Joint Research Centre
the European Commission's in-house science service

Serving society
Stimulating innovation
Supporting legislation
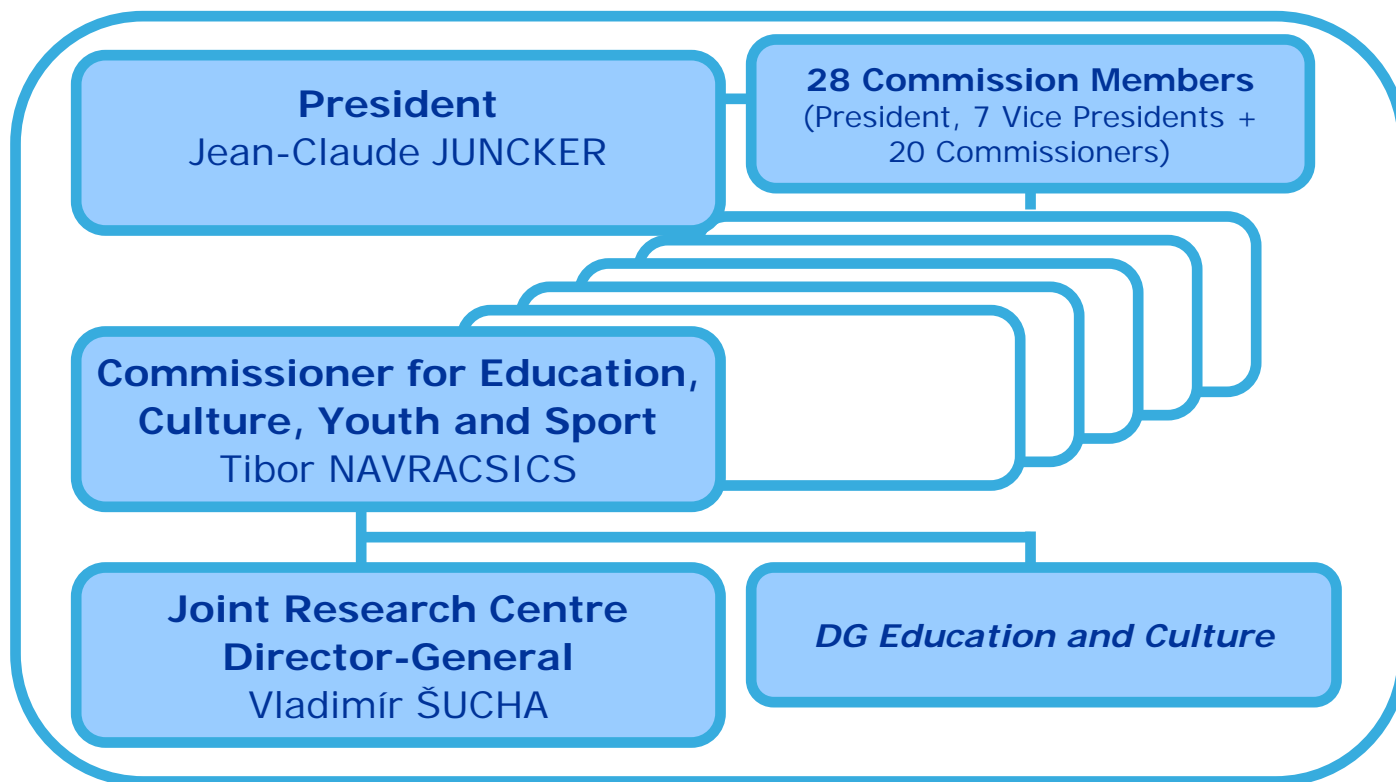
JCTLM Member Activities: IRMM

H. Schimmel, G. Auclair, I. Zegers
E. Monogioudi, J. Kuhlmann
SID Unit

Sèvres, November 30th 2015

www.ec.europa.eu/jrc

European Commission

The JRC inside the European Commission

President
Jean-Claude JUNCKER

28 Commission Members
(President, 7 Vice Presidents +
20 Commissioners)

**Commissioner for Education,
Culture, Youth and Sport**
Tibor NAVRACSICS

**Joint Research Centre
Director-General**
Vladimír ŠUCHA

DG Education and Culture

European Commission

The Joint Research Centre (JRC)

JRC Sites

IRMM
Institute for Reference Materials and Measurements

IET
Institute for Energy and Transport

ITU
Institute for Transuranium Elements

Headquarters

PETTEN
GEEL
BRUSSELS
KARLSRUHE

ISPRA

IPTS
Institute for Prospective Technological Studies

SEVILLE

IHCP
Institute for Health and Consumer Protection

IPSC
Institute for the Protection and Security of the Citizen

ISM
Ispra Site Management

IES
Institute for Environment and Sustainability

European Commission's in-house science service

Established in 1957 (Euratom Treaty)

Supporting EU policies with independent, evidence-based scientific and technical support

7 Institutes (Directorates) in 6 locations

~ 3.000 staff (2015)

