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DOUGLASS HANLY MOIR PATHOLOGY

RCPAQAP
RCPA Quality Assurance Programs

Supporting the Validity of Vitamin D Assays Using Reference Values and CRMs

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National Measurement Institute

Australian Government Department of Industry

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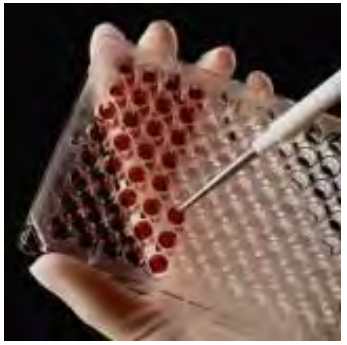
Pathology Testing in Australia

- Pathology testing provides patients and doctors with:
  - 70% of all medical diagnoses
  - every cancer diagnosis



- Pathology testing enables the diagnosis, management and prevention of many diseases
  - diabetes, cardiovascular disease, arthritis, hepatitis and HIV

Australian Association of Pathology Practices,
"Review of Funding Arrangements of Pathology Services", May 2010

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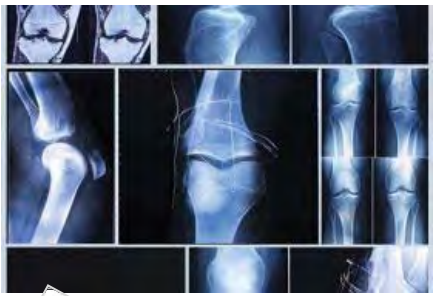
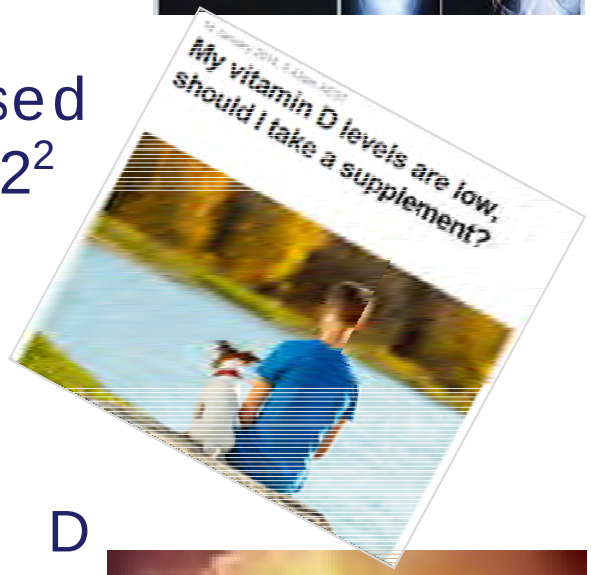
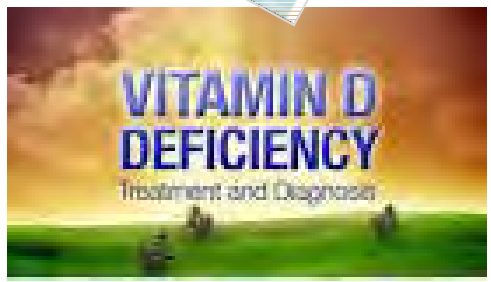
Vitamin D: A Public Health Issue

- The cost of vitamin D testing to Medicare rose from $1.02 million to over $140 million between 2000 and 2012[1]
  - ~4 million Australians were diagnosed with vitamin D deficiency in 2011-2012[2]





- Concerns have been raised over:
  - **the accuracy of testing methods**[2, 3]
  - the true prevalence of vitamin D deficiency
  - the cost-effectiveness of vitamin D tests



1. R. Lucas and R. Neale, Neurology, 2014, 43[3]: 119-122
2. http://www.medicalobserver.com.au/news/four-million-australians-deficient-in-vitamin-d (www.medicalobserver.com.au/news)
3. The Royal College of Pathologists of Australasia, Position Statement, May 2013, Chemical Pathology Advisory Committee, No.: 1/2013

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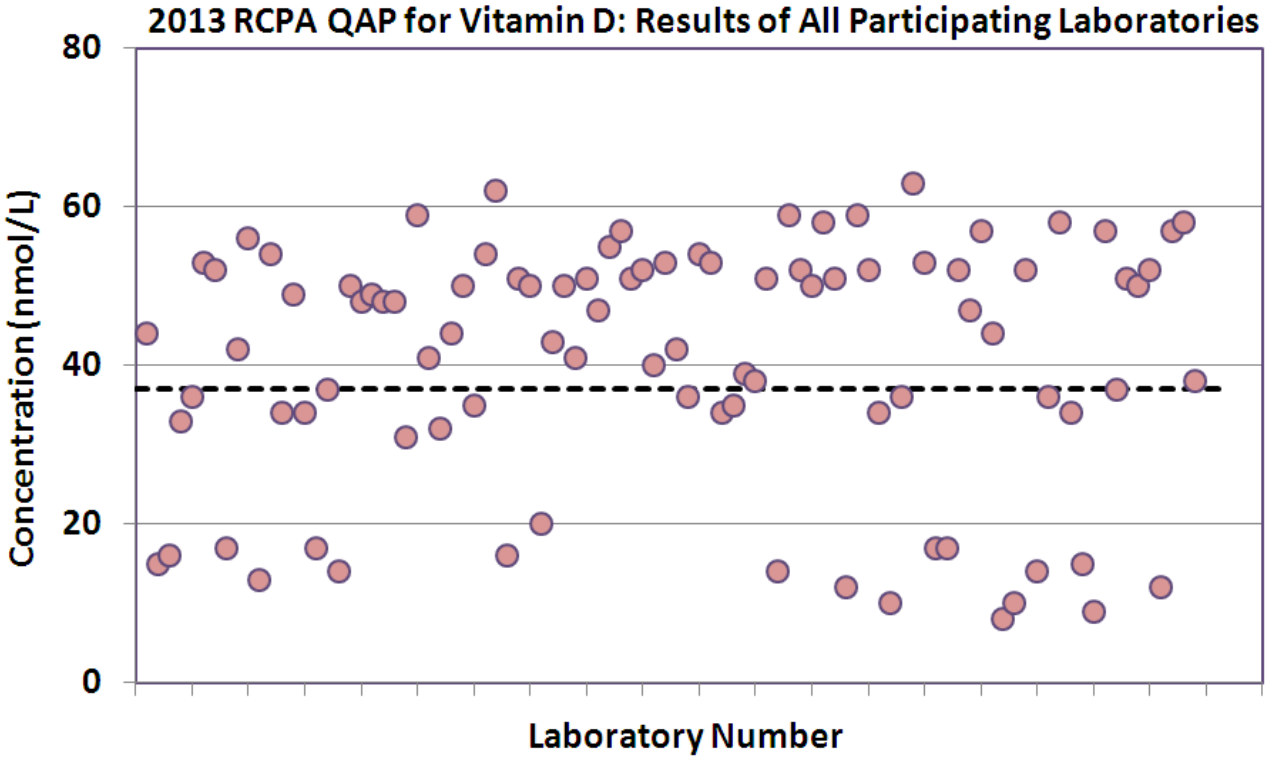
Measuring Vitamin D

