European Commission

Clinical reference materials at IRMM

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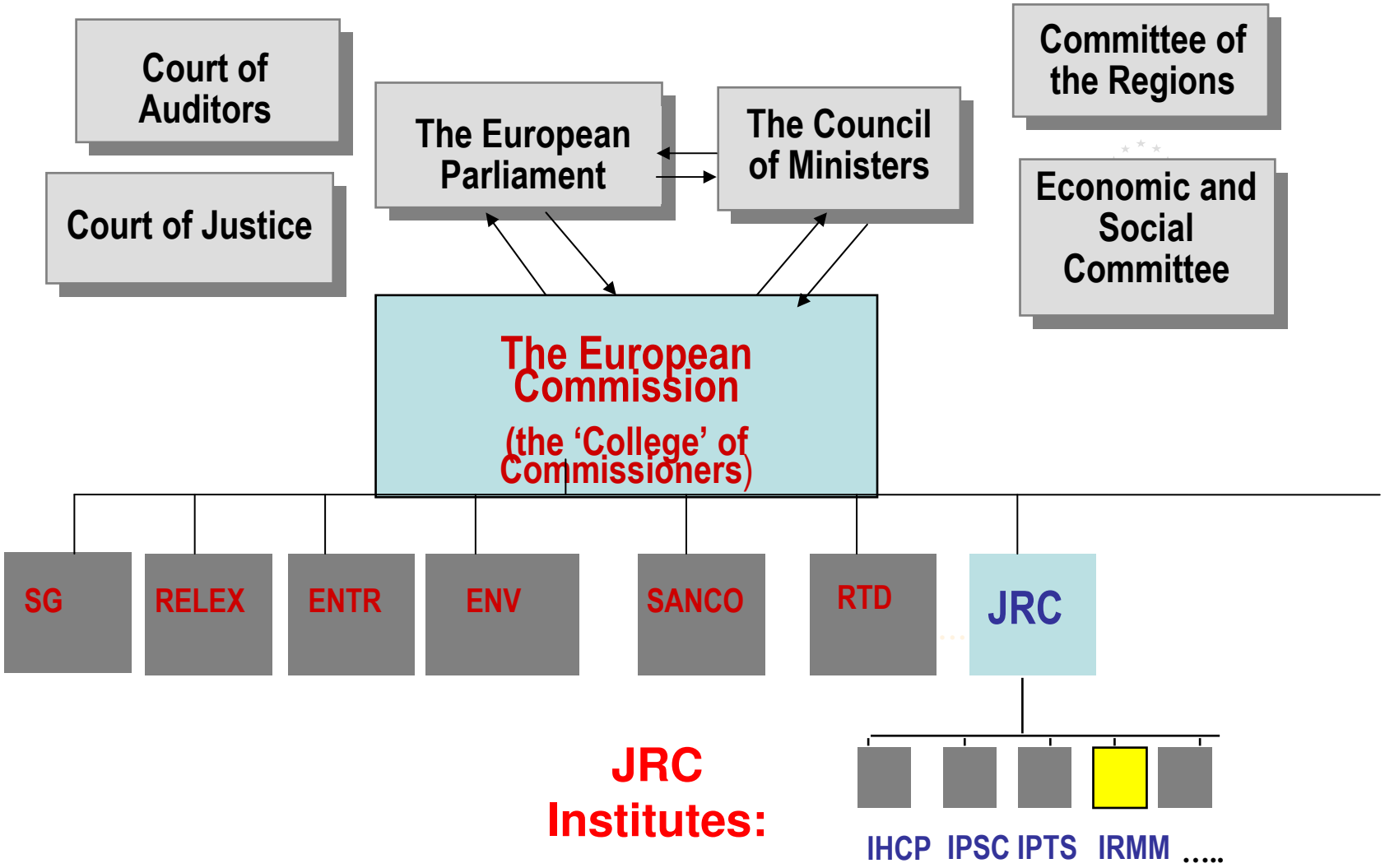
Joint Research Centre
IRMM

