signal stability—appropriate internal standards
- Potential interference by molecules/metabolites with identical MW
- Matrix effects
  - ➢ Reduced/enhanced ionization intensity from co-eluted impurities or matrix
  - ➢ Optimal internal standards
- Cross talk
- Insource transformation

Operational Challenges

- High initial capital investment
  - ➢ Low reagent cost
  - ➢ Long-term investment
- Challenge to maintain high performance of the instruments
  - ➢ Hardware
  - ➢ Methodology
- Manual operation
- No commercial interface
- No strong service support
- No consensus on performance characteristics suitable for clinical use

Clinical and Laboratory Standards Institute

- C50-A: Mass Spectrometry in Clinical Lab
- EP14-A2: Evaluation of matrix effect
- EP7-A2: Interference testing
- EP6-A: Evaluation of linearity
- EP17-A: Limits of detection and quantitation
- EP10-A3: Preliminary evaluation
- EP9-A2: Method comparison

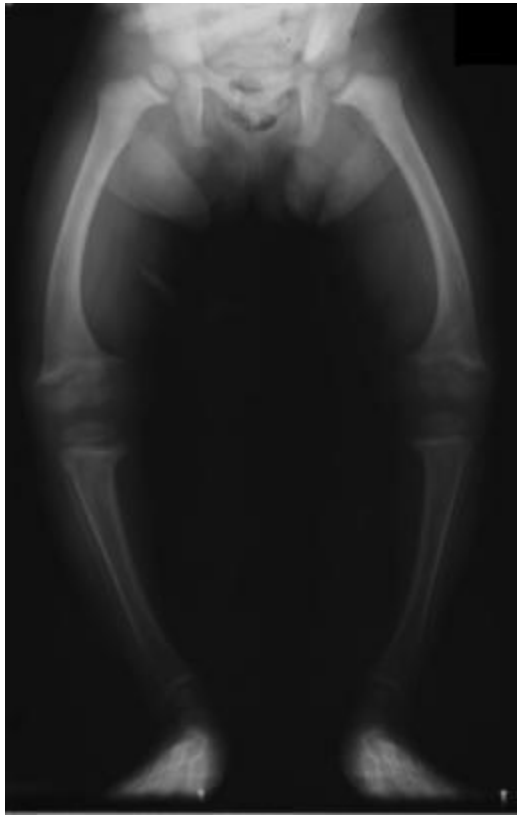
Assay Validation

- Quality of chromatograms
- Matrix effects
- Interferences
- Analytical measurable range
- Accuracy (analytical recovery)
- Carry-over
- Precision
- Method comparison
- Robustness

- **Examples—Vitamin D Metabolites**

Vitamin D Discovery

- In the early 20th century, it was discovered that cod liver oil and sunshine exposure had antirachitic effect
- The antirachitic substance was the 4th vitamin discovered and was called vitamin D
- - Cholecalciferol (D3)
- - Ergocalciferol (D2)



Measure of Vitamin D Nutritional Status

- Lack of data showing free 25(OH)D or 1,25(OH)2D are better indicator of vitamin D status
- - Vitamin D-DBP can be up-taken by cells
- By consensus, the total 25(OH)D is the accepted indicator of D status
- - 25(OH)D is not much regulated and primarily dependent on substrate concentration

- Easily measured (higher concentration)

- Longer half-life (~3 wk) vs 1,25(OH)2D (~4h) and D (~1 d)

Vitamin D in Health and Disease

- Bone metabolism
- Muscle strength
- Cancer
- Cardiovascular disease
- Autoimmune disease
- Diabetes (both type I and II)
- Neurological disorders
- Kidney disease
- Bacterial and viral infections
- Pregnancy outcome
- All-cause mortality

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Dietary Reference Intakes for Calcium and Vitamin D

To help clarify this issue, the U. S. and Canadian governments asked the Institute of Medicine (IOM) to assess the current data on health outcomes associated with calcium and vitamin D. The IOM tasked a committee of experts with reviewing the evidence, as well as updating the nutrient reference values, known as Dietary Reference Intakes (DRIs). These values are used widely by government agencies, for example, in setting standards for school meals or specifying the nutrition label on foods. Over time, they have come to be used by health professionals to counsel individuals about dietary intake.

The committee provided an exhaustive review of studies on potential health outcomes and found that the evidence supported a role for these nutrients in bone health but not in other health conditions. Further, there is emerging evidence that too much of these nutrients may be harmful.

TABLE: Dietary Reference Intakes for Calcium and Vitamin D

	Calcium			Vitamin D		
Life Stage Group	Estimated Average Requirement (mg/day)	Recommended Dietary Allowance (mg/day)	Upper Level Intake (mg/day)	Estimated Average Requirement (IU/day)	Recommended Dietary Allowance (IU/day)	Upper Level Intake (IU/day)
Infants 0 to 6 months	*	*	1,000	**	**	1,000
Infants 6 to 12 months	*	*	1,500	**	**	1,500
1–3 years old	500	700	2,500	400	600	2,500
4–8 years old	800	1,000	2,500	400	600	3,000
9–13 years old	1,100	1,300	3,000	400	600	4,000
14–18 years old	1,100	1,300	3,000	400	600	4,000
19–30 years old	800	1,000	2,500	400	600	4,000
31–50 years old	800	1,000	2,500	400	600	4,000
51–70 year old males	800	1,000	2,000	400	600	4,000
51–70 year old females	1,000	1,200	2,000	400	600	4,000
>70 years old	1,000	1,200	2,000	400	800	4,000
14–18 years old, pregnant/lactating	1,100	1,300	3,000	400	600	4,000
19–50 years old, pregnant/lactating	800	1,000	2,500	400	600	4,000

*For infants, Adequate Intake is 200 mg/day for 0 to 6 months of age and 260 mg/day for 6 to 12 months of age.
**For infants, Adequate Intake is 400 IU/day for 0 to 6 months of age and 400 IU/day for 6 to 12 months of age.