- **Immunoassays** (IA) are primarily used for vitamin D analysis
  - laboratories moving towards **LC-MS/MS** technology

High degrees of accuracy and precision are not always met with existing assays



Sample E13-2 @ 37 nmol/L

Lowest result: 8 nmol/L
Highest result: 63 nmol/L

Results taken from 2013 RCPAQAP Cycle EN40

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Challenges in Vitamin D Analysis

- **25-hydroxyvitamin D3 (25OHD3)** is the most abundant, circulating metabolite of vitamin D in blood serum

- Multiple homologous metabolites (e.g. 3-epi-25-hydroxyvitamin D3) can cause **cross-reactivity** in IA and **matrix interferences** in LC-MS/MS

- Lipids in serum can cause **matrix effects** in IA and LC-MS/MS

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Global Standardisation of Vitamin D Tests

- **Vitamin D Standardisation Program (VDSP)**
  - National Institutes of Health (**NIH**), Centers for Disease Control and Prevention (**CDC**), National Center for Environmental Health (**NCEH**), National Institute of Standards and Technology (**NIST**) and **Ghent University**, Belgium

    - ensure that measurements are accurate and equivalent regardless of the analysis method or laboratory used

- The **ANU**, **ABS**, **UWA**, **Douglass Hanly Moir Pathology** and the **NMI** are VDSP participants

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Standardising Vitamin D Measurements in Australia

- A collaborative project between:
  - **National Measurement Institute (NMI)**
  - **Douglass Hanly Moir Pathology (DHM)**
  - **RCPA QAP**

- **Part 1:** Development of a Reference Measurement Procedure (RMP) for vitamin D in serum
- **Part 2:** Application of RMPs in the validation of routine laboratory methods
- **Part 3:** Reference values for target-setting in external quality assurance programs using RMPs

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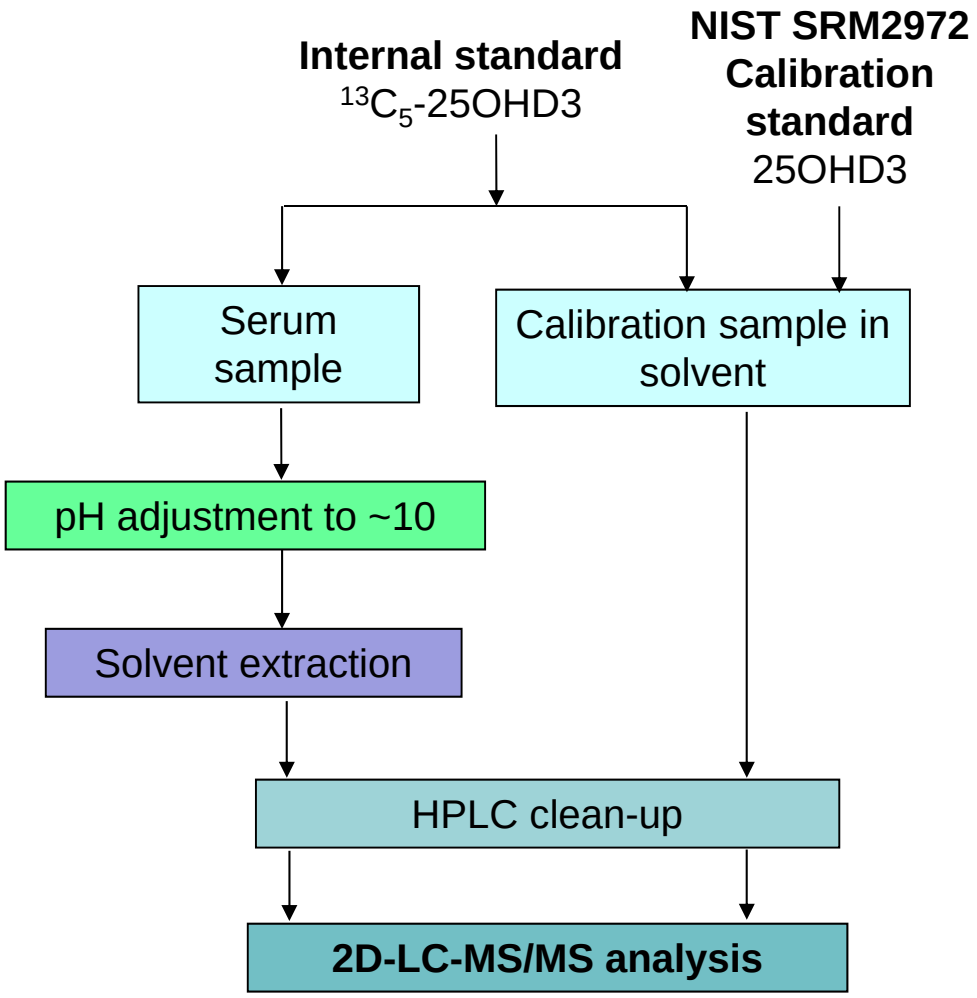
Part 1: A Reference Measurement Procedure (RMP) for Vitamin D Analysis

- A benchmark method that represents the best analytical capability available
  - measurements with experimentally demonstrated negligible systematic error and high precision that are the best estimate of the "true value"



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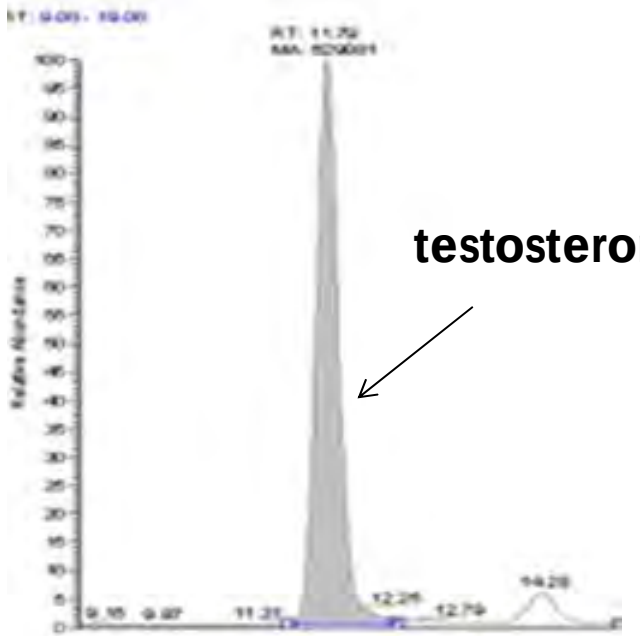
Achieving High Accuracy in RMPs