Budget €393 million annually, plus €73 earned income

European Commission

EU Legislative procedure

European Commission

Proposes legislation

Co-decide

EUROPEAN PARLIAMENT

Council of the European Union

Accept
Amend

EU LEGISLATION

European Commission
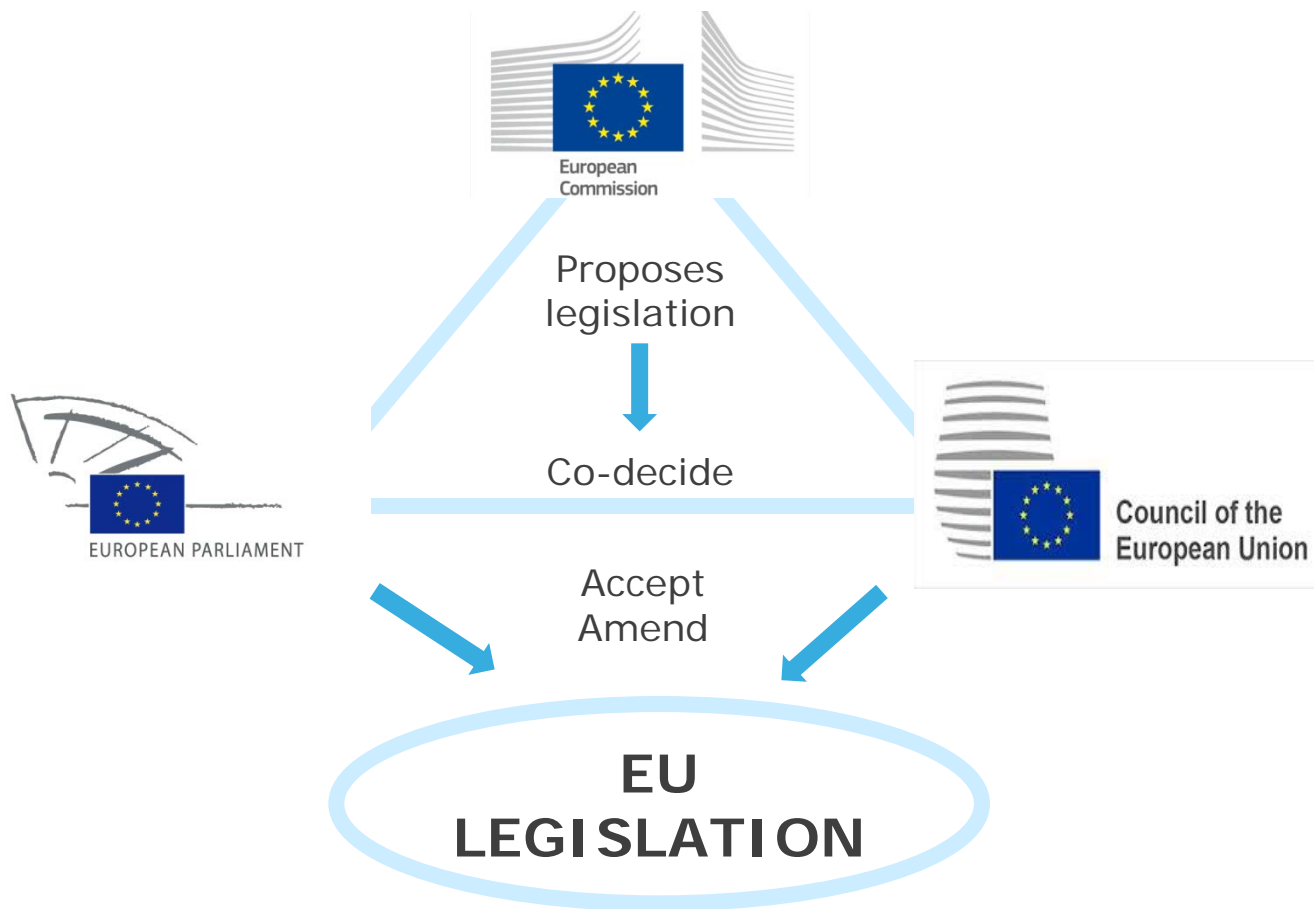
Revision and implementation of IVD Directive (98/79/EC) / Regulation

- *Proposal published 2012, IRMM consulted.*
- *Since then under scrutiny and amended by the Council of the European Union, European Parliament and European Commission (information see http://ec.europa.eu/growth/sectors/medical-devices/regulatory-framework/revision/index_en.htm).*
- *In 2015 several 'trilogue' negotiations between Council of the European Union, European Parliament and European Commission to sort out remaining issues (progress being made).*
- *IRMM represented in IVD technical group, consisting of competent authorities representatives addressing technical issues concerning implementation of the IVD Directive \ Regulation*

European Commission

Overview activities

- *CRM development concentrating on:*
  - *CRMs for enzymatic catalytic activity (renewals)*
  - *HbA1c calibration solutions (changed format)*
  - *Serum proteins (B2M)*
  - *CRMs for Alzheimer's disease diagnostics (Abeta42)*
  - *CRMs for autoimmune disease diagnostics*

- *Provide advice to IFCC and its committees and working groups*
- *Provide input to ISO TC212 (revision ISO 17511)*

European Commission

CRMs for HbA1c

- *At the final stage*
- *Gravimetric mixtures of pure HbA1c and HbA0*
- *6 levels at approximately 0, 30, 60, 90, 120 and 150 mmol/mol*
- *Can be directly used for the calibration of the IFCC reference measurement procedure for HbA1c*

European Commission
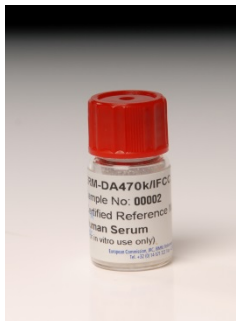
CRM for B2M

ERM-DA470k/IFCC
Released in 2008 and certified for 12 proteins, freeze-dried

β-2-microglobulin
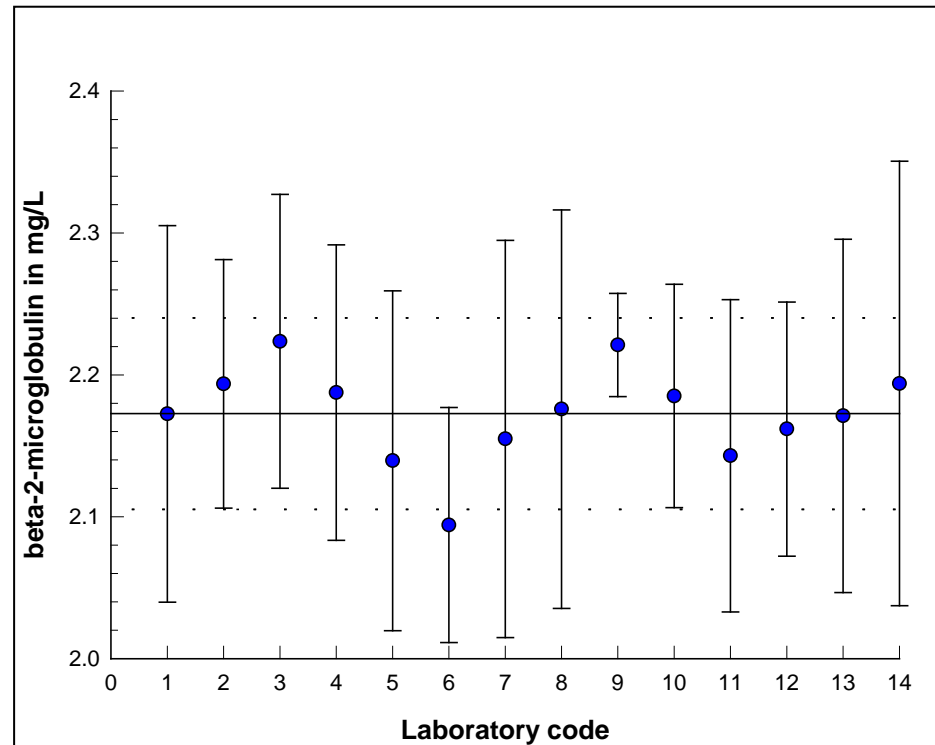
marker for multiple myeloma and lymphoma