JCEM THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know

A. Catharine Ross, JoAnn E. Manson, Steven A. Abrams, John F. Aloia, Patsy M. Brannon, Steven K. Clinton, Ramon A. Durazo-Arvizu, J. Christopher Gallagher, Richard L. Gallo, Glenville Jones, Christopher S. Kovacs, Susan T. Mayne, Clifford J. Rosen and Sue A. Shapses

Serum 25OHD Levels and Screening

Guidelines regarding the use of serum markers of vitamin D status for medical management of individual patients and for screening were beyond the scope of the Committee's charge, and evidence-based consensus guidelines are not available. However, these issues should be addressed by appropriate federal agencies and professional organizations in light of the findings in this report. As noted above, the Committee recognized that serum 25OHD is a useful integrated marker of vitamin D exposure, incorporating endogenous synthesis from solar exposure, dietary intake from foods, fortified products, and/or supplements, and other factors. However, the Committee also recognized that observational studies of correlations between tus and affected by kidney function. After a careful review of available literature, the Committee concluded that serum 25OHD levels of 16 ng/ml (40 nmol/liter) cover the requirements of approximately half the population, and levels of 20 ng/ml (50 nmol/liter) cover the requirements of at least 97.5% of the population. These levels will be useful to clinicians as they consider management of patients under their care. For upper levels of serum 25OHD, sparse data are available, particularly regarding long-term effects of chronically high concentrations, and a margin of safety for public health recommendations is prudent. Thus, serum 25OHD levels above 50 ng/ml (125 nmol/liter) should raise concerns among clinicians about potential adverse effects.

Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline

Michael F. Holick, Neil C. Binkley, Heike A. Bischoff-Ferrari, Catherine M. Gordon, David A. Hanley, Robert P. Heaney, M. Hassan Murad, and Connie M. Weaver

Boston University School of Medicine (M.F.H.), Boston, Massachusetts 02118; University of Wisconsin (N.C.B.), Madison, Wisconsin 53706; University Hospital Zurich (H.A.B.-F.), CH-8091 Zurich, Switzerland; Children's Hospital Boston (C.M.G.), Boston, Massachusetts 02115; University of Calgary Faculty of Medicine (D.A.H.), Calgary, Alberta, Canada T2N 1N4; Creighton University (R.P.H.), Omaha, Nebraska 68178; Mayo Clinic (M.H.M.), Rochester, Minnesota 55905; and Purdue University (C.M.W.), West Lafayette, Indiana 47907

Summary of Recommendations

1.0 Diagnostic procedure

1.1 We recommend screening for vitamin D deficiency in individuals at risk for deficiency. We do not recommend population screening for vitamin D deficiency in individuals who are not at risk (1|⊕⊕⊕⊕).

1.2 We recommend using the serum circulating 25-hydroxyvitamin D [25(OH)D] level, measured by a reliable assay, to evaluate vitamin D status in patients who are at risk for vitamin D deficiency. Vitamin D deficiency is defined as a 25(OH)D below 20 ng/ml (50 nmol/liter), and vitamin D insufficiency as a 25(OH)D of 21–29 ng/ml (525–725 nmol/liter). We recommend against using the serum 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] assay for this purpose and are in favor of using it only in monitoring certain conditions, such as acquired and inherited disorders of vitamin D and phosphate metabolism (1|⊕⊕⊕⊕).