- Gravimetric sample preparation
  - Isotope-dilution mass spectrometry (IDMS)
    - Exact-matching single-point calibration
      - **Two-dimensional liquid chromatography**

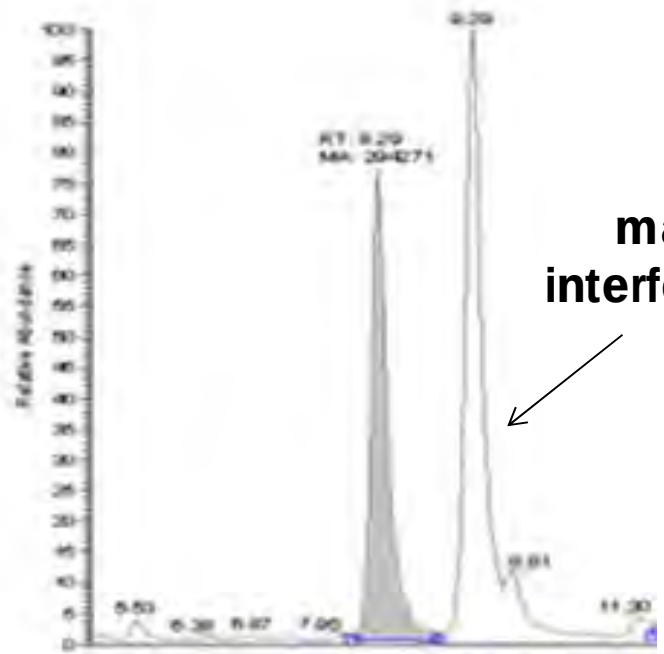
Matrix Interferences

- **Isomeric and isobaric matrix interferences** cannot be resolved by mass spectrometry
  - they produce the same fragment ions as the target compound in the mass spectrometer

BEH Shield: 1st column



BEH Phenyl: 2nd column



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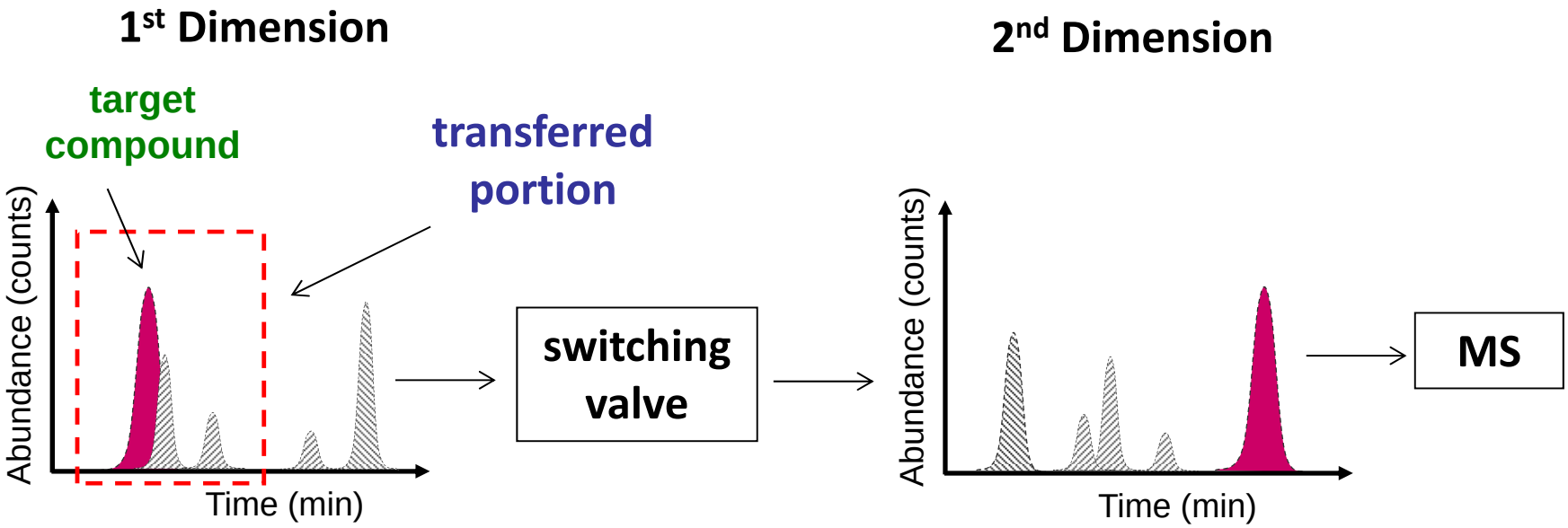
Two-Dimensional Liquid Chromatography

- Coupling of two chromatography systems of very different selectivity
- Compounds elute in a different order in the 1st and 2nd dimensions



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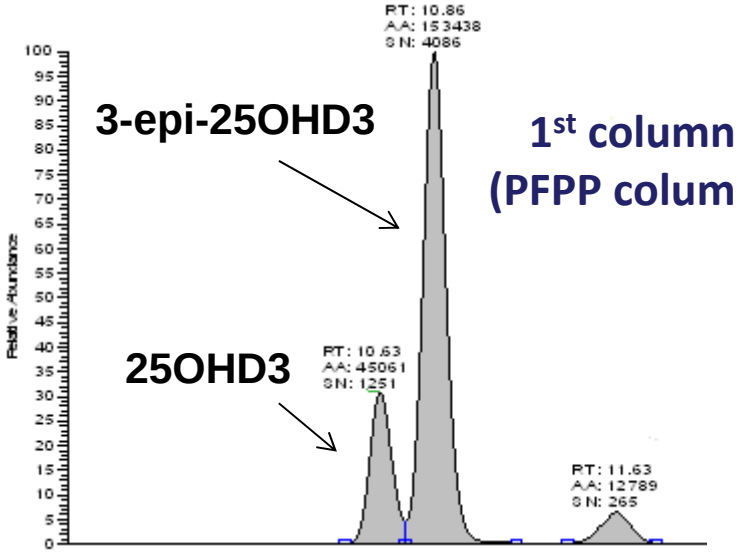
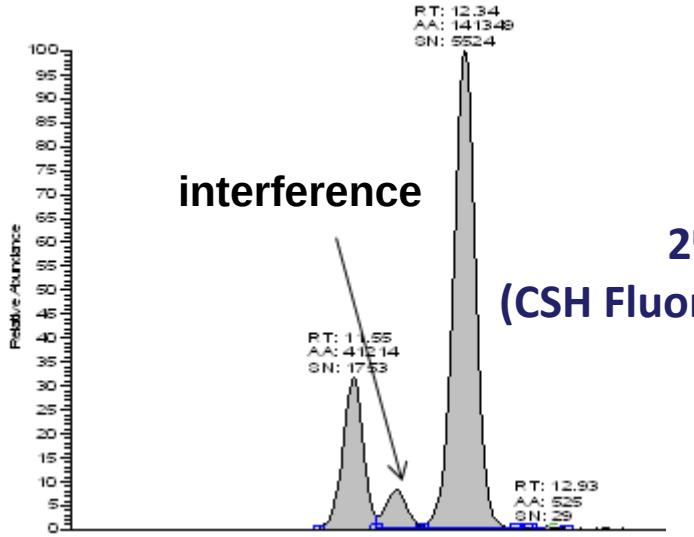
Separation of Interferences by 2D-LC