Serving society
Stimulating innovation
Supporting legislation

European Commission



Research Centre

EC: Joint Research Centre

European Commission

7 Institutes in 5 Member States

IRMM - *Geel, Belgium*
Institute for Reference Materials and Measurements

ITU - *Karlsruhe, Germany*
Institute for Transuranium Elements

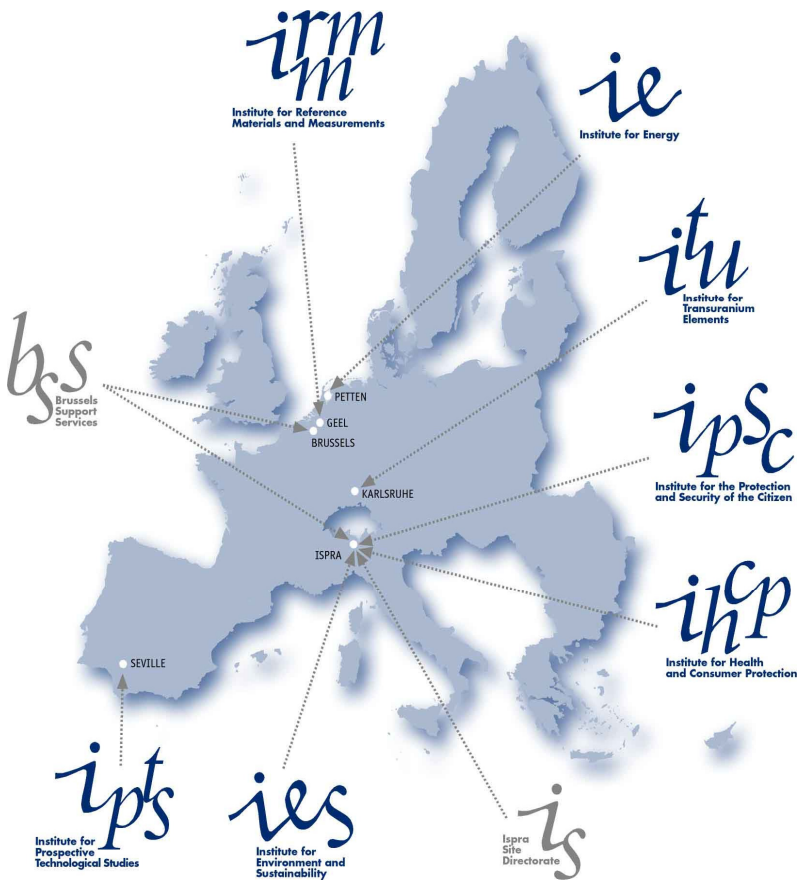
IE - *Petten, The Netherlands*
Institute for Energy

IPSC - *Ispra, Italy*
Institute for the Protection and Security of the Citizen

IES - *Ispra, Italy*
Institute for Environment and Sustainability

IHCP - *Ispra, Italy*
Institute for Health and Consumer Protection

IPTS - *Seville, Spain*
Institute for Prospective Technological Studies



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RM Programmes

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Vitamins

Proximates

Veterinary drugs

Mycotoxins

PCBs

Food CRMs

Enzymes

Prions

TSE QCMs

Clinical CRMs

Hormones

Microorganisms

Proteins

PAHs

Microbiol. CRMs

GMO CRMs

Hydrocarbons

DNA

Soil/ Sediment CRMs

Dioxins

Engin. Mat. CRMs

Size

Water CRMs

Elements

Isotopes

Ions

Metal species

Impact toughness

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Collaborations for clinical RMs