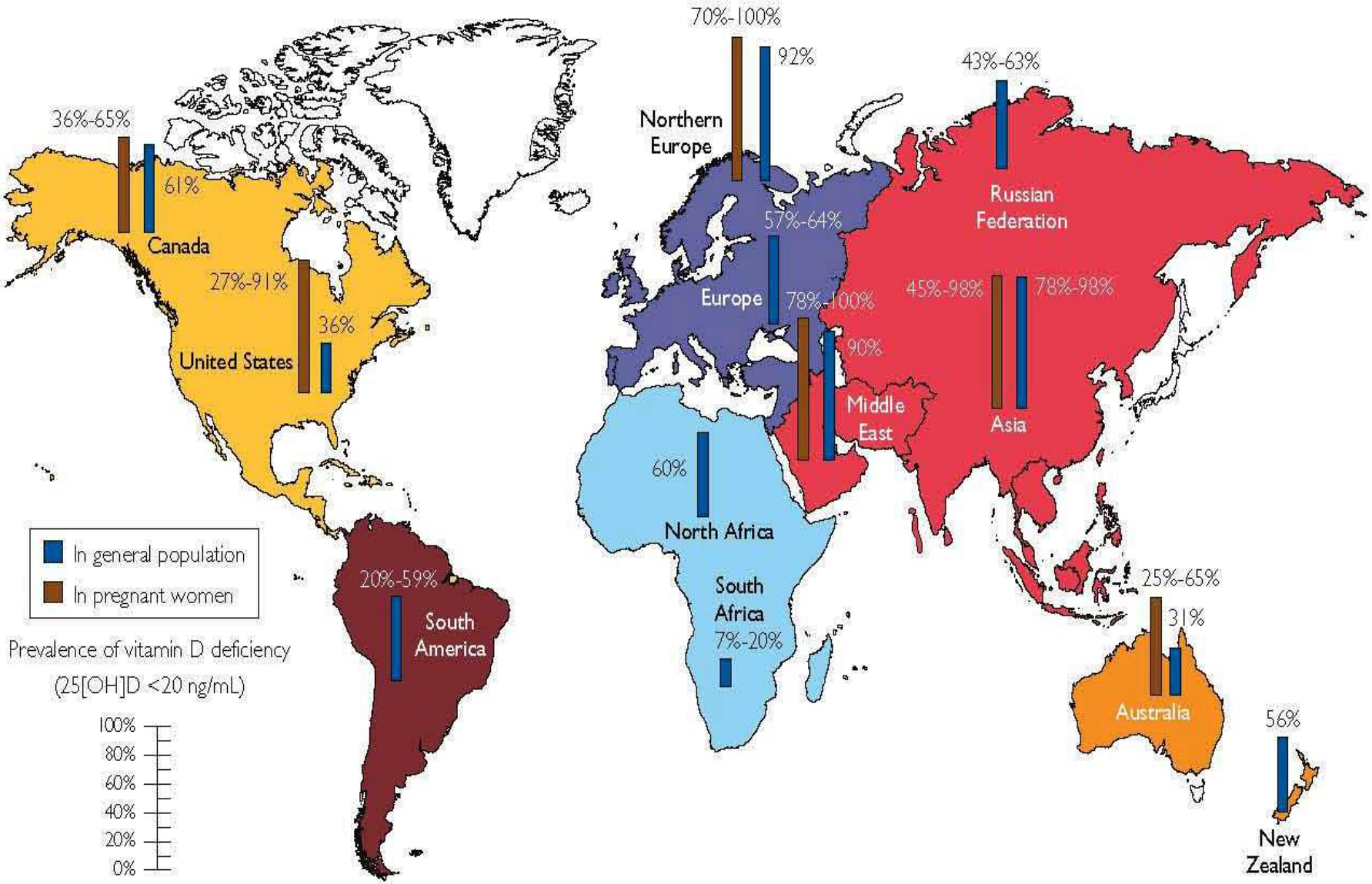


FIGURE 5. Reported incidence of vitamin D deficiency defined as a 25-hydroxyvitamin D (25[OH]D) level below 20 ng/mL around the globe in pregnant women and the general population. To convert 25(OH)D values to nmol/L, multiply by 2.496. Copyright Holick 2013, reproduced with permission.

Available Methods for Measuring Serum 25(OH)D

- Immunoassay
  - Radioimmunoassay (RIA)
  - Enzyme Immunoassay (EIA)
- Protein binding assay
- HPLC
- LC-MS/MS
  - 25(OH)D2 and 25(OH)D3
  - Gold standard

Method Comparison—IA and LC-MS/MS

Scandinavian Journal of Clinical & Laboratory Investigation, 2013; 73: 82–86

informa
healthcare

ORIGINAL ARTICLE

Lack of transferability between different immunoassays and LC-MS/MS for total 25-hydroxyvitamin D measurement and disagreement defining deficiency

IRATXE AJURIA-MORENTIN[1], CARMEN MAR-MEDINA[1], EDURNE BERECIARTUA-URBIETA[1], FERNANDO IZQUIERDO-QUIRCE[1], CARMEN VALLADARES-GÓMEZ[1], ELIA CRESPO-PICOT[1] & TORRRES JAUME[2]

[1]*Clinical Analysis Laboratory, Galdakao-Usansolo Hospital, Galdakao, Bizkaia, Spain, and*
[2]*Spanish National Reference Laboratory, Barcelona, Spain*

Abstract

Background. Over the last few years, it has become much more common to measure concentrations of vitamin D, as its deficiency has been associated with an increasing number of health problems. Recently, a number of new immunoassays for measurement of total 25-hydroxyvitamin D (25OH-D) concentration have been released but their results may not be transferable. ***Methods.*** Our main objective was to compare results from the Cobas® e411 (Roche Diagnostics), Advia Centaur® (Siemens), Architect (Abbott), IDS-iSYS (Vitro S.A.), and Liaison® (Diasorin) immunoassay systems with each other and with liquid chromatography-tandem mass spectrometry (LC-MS/MS). We obtained 184 routine serum samples, covering the whole measuring range, for these methods. ***Results.*** Kappa values above 0.8 were considered to indicate excellent agreement. With a cut-off of 50 nmol/L Architect and Cobas were the only immunoassay methods able to identify patients with deficiencies consistent with the findings of the reference method LC-MS/MS. On the other hand, using a cut-off of 37.5 nmol/L for Liaison and 75 nmol/L for IDS-iSYS, while maintaining the value of 50 nmol/L for the LC-MS/MS method, kappa values of 0.80 and 0.83 respectively were obtained. ***Conclusions.*** Choosing the best method for each laboratory is challenging due to methodological differences between them and 50 nmol/L cannot be considered as a general cut-off for defining hypovitaminosis.

Key Words: *Deficiency, immunoassay, methods, reference levels, serum, vitamin D*

Table I. Passing-Bablok regression results for 178 samples. Results listed in each cell correspond (from top to bottom) to the correlation coefficient, slope (95% CI), intercept (95% CI) and kappa inde for comparisons between all systems.

Reference method	Under study					
	LC MS/MS	Architect	Centaur	Cobas	Liaison	IDS-iSYS
LC–MS/MS						
r		0.900	0.874	0.847	0.839	0.917
slope		0.888 (0.820–0.948)	0.673 (0.614–0.740)	1.159 (1.070–1.260)	0.779 (0.714–0.857)	1.138 (1.057–1.215)
intercept		3.35 (2.20–4.40)	5.028 (3.855–6.024)	−1.525 (−2.926 to −0.097)	0.1 (−1.2–1.1)	4.85 (3.45–6.46)
kappa		**0.861**	0.724	**0.827**	0.633	0.548
Architect						
r	0.900		0.849	0.887	0.903	0.948
slope	1.127 (1.055–1.220)		0.787 (0.718–0.861)	1.323 (1.233–1.421)	0.938 (0.875–1.000)	1.331 (1.261–1.403)
intercept	−3.8 (−5.4 to −2.3)		2.388 (1.124–3.603)	−5.712 (−7.470 to −4.104)	−3.3 (−4.4 to −2.2)	0.97 (−0.51–2.21)
kappa	**0.861**		0.724	**0.848**	0.626	0.605
Centaur						
r	0.874	0.849 1.271 (1.161–1.393)		0.805	0.817	0.857
slope	1.487 (1.351–1630)	−3.04 (−5.02 to −1.31)		1.708 (1.548–1.889)	1.139 (1.042–1.269)	1.688 (1.543–1.856)
intercept	−75 (−9.8 to −5.2)	0.724		−10.026 (−13.379 to −7.446)	−5.5 (−7.5 to 3.8)	−3.60 (−644 to −0.88)
kappa	0.724			0.693	0.651	0.477
Cobas						
r	0.847	0.887	0.805		0.799	0.856
slope	0.863 (0.793–0.935)	0.756 (0.704–0.818)	0.586 (0.529–0.646)		0.682 (0.623–0.755)	1.022 (0.947–1.105)
intercept	1.3 (0.1–2.3)	452 (3.30–5.20)	0.871 (4.811–7.083)		0.8 (−0.3–2.1)	0.37 (4.02–7.55)
kappa	**0.827**	**0.848**	0.693		0.569	0.582
Liaison						
r	0.839	0.903	0.817	0.799		0.833
slope	1.286 (1.167–1400)	1.067 (1.000–1.143)	0.878 (0.788–0.960)	1.465 (1.324–1.604)		1.427 (1.327–1.540)
intercept	−0.1 (−15–1.4)	3.57 (2 54–440)	4.842 (3.695–5.941)	−1.178 (−3.297–0.428)		5.68 (3.92–6.78)
kappa	0.633	0.626	0.651	0.569		0.324
IDS-iSYS						
r	0917	0.948	0.857	0.856	0.883	
slope	0.879 (0.823–0.946)	0.751 (0.713–0.793)	0.592 (0.339–0.648)	0.979 (0.905–1.056)	0.701 (0.649–0.753)	
intercept	4.3 (−6.1 to −2.8)	−0.73 (−1.76–0.36)	2.133 (0.571–3.469)	−6.230 (−7.970 to −4.180)	−4.0 (−5.1 to −2.5)	
kappa	0.548	0.605	0.477	0.582	0.324	