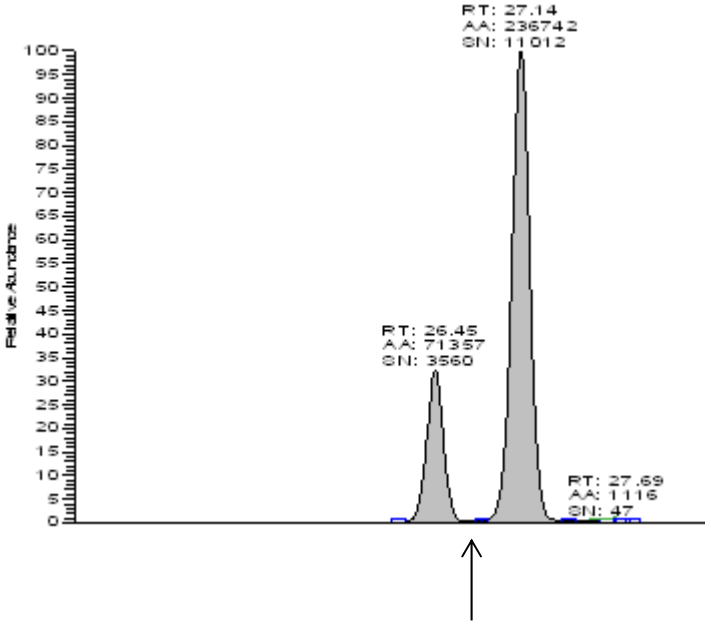


1st column
(PFPP column)



2nd column
(CSH Fluoro-Phenyl column)

2D-LC analysis
(PFPP (1st dimension) & CSH
Fluoro-Phenyl (2nd dimension)



interference removed
with complete separation
of vitamin D metabolites!

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Analysis of Matrix CRMs (NIST SRM972a)

SRM972a consists of 4 serum materials: Levels 1 to 4

	Concentration (nmol/L)			
Level	**Measured value**	**Certified value**	**Mean difference**	**Significant difference?**
1	73.9	71.8 ± 2.7	3.1%	**No**
2	45.3	45.1 ± 1.1	0.3%	**No**
3	49.9	49.4 ± 1.1	0.7%	**No**
4	75.2	73.4 ± 2.4	2.6%	**No**

Differences were assessed for statistical significance at a level of confidence of 95%
Three replicate analyses performed

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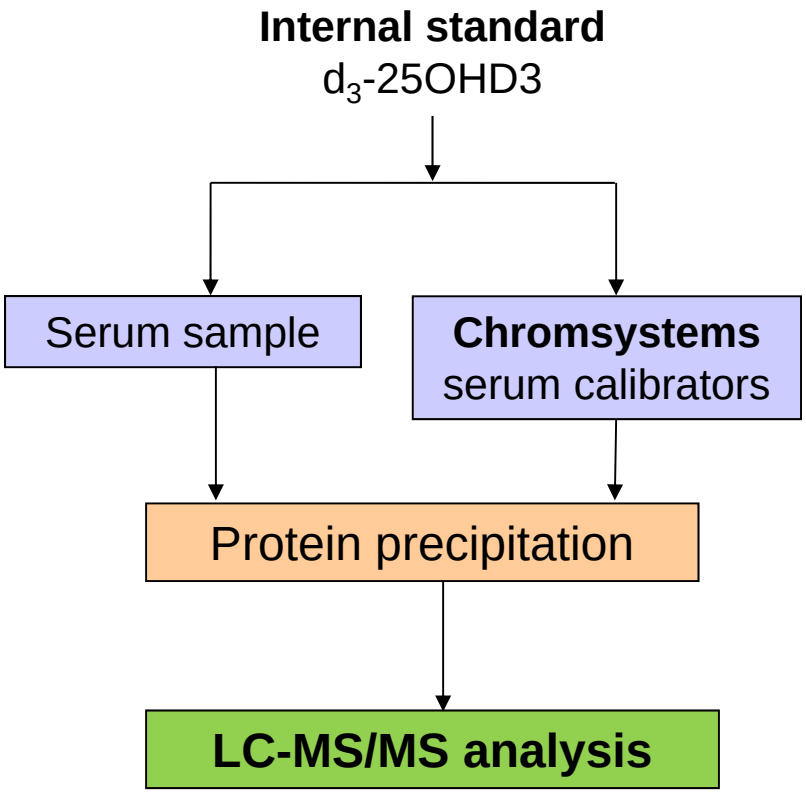
Part 2: RMPs and Method Validation

- **Douglass Hanly Moir Pathology**
  - 1of 7 laboratories in Australia performing vitamin D analysis by LC-MS/MS
  - Provider of LC-MS/MS measurements for the 2011-2013 Australian Health Survey



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Validation of In-House Methods

NPAAC Performance Standard

- **Assessment of accuracy and precision by:**
  - analysis of CRMs or in-house reference materials
  - recovery studies
  - participation in external quality assurance programs
  - comparison with a RMP

The **National Pathology Accreditation Advisory Council (NPAAC)** performance standard, *"Requirements for the Development and Use of In-House In-Vitro Diagnostic Devices (IVDs) (S4.4), June 2014*

DHM's Method Validation

- Analysis of serum quality control materials
  - *Chromsystems MassCheck*
  - *UTAK Serum Controls*
- Analysis of CRMs
  - *NIST SRM968e*
  - *NIST SRM972a*
- **Analysis of 30 patient samples by:**
  - *DHM LC-MS/MS method*
  - *Immunoassay method (DiaSorin Liaison)*
  - *NMI RMP*