2.17 ± 0.07 mg/L (k=2)

CRM for B2M

ERM-DA470k/IFCC using kit calibrators
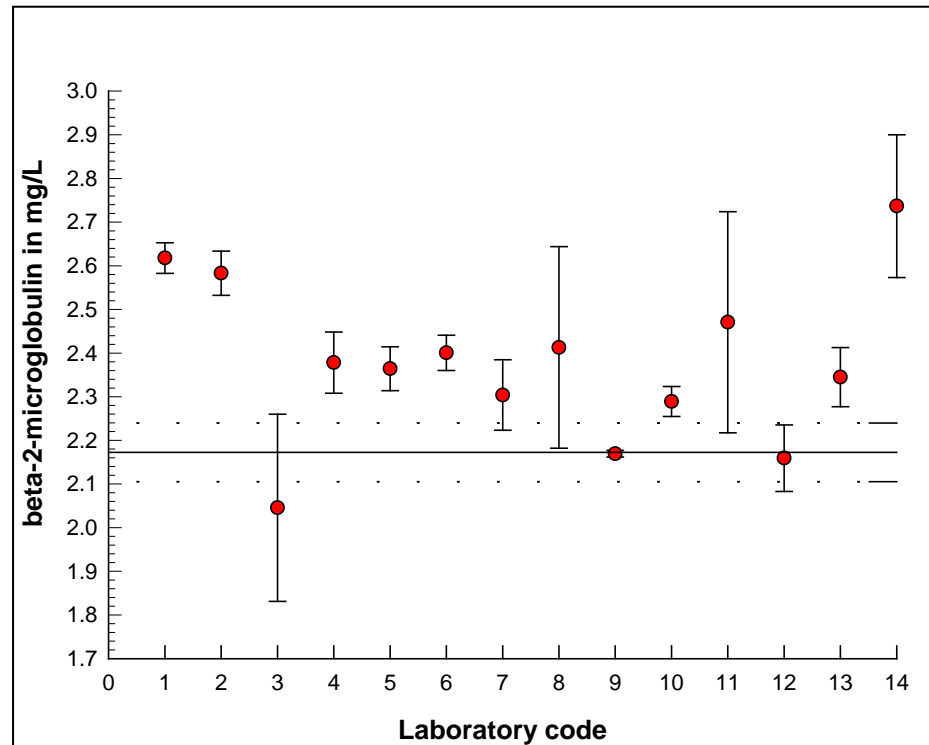
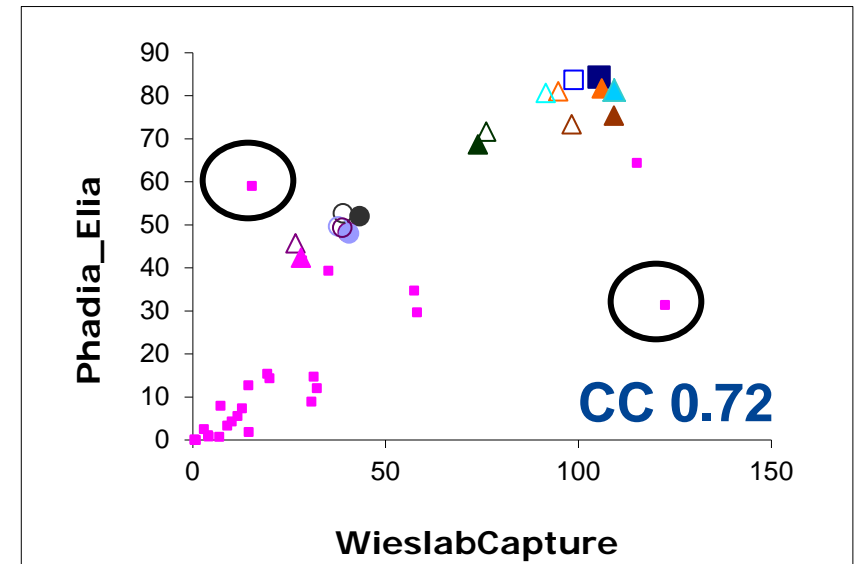**ERM-DA470k/IFCC using calibrant provided by IRMM**