Clin Chem Lab Med 2011;49(3):555–558 © 2011 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2011.072

Letter to the Editor

Cross-reactivity of 25-hydroxy vitamin D2 from different commercial immunoassays for 25-hydroxy vitamin D: an evaluation without spiked samples

- Healthy volunteers from the laboratory staff
- 100,000 IU of vitamin D3 as four ampoules of Dcure (S.M.B., Brussels, Belgium) (Liege group; n=7). Samples were collected at day 1, 7
- 600,000 IU of vitamin D2 as a single vial of Sterogyl 15 (DB Pharma, La Varenne Saint-Hilaire, France) (Paris group; n=11). Samples were collected at day 0, 7, and 28

25-Hydroxyvitamin D2 Recovery

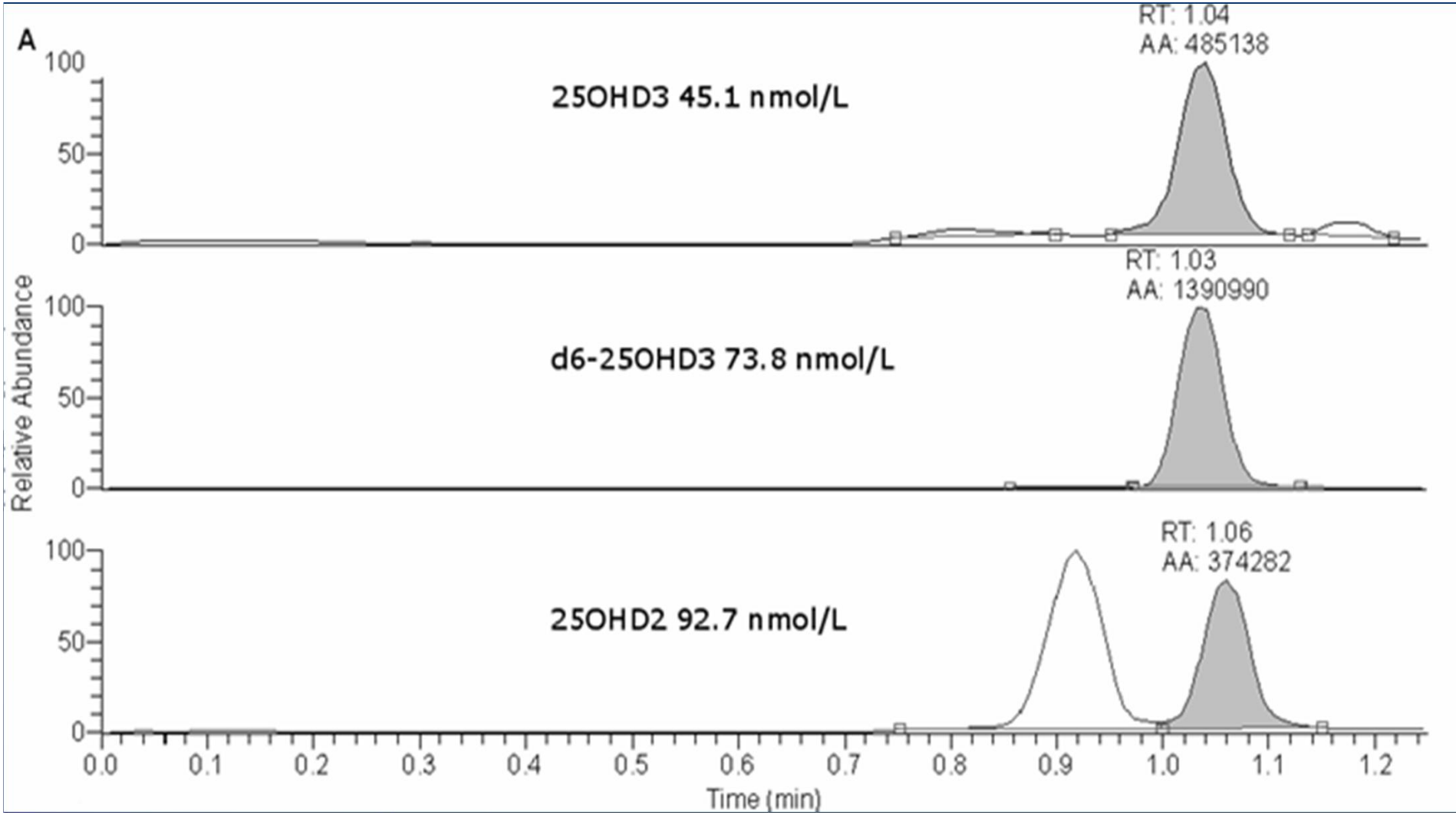
Table 2 25(OH)D concentrations measured with the seven methods in the various groups of subjects. The percent increase in the 25(OH)D concentration after supplementation with 600,000 IU vitamin D2 (columns five and six) are calculated as [25(OH)D at day 7 or day 28 − 25(OH)D at day 0]/25(OH)D at day 0. The percent cross-reactivity for 25(OH)D2 is the sum of the $r'_m = (y_m - R_m X_3)/X_2$ calculated in each sample of the D2 group (see text).