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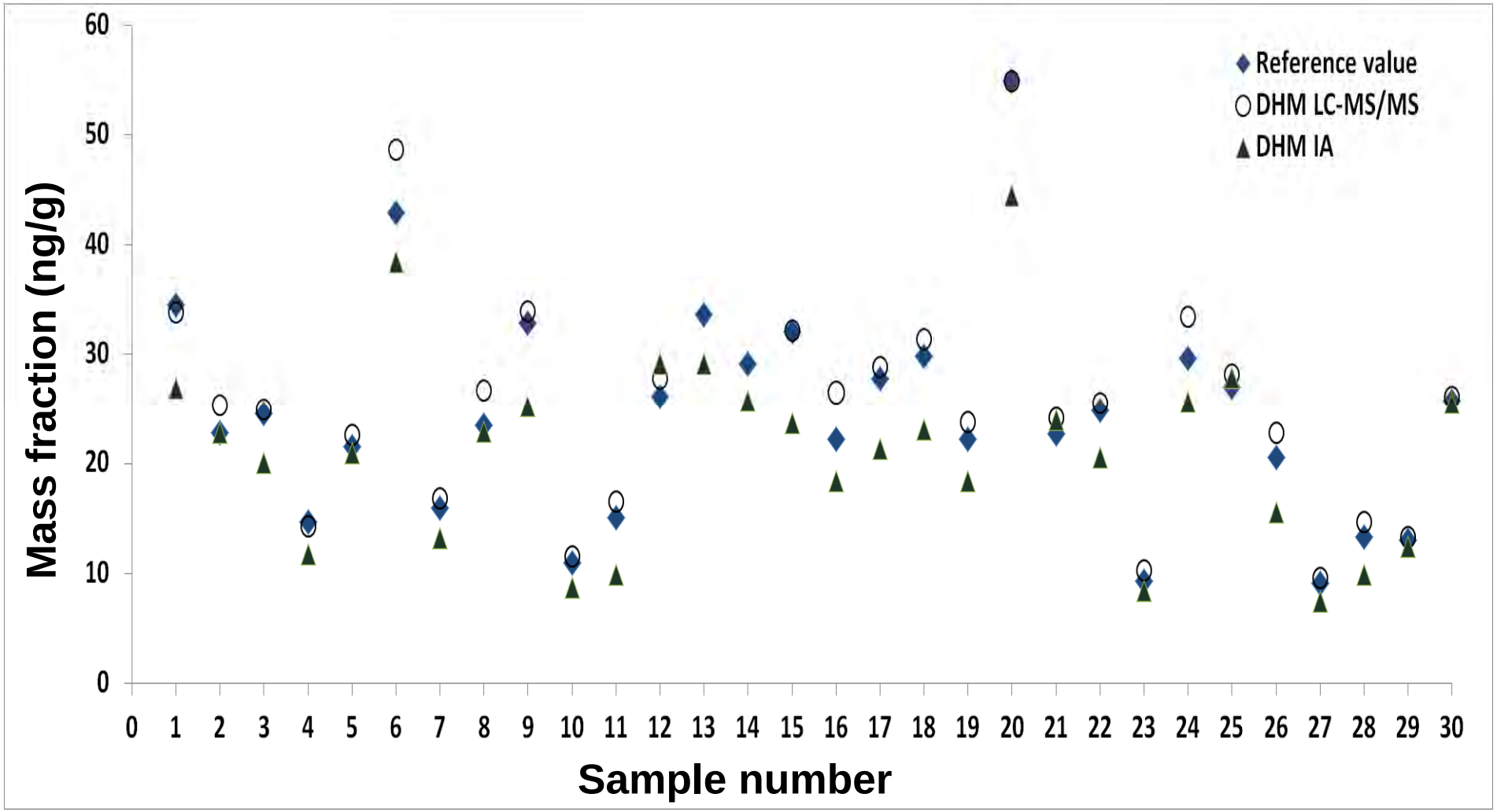
Comparison of IA and LC-MS/MS Results Versus Reference Values



Note: DHM LC-MS/MS results not reported for Samples 13 and 14 due to insufficient sample volumes

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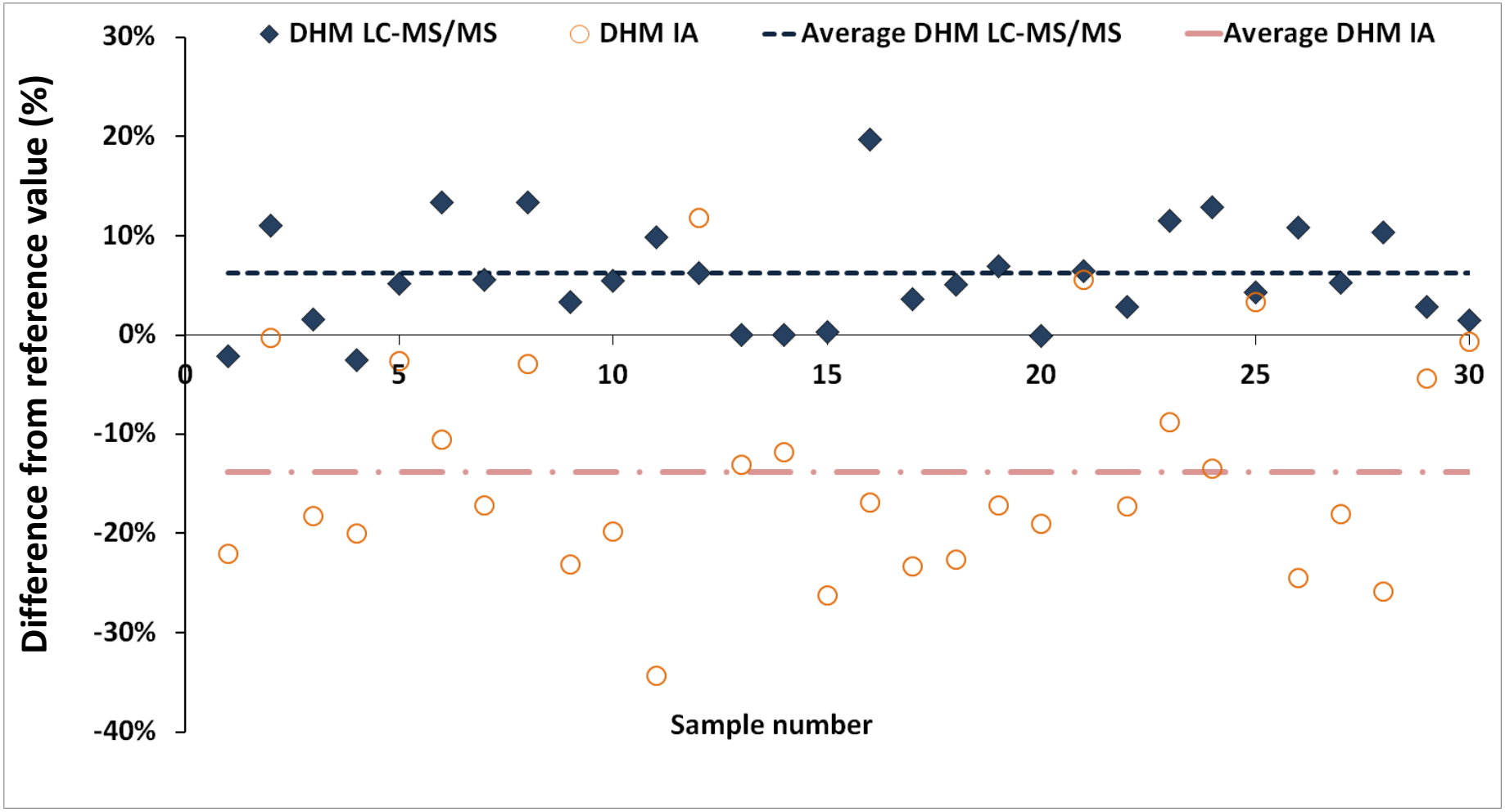
Differences in IA and LC-MS/MS Results Versus Reference Values