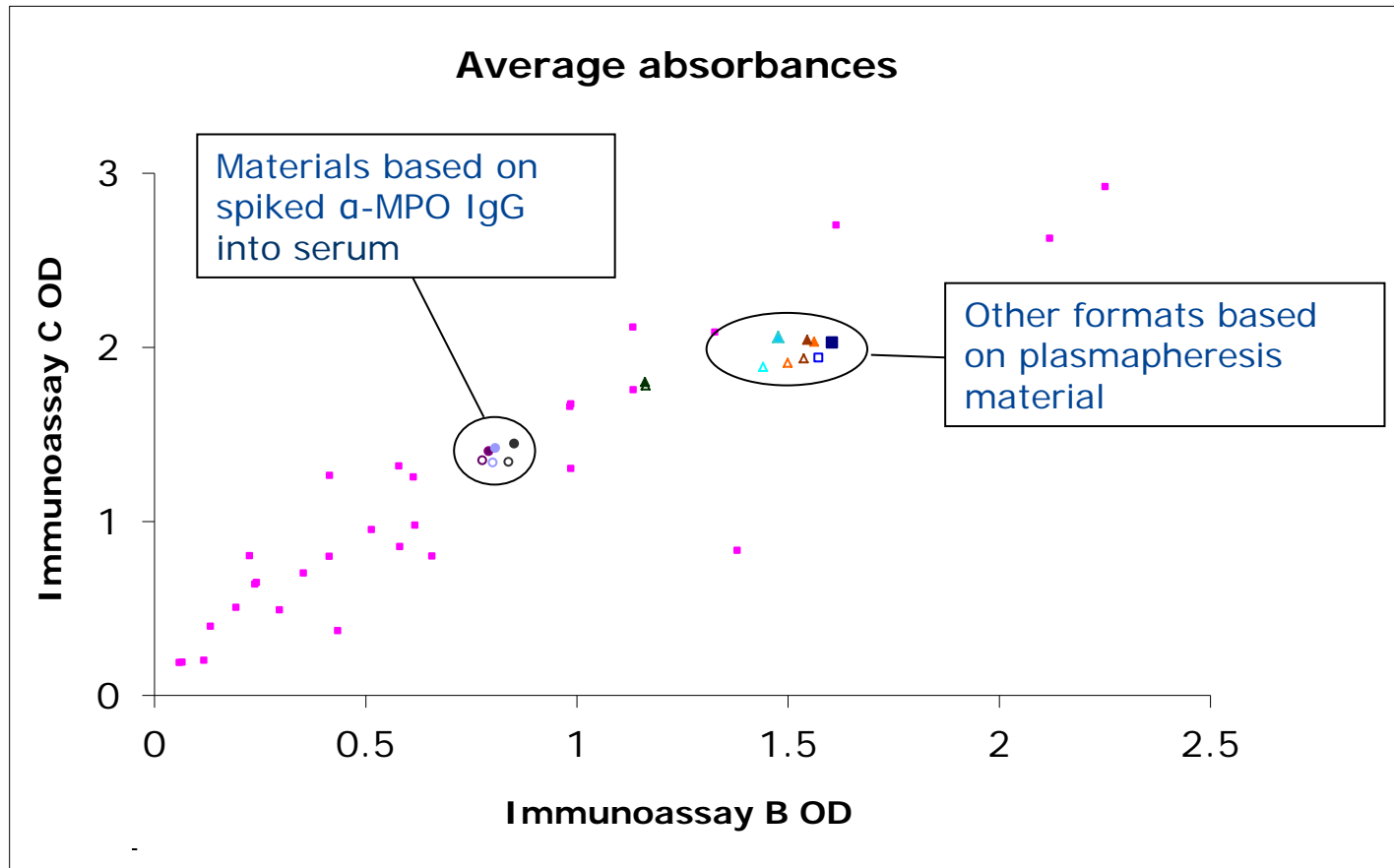# CRMs for autoimmune diagnostics (IFCC WG-HAT)

- *Two layer standardisation problem:*
  - *heterogeneous response between individuals to variable antigens (at least antigen to be defined)*
  - *standardisation of the quantitative measurement of the IgG fraction binding to a defined antigen*
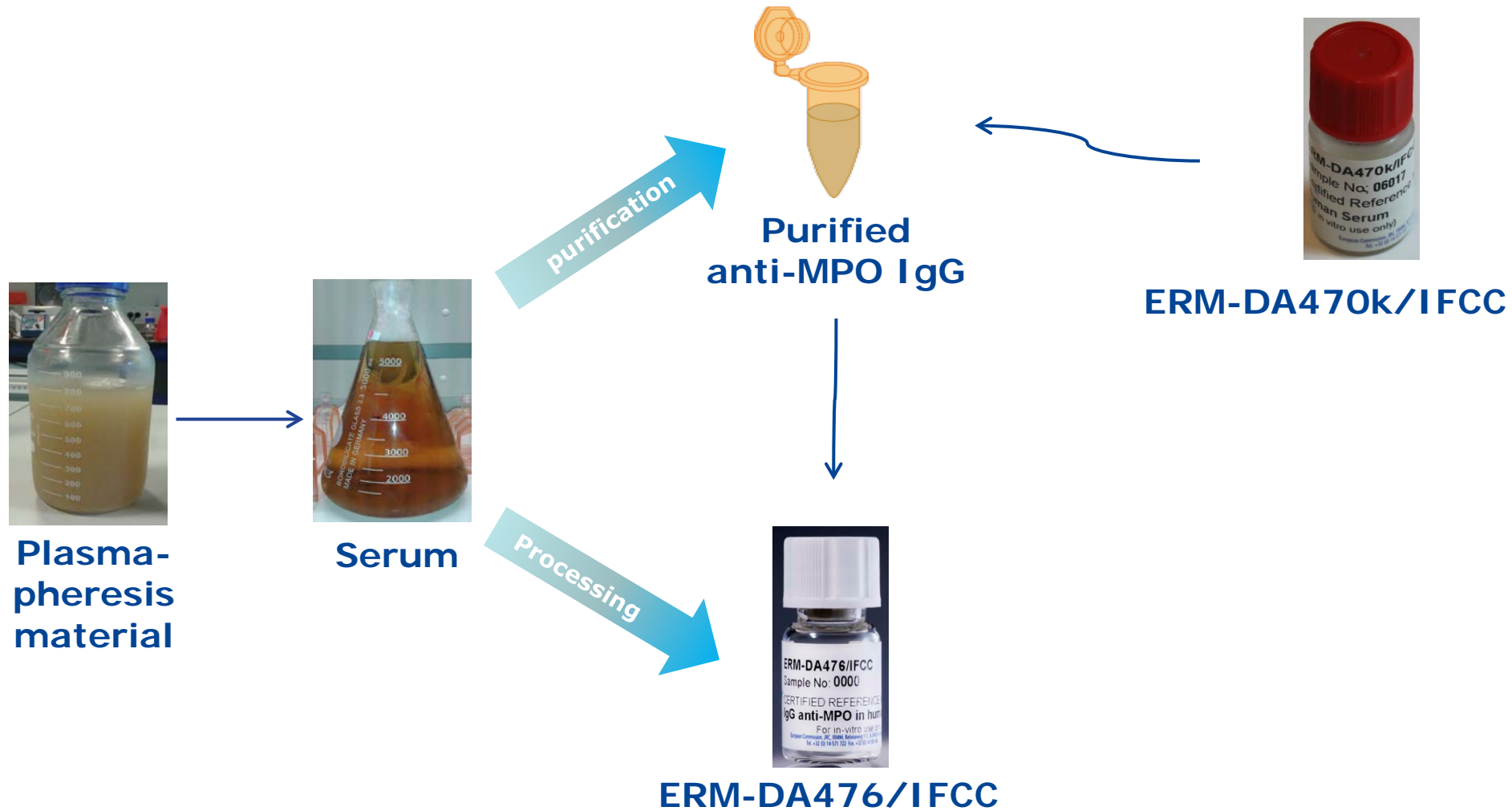
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CRMs for autoimmune diagnostics – correlation studies