	25(OH)D in ng/mL in the D3 group mean ± SD n = 24	25(OH)D in ng/mL in the D2 group mean ± SD n = 19	25(OH)D in ng/mL in the Paris group at day 0 mean ± SD n = 11	25(OH)D in ng/mL in the Paris group at day 7 mean ± SD (mean% increase from day 0) n = 11	25(OH)D in ng/mL in the Paris group at day 28 mean ± SD (mean% increase from day 0) n = 9	Percent cross-reactivity for 25(OH)D2 mean ± SD in % (inter-quartile range)
LC-MS/MS total	36.3 ± 10.3	64.2 ± 23.0	27.8 ± 6.0	73.1 ± 11.9 (+162.9%)	51.4 ± 8.0 (+84.9%)	NA
Liaison	33.6 ± 9.4	67.9 ± 31.6	27.5 ± 7.8	81.3 ± 21.1 (+195.6%)	44.0 ± 7.1 (+60%)	100.5 ± 33.9 (78.4–113.6)
DiaSorin RIA	35.3 ± 11.9	63.7 ± 25.2	29.3 ± 6.8	77.0 ± 19.7 (+162.8%)	44.4 ± 7.4 (+51.5%)	95.8 ± 29.0 (76.3–119.6)
Elecsys	33.9 ± 7.6	24.6 ± 5.8	30.2 ± 6.0	27.4 ± 6.0 (–9.3%)	19.8 ± 4.0 (–34.4%)	14.8 ± 9.6 (–5.9–24.6)
IDS RIA	39.9 ± 10.5	64.3 ± 17.8	32.5 ± 7.8	78.1 ± 5.3 (+140.0%)	50.3 ± 3.6 (+54.8%)	93.4 ± 17.7 (77.8–104.2)
IDS EIA	36.1 ± 8.4	75.5 ± 26.0	30.8 ± 7.8	98.3 ± 19.9 (+219.2%)	54.8 ± 4.8 (+77.9%)	130.4 ± 29.1 (103.8–147.3)
iSYS	38.6 ± 9.4	65.1 ± 21.6	33.1 ± 8.5	83.1 ± 16.5 (+151.1%)	48.0 ± 3.8 (+45.0%)	107.8 ± 26.0 (91.1–128.2)

Cleveland Clinic LC-MS/MS Method

- Minimal sample preparation
  - Protein precipitation with acetonitrile
  - Online turbulent flow extraction
- Gradient LC method on a polar end-capped C18 column in 5.5 min
- MS in positive APCI mode
  - 401.3→383.2 m/z for 25OHD3, 413.3→395.0 for 25OHD2, and 407.3→389.2 m/z for d6-25OHD3