IA Differences: -34% to 12% (**average = -14%**)

LC-MS/MS Differences: -3% to 20% (**average = 6%**)

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Chromsystems and RECIPE Calibrators

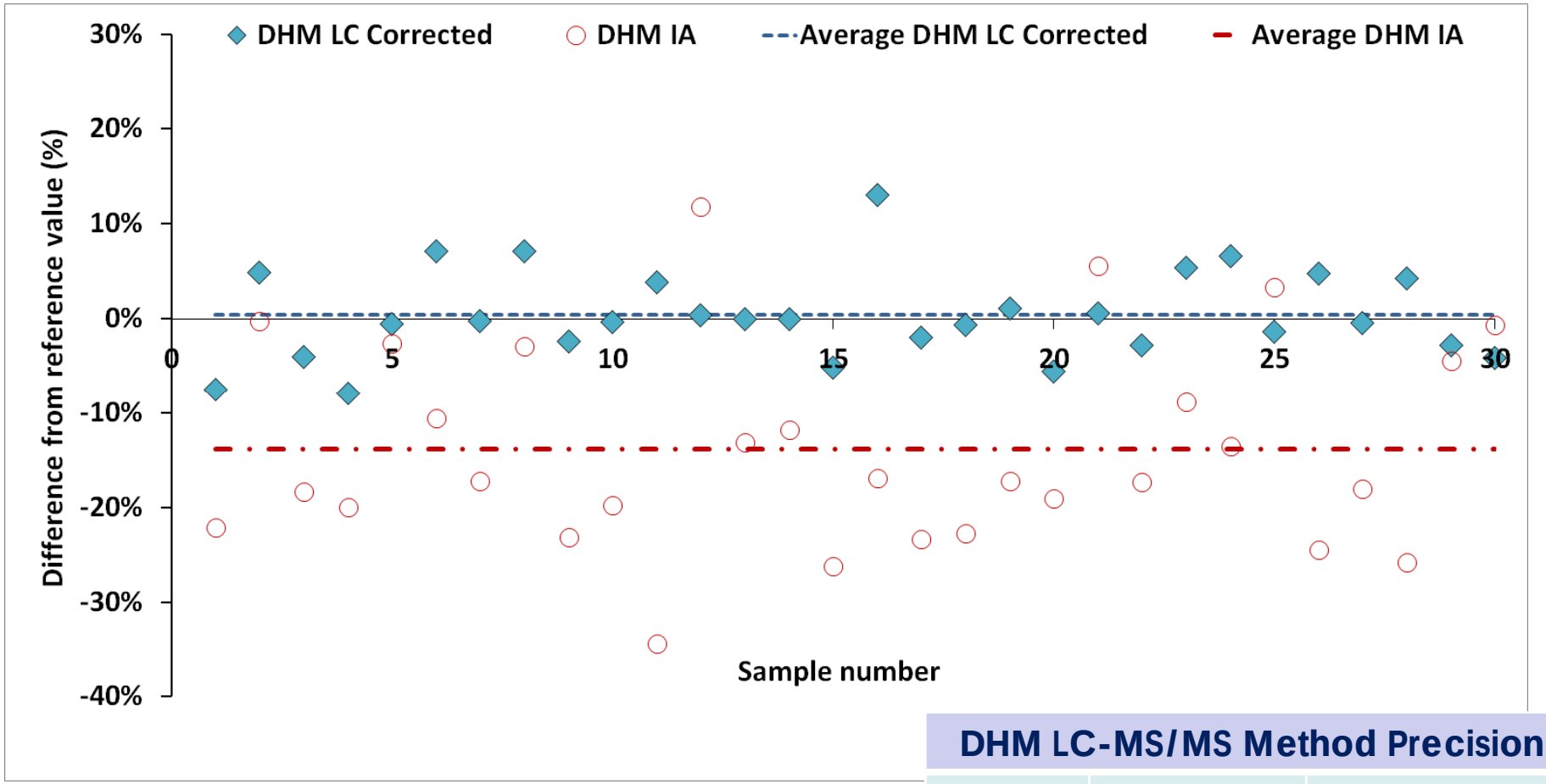
CHROMSYSTEMS CALIBRATORS (Lot: 5012)			
Calibrator	**Manufacturer's value (ng/mL)**	**Mass fraction by RMP (ng/g)**	**Difference**
Low Level	4.3	4.2	3%
Level 1	19.5	17.9	8%
Level 2	33.7	31.8	6%
Level 3	66.3	63.0	5%

Certified Reference Material

NIST SRM2972: 25OHD3 in ethanol

RECIPE CALIBRATORS (Lot: 238)			
Calibrator	**Manufacturer's value (ng/mL)**	**Mass fraction by RMP (ng/g)**	**Difference**
Level 1	9.61	9.01	6%
Level 2	27.4	28.0	2%
Level 3	73.4	76.4	4%

Applying a Correction Factor for the Chromsystems Calibrators



LC-MS/MS Differences after correction:
-0.3% to 8% (**average = 0.4%**)

Correction factor: 5.5%

DHM LC-MS/MS Method Precision		
	Level 1	**Level 2**
Mean	43.3	98.8
%CV	6.9	5.4
Count	149	149

CHROMSYSTEMS MassCheck Controls (Lot: 1513)

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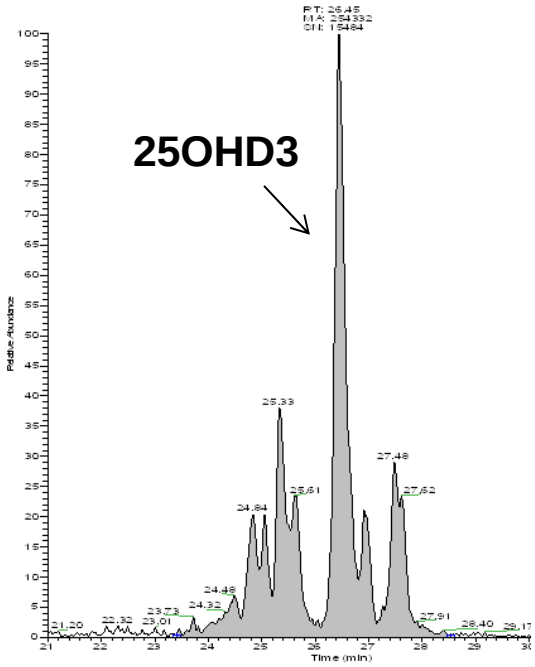
Part 3: Reference Values for EQA Programs

- EQA programs offer a practical opportunity for laboratories to judge their performance against a RMP