Manufacturers	Platforms & Kits
AESKU	AESKULISA® MPO
Bio-Rad	Anti-MPO EIA
	Bioplex 2200
Eurodiagnostica	MPO ANCA DIASTAT®
	Capture MPO-ANCA Wieslab®
	MPO-ANCA Wieslab®
Euroimmun	Anti-MPO ELISA
	Myeloperoxidase (MPO) (pANCA) (IgG)
IMMCO	Anti-MPO ELISA
Inova	QUANTA Lite MPO SC ELISA
	MPO BIOFLASH
	QUANTA Lite® MPO IgG
Menarini	Menarini
Orgentec	ORG 519 Anti-MPO (pANCA
Phadia	Varelisa™ MPO ANCA
	EliA MPO^S
Orgentec	Anti-MPO ORG 519
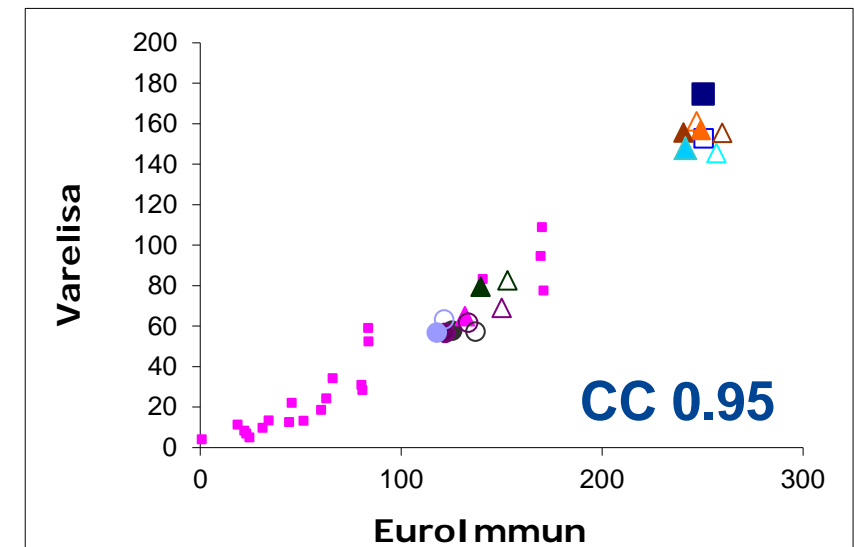
CRMs for autoimmune diagnostics - commutability studies

Average absorbances

Materials based on spiked α-MPO IgG into serum

Other formats based on plasmapheresis material

Immunoassay C OD

Immunoassay B OD

European Commission

CRMs for autoimmune diagnostics – value assignment

Plasma-pheresis material

Serum

purification

Purified anti-MPO IgG

Processing

ERM-DA470k/IFCC

ERM-DA476/IFCC

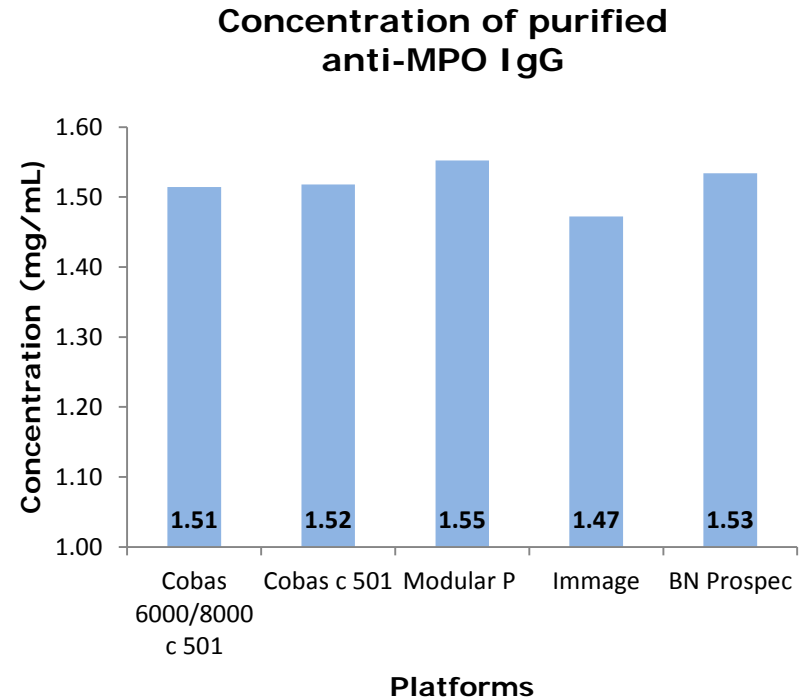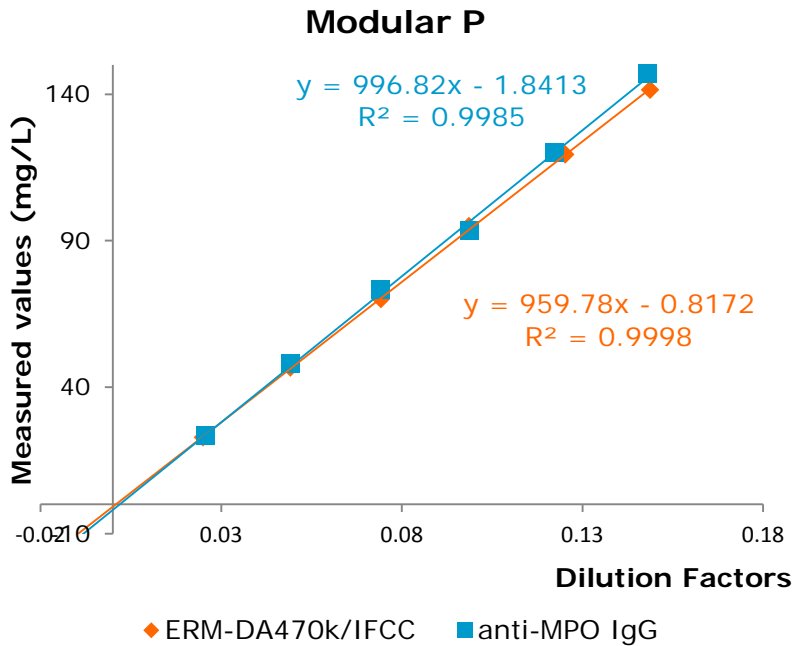# CRMs for autoimmune diagnostics – value assignment to calibrant