Chromatogram

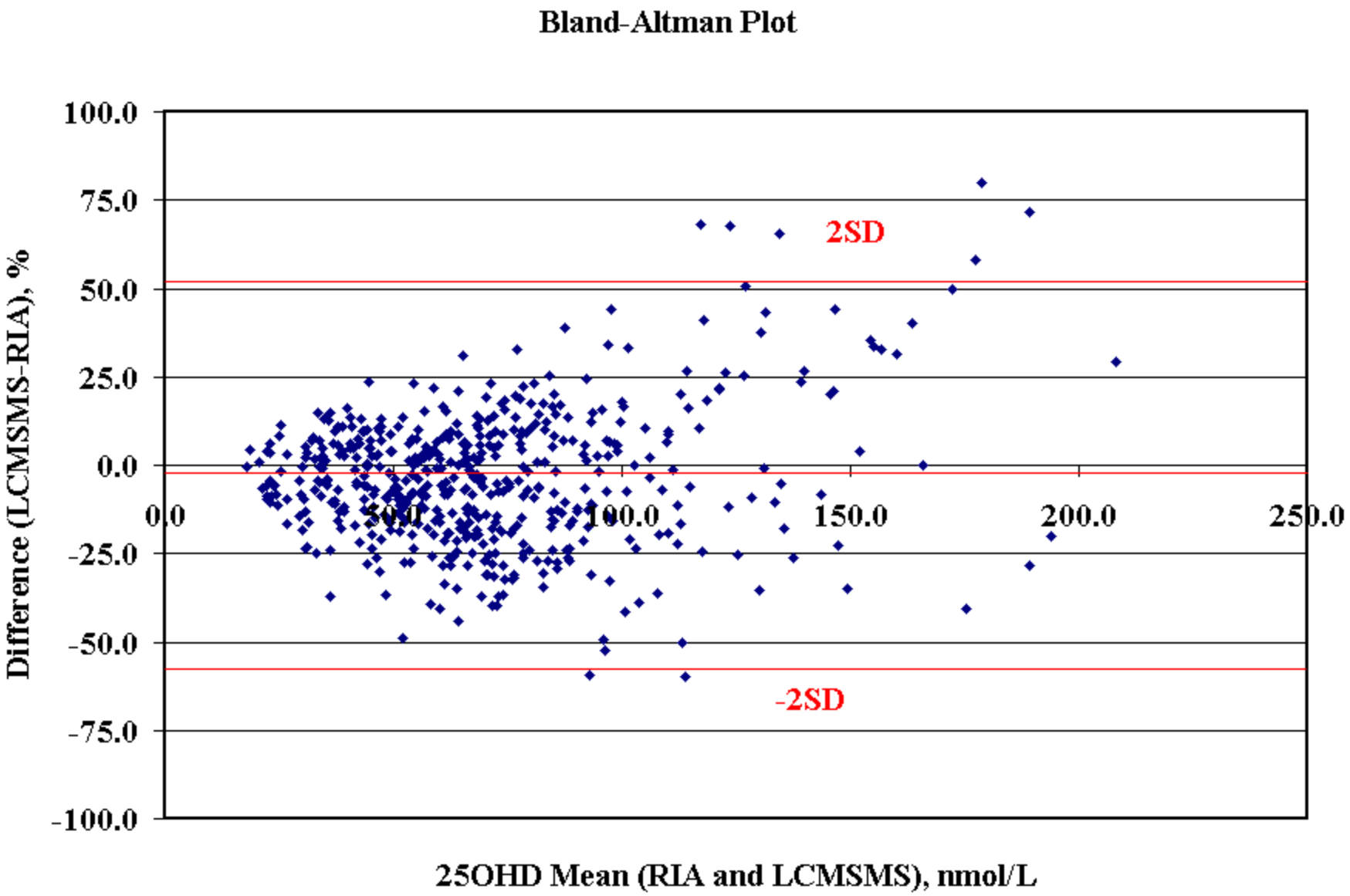


Analyte	Add-In (nmol/L)	Expected (nmol/L)	Mean (nmol/L)	Analytical Recovery	%CV
25OHD2	2.36	2.39	2.98	125.0%	29.2%
	4.73	4.78	4.60	96.2%	4.3%
	7.10	7.18	7.20	100.3%	7.5%
	9.46	9.57	9.45	98.8%	6.9%
	18.93	19.13	18.78	98.1%	5.3%
	37.86	38.26	36.66	95.8%	5.0%
	75.72	76.53	72.57	94.8%	4.8%
	151.44	153.06	147.15	96.1%	3.0%
	302.88	306.12	277.92	90.7%	1.8%

25OHD3	2.44	2.49	2.95	118.7%	14.5%
	4.87	4.98	5.42	109.0%	10.2%
	7.31	7.47	7.81	104.7%	3.1%
	9.75	9.96	10.43	104.9%	2.3%
	19.50	19.92	18.89	94.7%	3.9%
	39.00	39.84	37.94	95.1%	1.4%
	78.00	79.68	72.63	91.0%	1.1%
	156.00	159.37	144.82	90.7%	2.3%
	312.00	318.74	283.55	88.7%	2.3%

Precision (CLSI EP-10A2)

	25OHD3			25OHD2		
	LOW	MID	HIGH	LOW	MID	HIGH
Mean (nmol/L)	**33.4**	**61.0**	**120.5**	**18.5**	**70.6**	**139.9**
Total SD	**3.0**	**4.6**	**11.4**	**3.1**	**7.8**	**15.6**
Intra-assay SD	**1.6**	**2.3**	**7.4**	**1.7**	**3.2**	**9.0**
Inter-assay %CV	**7.7**	**6.6**	**7.1**	**14.2**	**10.0**	**9.2**
Intra-assay %CV	**4.9**	**3.8**	**6.1**	**9.3**	**4.6**	**6.4**


Bland-Altman Plot
100.0
75.0
50.0
25.0
0.0
-25.0
-50.0
-75.0
-100.0
0.0
50.0
100.0
150.0
200.0
250.0
2SD
-2SD
Difference (LCMSMS-RIA), %
25OHD Mean (RIA and LCMSMS), nmol/L

When to Measure 1,25-Dihydroxyvitamin D Clinically?

- Hyper- or hypo-parathyroidism
- Chronic kidney disease
  - PTH suppression
  - Compliance with 1,25(OH)D therapy
- Vitamin D-dependent rickets
  - Types I: low conversion of vitamin D to 1,25(OH)D
  - Type II: resistance to 1,25(OH)D
- Others
  - Sarcoidosis

Radioimmunoassay for Measuring 1,25-Dihydroxyvitamin D

- Extract sample with acetonitrile, centrifuge and decant
- Purify pretreatment solution by C18 column
- RIA procedure including pipetting, centrifugation, decanting and reading on the gamma counter
- Large variation
- Interference
- At least 1-day long procedure

Cleveland Clinic LC-MS/MS Method

- Triple quadrupole mass spectrometer
- Lithium adduct monitored (0.5 mM lithium acetate in mobile phase)
- Onyx monolithic C18 columns (100 x 3.0mm)
- Total run time 10 minutes
- Calibrators in charcoal stripped serum down to LOQ level
- Validation performed in real samples

Removing Interferences

Things we tried and failed

- LC gradient
- Selection of other MRMs
- Two Onyx monolithic

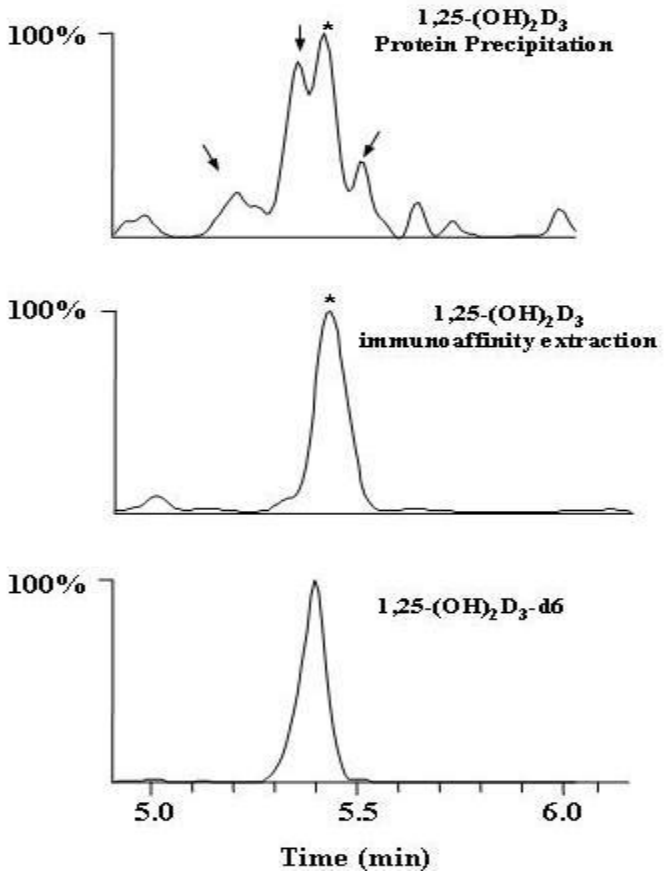
columns in tandem

- Addition of a SPE
- Derivatization with PTAD
- Turbulent flow online extraction