Sample	Reference value (nmol/L)	Relative expanded uncertainty
E13-1	1.7*	-
E13-2	37.3	3.2%
E13-3	71.5	4.2%
E13-4	108.5	3.7%
E13-5	142.4	3.8%
E13-6	175.1	4.1%

**Note: Result provided for E13-1 is indicative as matrix interferences were observed in sample chromatograms*
Six replicate analyses of each material were performed
Expanded uncertainties determined at 95% confidence level



Matrix interferences in Sample E13-1

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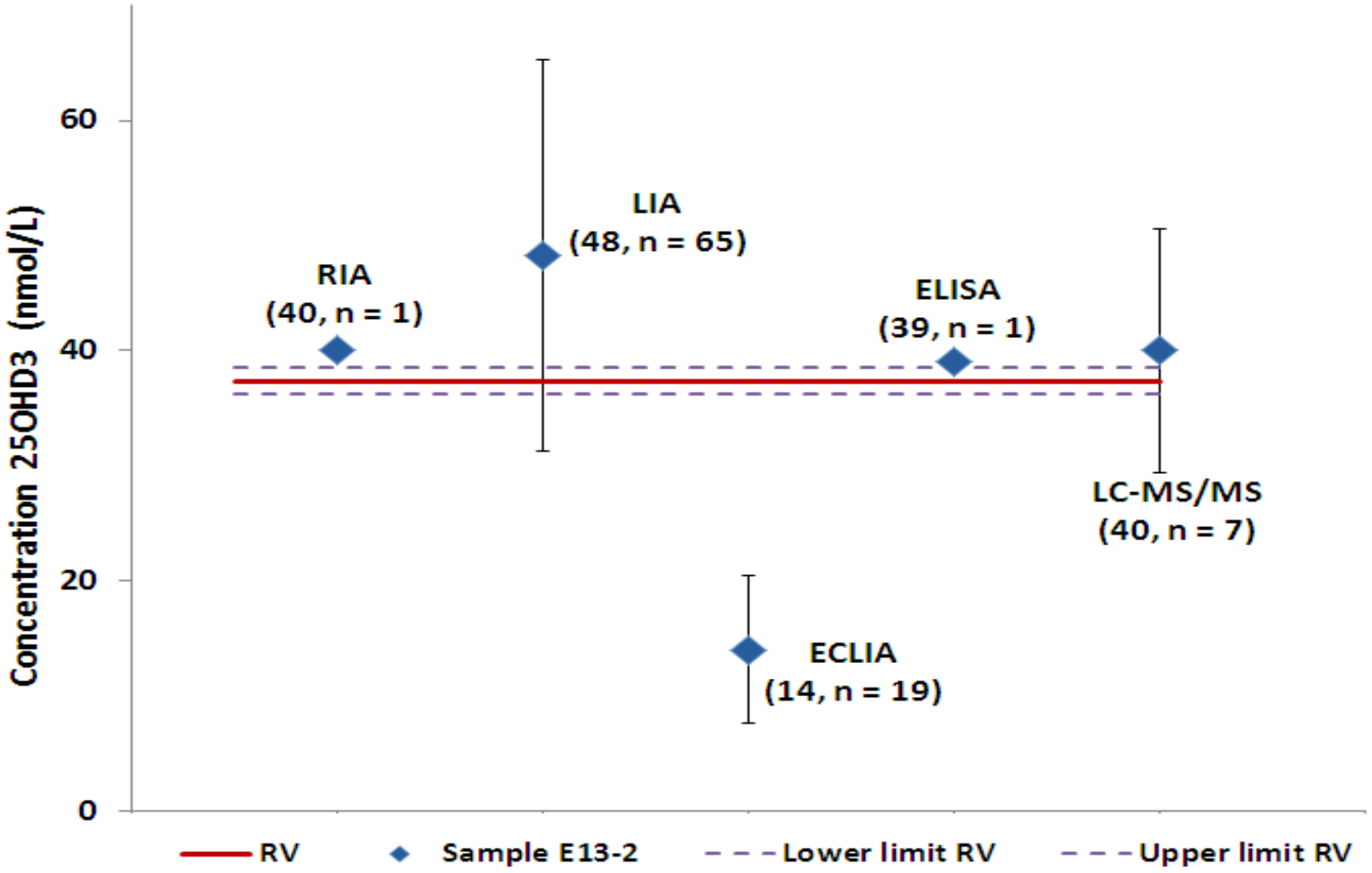
Sample E13-2 @ 37 nmol/L



RV: reference value
RIA: Radioimmunoassay
LIA: Luminescent immunoassay
ECLIA: Electrochemiluminescence immunoassay

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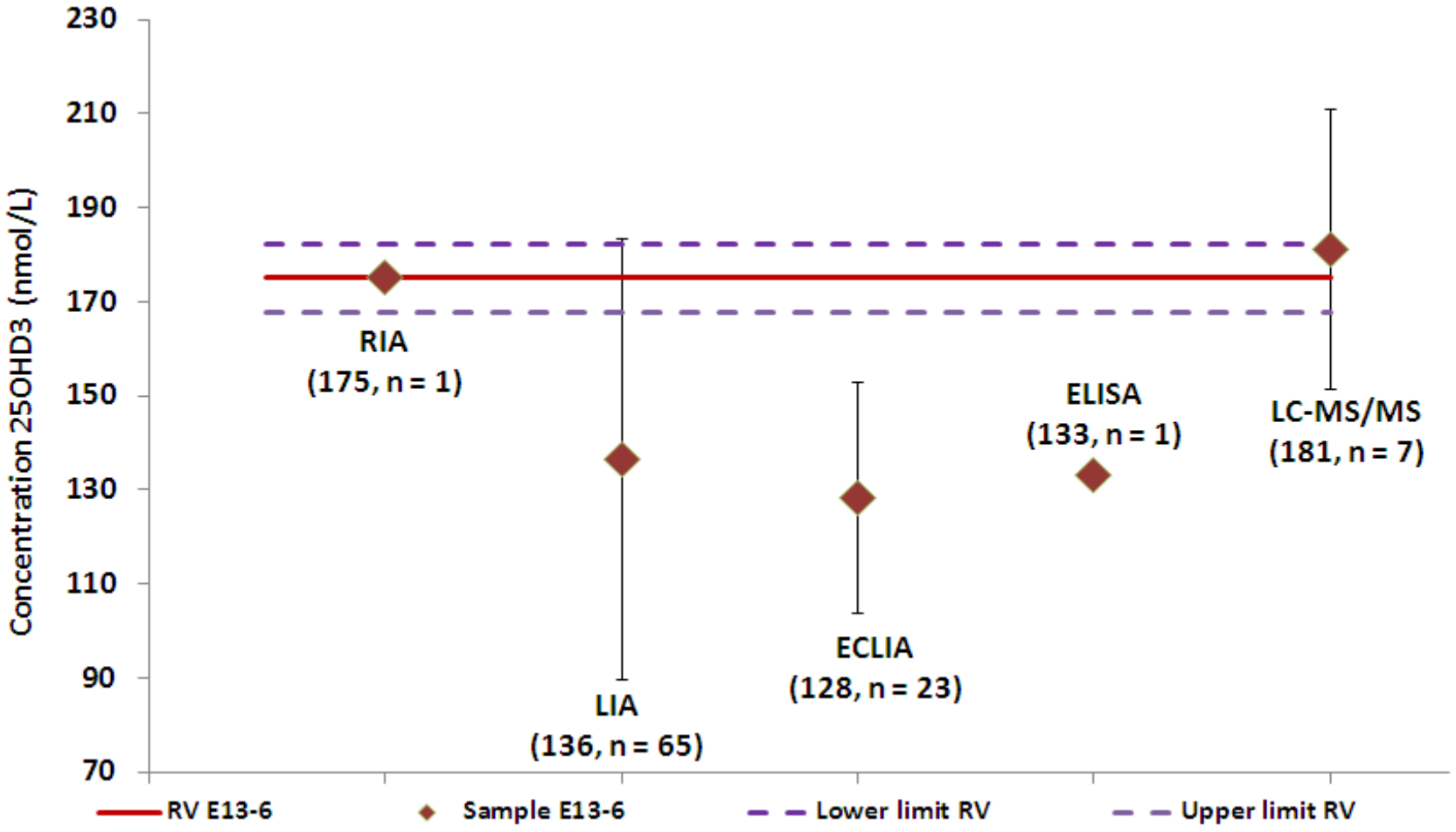
Sample E13-6 @ 175 nmol/L