ERM-DA470k/IFCC

Nephelometry
Turbidimetry →
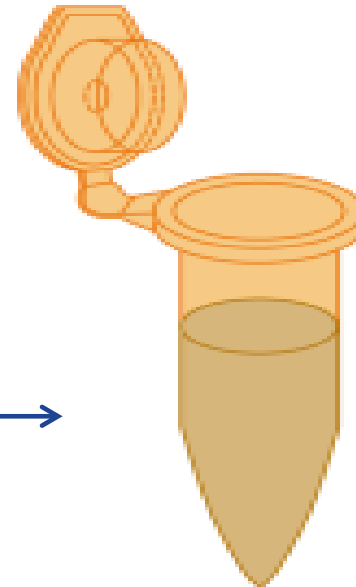
**Purified
anti-MPO IgG**

CRMs for autoimmune diagnostics – value assignment to calibrant

Modular P

Measured values (mg/L)

y = 996.82x - 1.8413
R² = 0.9985

y = 959.78x - 0.8172
R² = 0.9998

Dilution Factors

◆ ERM-DA470k/IFCC   ■ anti-MPO IgG

Concentration of purified anti-MPO IgG

Concentration (mg/mL)

Platform	Value
Cobas 6000/8000 c 501	1.51
Cobas c 501	1.52
Modular P	1.55
Immage	1.47
BN Prospec	1.53

Platforms

$$[\text{anti-MPO IgG}] = \frac{\text{slope}_{\text{anti-MPO IgG}}}{\text{slope}_{\text{ERM-DA470k/IFCC}}} \times [\text{ERM-DA470k/IFCC}]$$

CRMs for autoimmune diagnostics – value assignment to matrix CRM
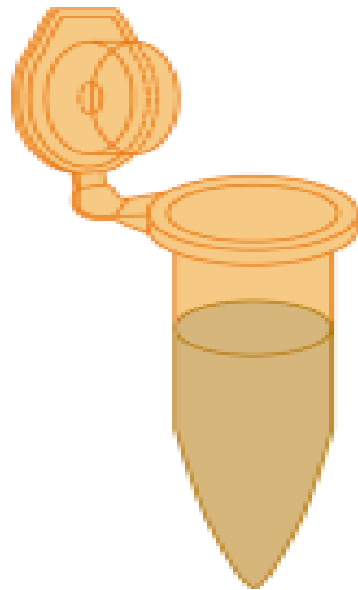
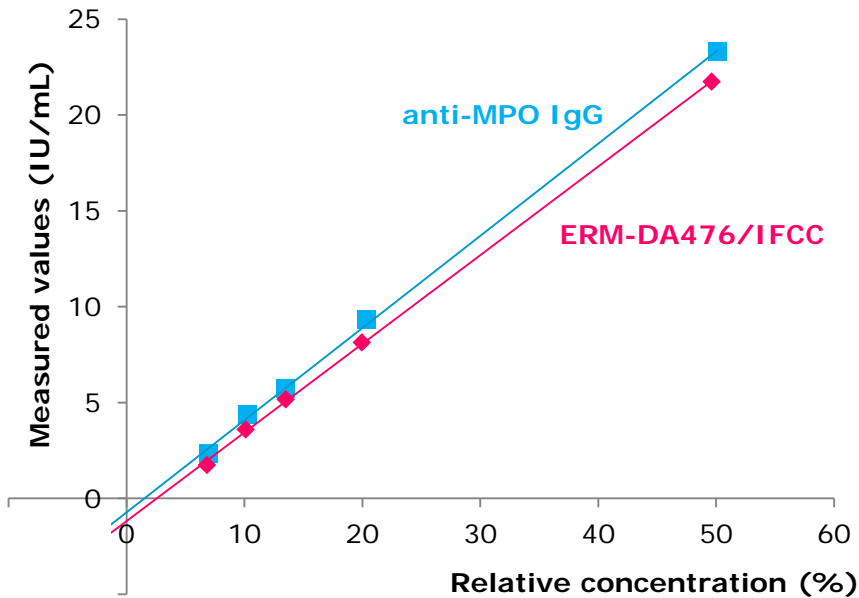**Purified anti-MPO IgG**

anti-MPO IgG methods

ERM-DA476/IFCC

CRMs for autoimmune diagnostics – value assignment to matrix CRM
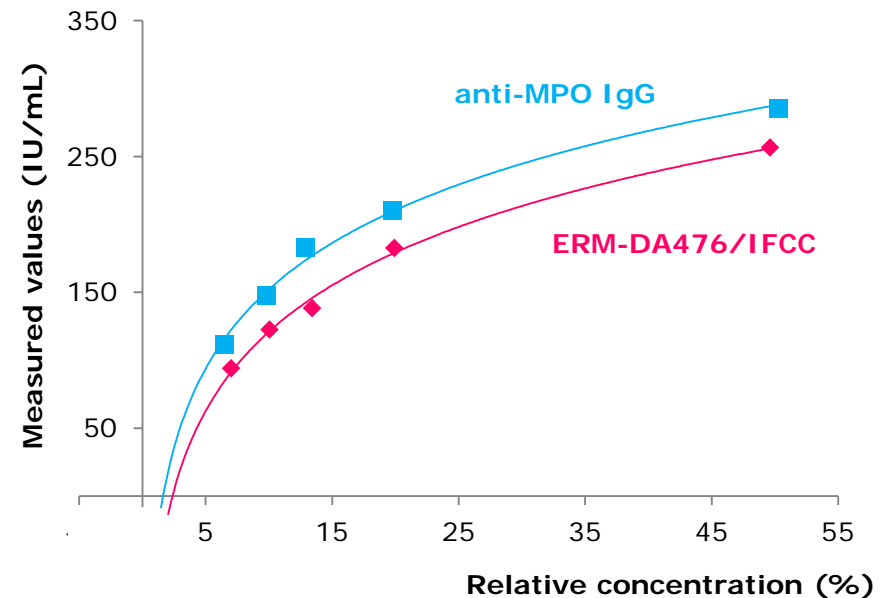
**Linear function
(IRMM- Wieslab CAP MPO IU)**