What worked robustly

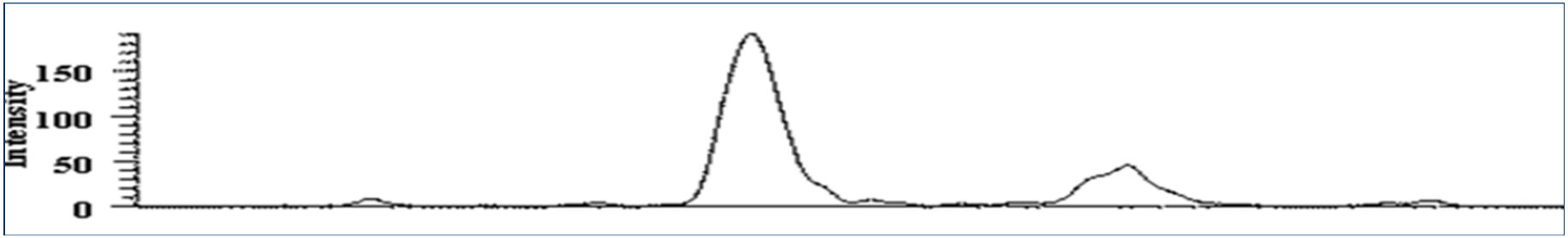
- 500 µL serum immunoaffinity extraction of serum samples

Example chromatograms (arrows: interference peaks)