RV: reference value
RIA: Radioimmunoassay
LIA: Luminescent immunoassay
ECLIA: Electrochemiluminescence immunoassay

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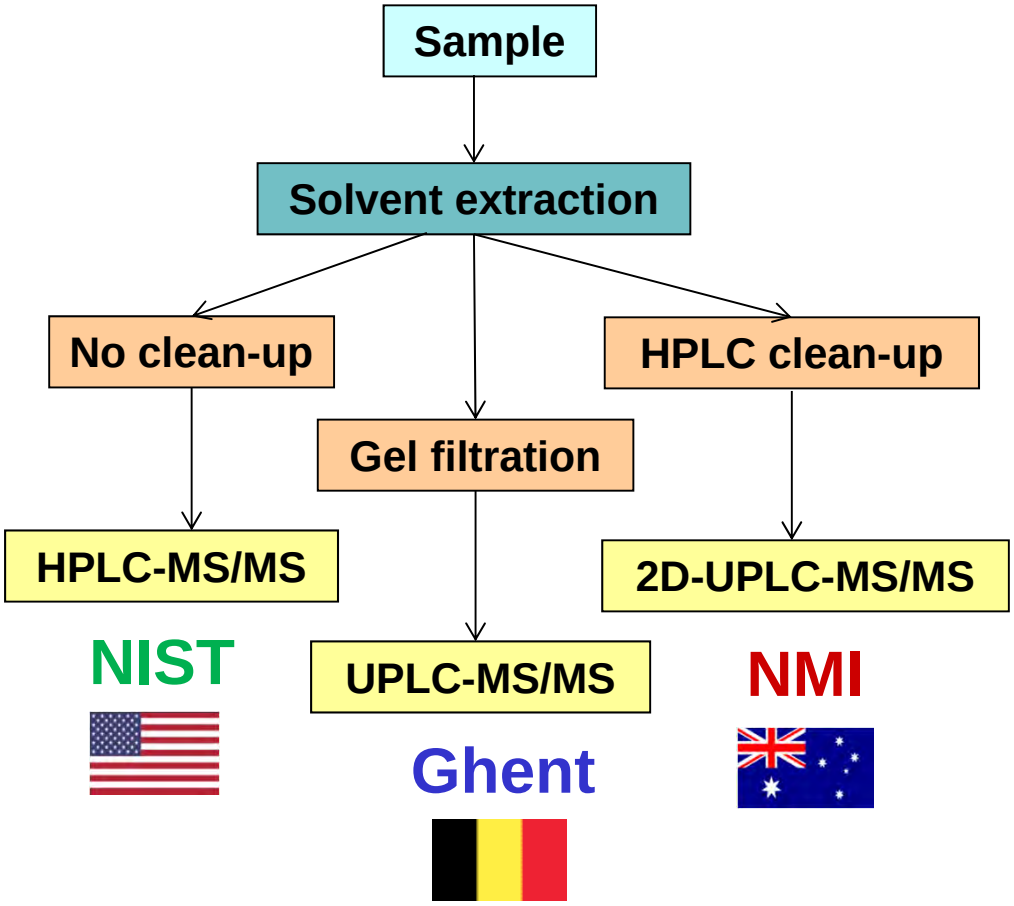
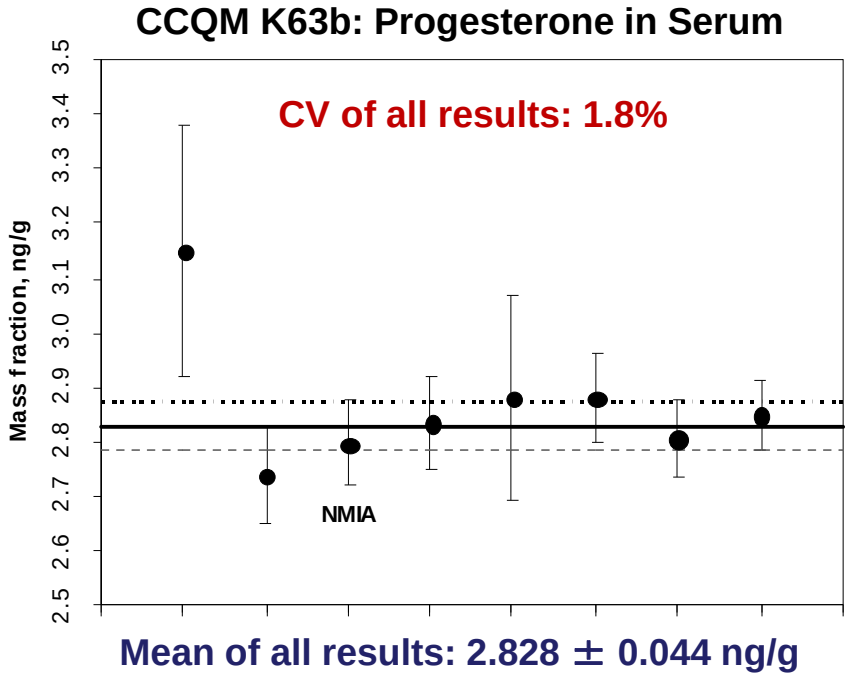
Demonstrating the Equivalence of RMPs Around the World



- **Consultative Committee for the Amount of Substance (CCQM)** international key comparison on vitamin D analysis planned for 2015



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- Lisa Jolly and Graham Jones (RCPAQAP)

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