**Logarithmic function
IMMCO**

Average Values

Average values

$$[ERM\text{-}DA476/IFCC] = \frac{slope_{ERM\text{-}DA476/IFCC}}{slope_{anti\text{-}MPO\,IgG}} \times [anti\text{-}MPO\,IgG]$$

CRMs for autoimmune diagnostics

Method	Method values		Common calibrant	
	Value	Unit	Value	Unit
Wieslab® Capture MPO-ANCA	40	IU/mL	92	mg/L
ImmuLisa™	356	IU/mL	68	mg/L
Wieslab® Capture MPO-ANCA	45	IU/mL	88	mg/L
Wieslab® MPO-ANCA	76	IU/mL	73	mg/L
EliA MPO[S]	77	IU/mL	93	mg/L
Autoimmune EIA Anti-Myeloperoxidase	78	U/mL	99	mg/L
AESKULISA MPO	205	U/mL	91	mg/L
Anti-Myeloperoxidase ELISA (IgG)	>200	RU/mL	75	mg/L
QUANTA Flash MPO	98	CU	78	mg/L

European Commission

CRMs for autoimmune diagnostics

Certified value [mg/L]	U_{CRM} (k = 2) [mg/L]
84	9

- **Will the reference materials solve all problems?**   **NO**
    - outliers, different selectivity, calibration strategies

- **What can the materials contribute to?**   **An anchor point**
    - reduction of batch to batch variation
    - long-term stability
   - better equivalence of measurement results

European Commission

CRMs for autoimmune diagnostics – IRMM and IFCC WG-HAT

|	anti-MPO	anti-PR3	anti-B2GP
Raw materials	✔	✔	✔
Correlation	✔	✔	✔
Commutability	✔	✔	✔
IgG purified	✔	✔	✔
Processing	✔	✔	✔
Homogeneity	✔		✔
Stability	✔		✔
Value assignment	✔		On-going

✔-done at IRMM
✔-done by other partners of the working group (Silvia Pierangeli, Rohan Willis)

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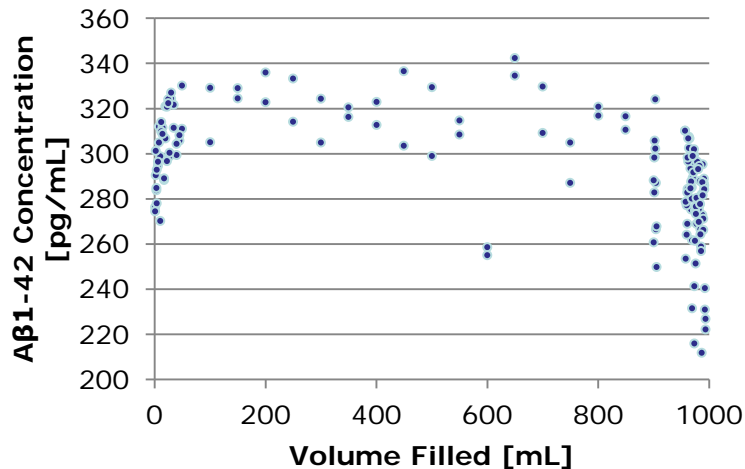# CRMs for Alzheimer's diagnostics – correlation and commutability
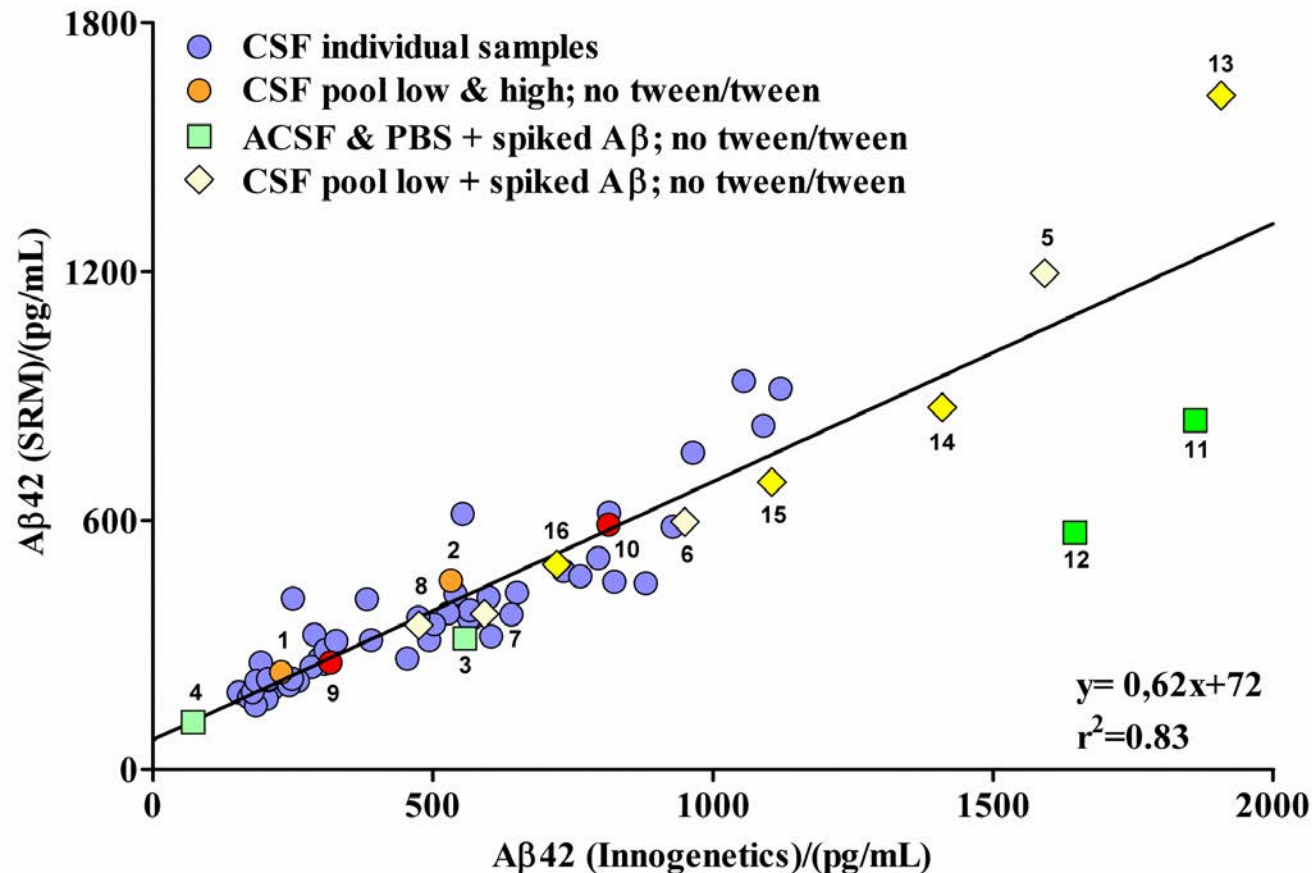
Bjerke et al, CCLM, in print