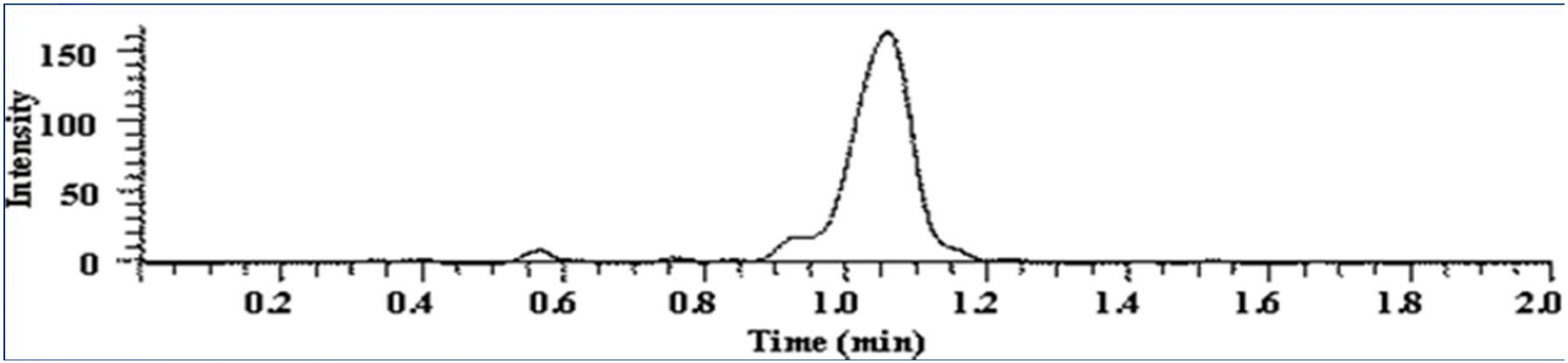


Representative Chromatograms of Samples with Low 1,25(OH)2D

- **Sample 26: 6.1 pg/mL 1,25(OH)2D3**

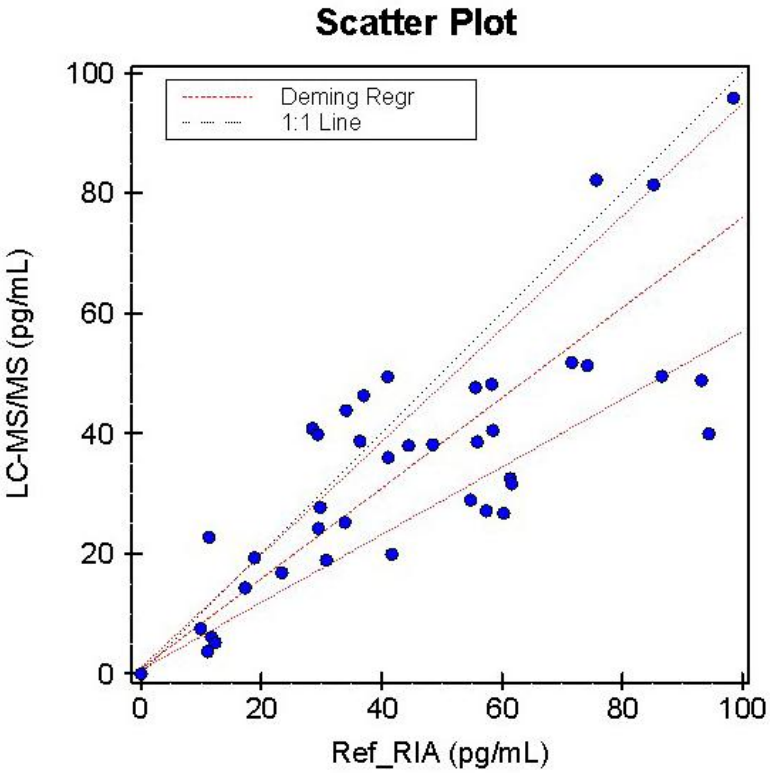


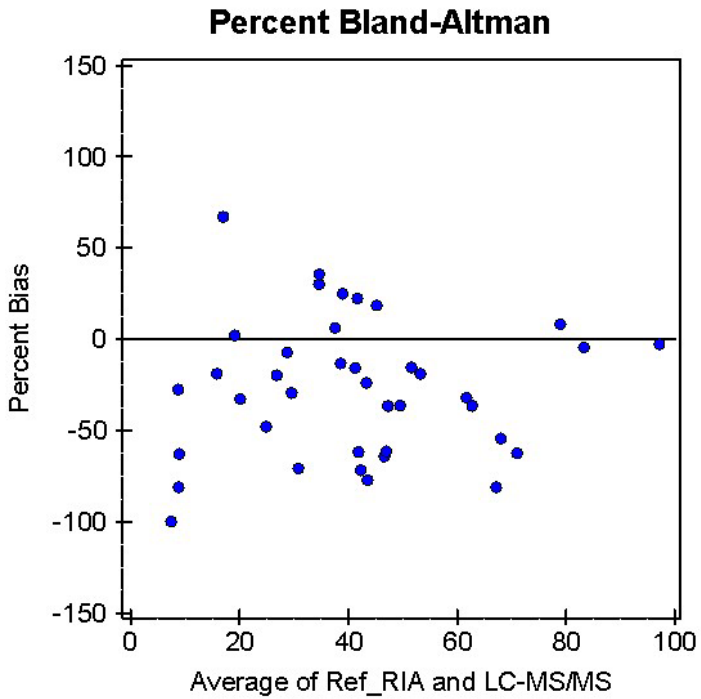
- **Sample 29: 12.1 pg/mL 1,25(OH)2D2**



Correlation with an RIA

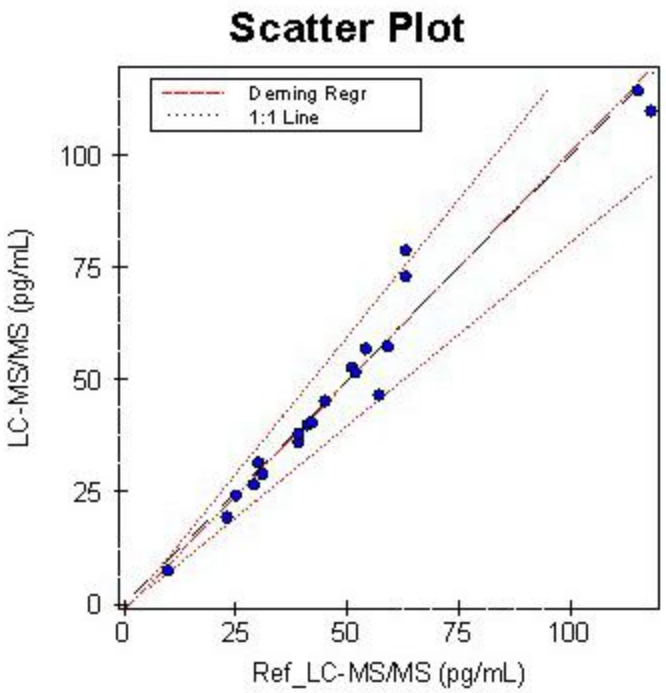
Slope=0.751 R=0.79 N=40

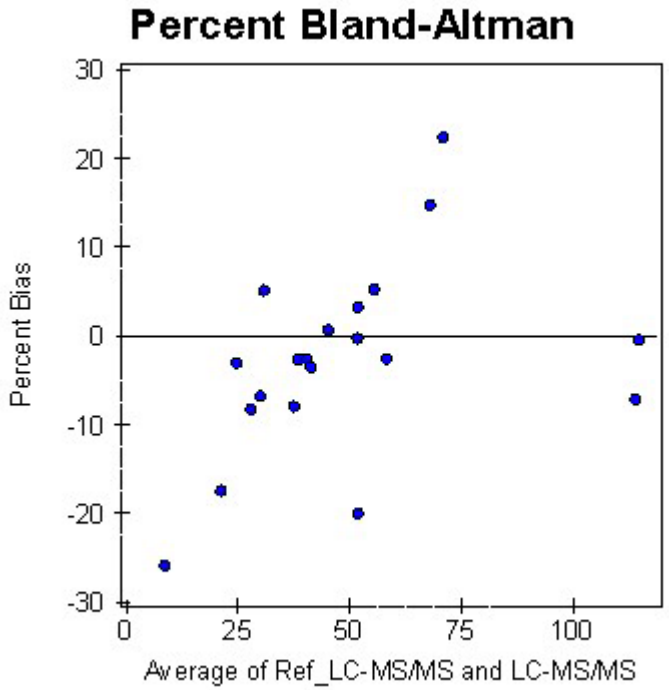




Correlation (Total) with a Commercial LC-MS/MS

Slope=1.02 R=0.98 N=20





Future of LC-MS/MS in Clinical Lab

- Automate sample preparation
  - ➢ Bar code reader
  - ➢ Hands off extraction
- Reduce solvent consumption, improve throughput
  - ➢ Low-flow LC coupled with MS of high sensitivity and scan speed
- Reduce transcription errors
  - ➢ Interface between sample preparation, LC-MS/MS, and LIS

Conclusion

- **LC-MS/MS has high sensitivity and specificity**
- **LC-MS/MS offers unique contributions to patient care**
- **There are challenges utilizing LC-MS/MS for clinical testing**
- **Vigorous validation of LC-MS/MS methods is warranted for patient care**
- **Important to collaborate with manufactures to improve the current technologies**

Chromatography & Separation Techniques Related Journals

- Journal of Bioanalysis & Biomedicine

- Journal of Analytical & Bioanalytical Techniques

Chromatography & Separation Techniques Related Conferences

- 6th International Conference and Exhibition on Analytical & Bioanalytical Techniques

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