CRMs for Alzheimer's diagnostics – material processing

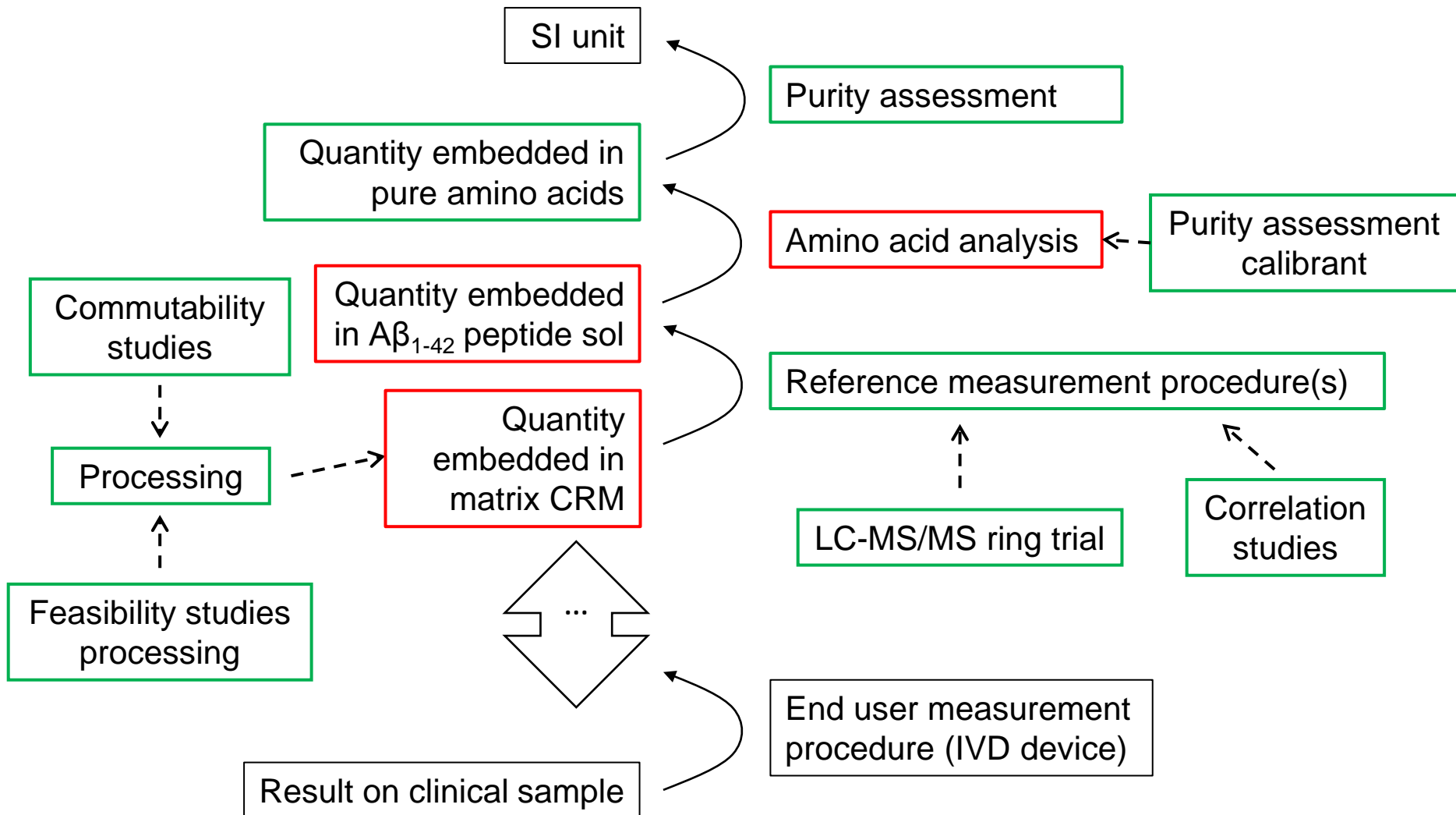• Manual Filled • Machine Filled
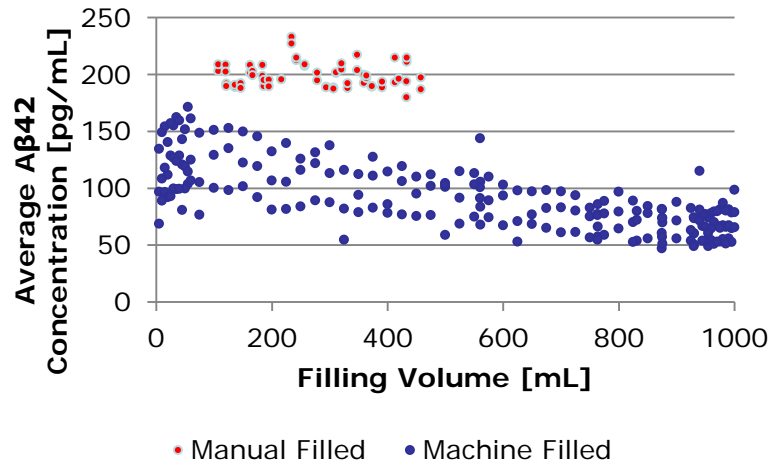
Drift during filling (aCSF)

Results from last trial (CSF)

$S_{between\ vial} = 1.9\ \%$
(between vial variability)

by optimizing processing conditions

problem at beginning and end of filling (aCSF)

European Commission

CRMs for Alzheimer's diagnostics – traceability chain

LC-MS/MS = Liquid chromatography-tandem mass spectrometry

Acknowledgements:

- *Many colleagues at IRMM who contributed (project responsibles, laboratory and processing staff)*
- *Members of the IFCC Working Groups and Committees IRMM is collaborating with*
- *Collaboration partners (researchers and IVD manufacturers)*

European Commission