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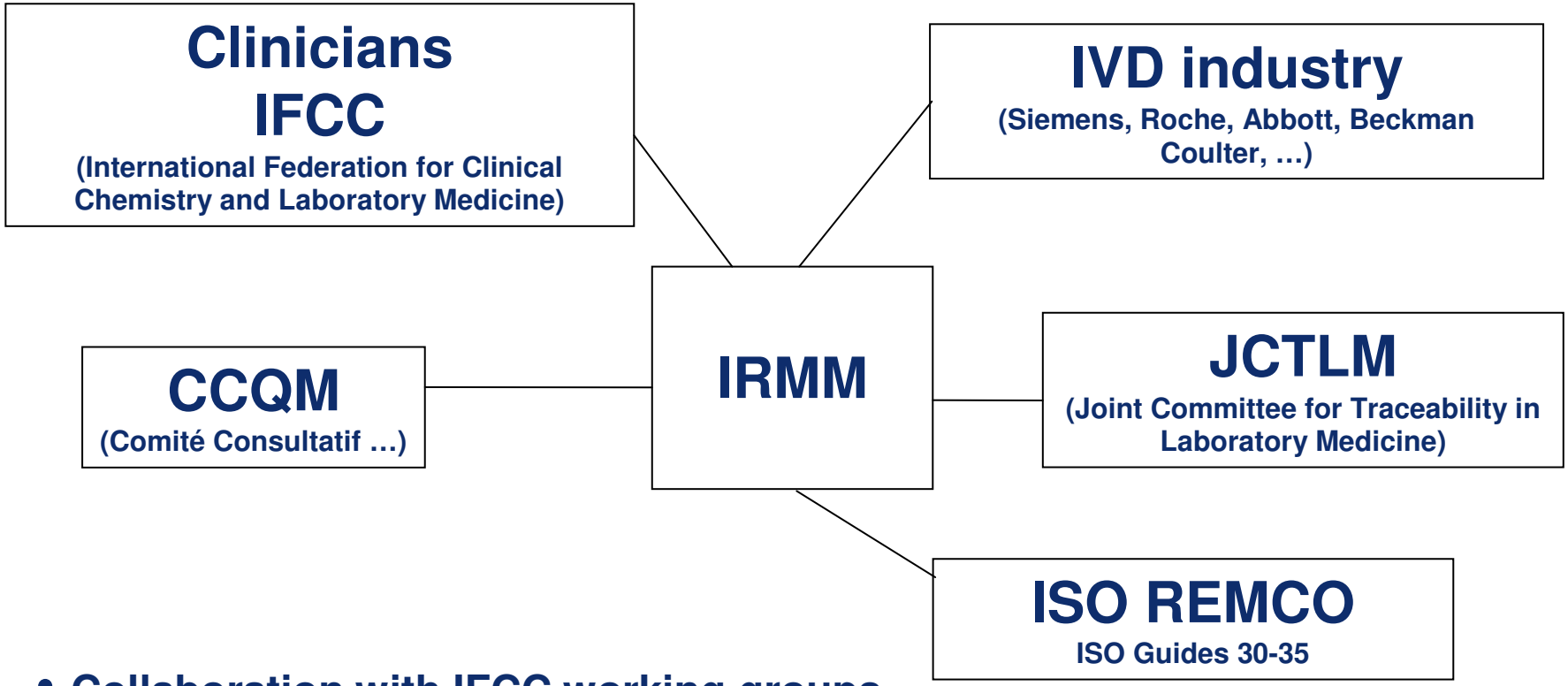

- Collaboration with IFCC working groups
- Production of reference materials
- Development of international standards (ISO)
- CCQM, JCTLM
- Biomolecular research and method development (only for needs not addressed elswhere than in IRMM)

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Reference Materials

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ISO

non-certified RMs

Certified RMs

non-certified RMs		Certified RMs
– homogeneous subsamples – appropriate stability	**material characteristics**	– homogeneous subsamples – appropriate stability
	additional investigations	metrologically valid establish-ment of property value(s)
• statements on homogeneity & stability	**accompanying information**	• property value(s) traceable to adequate reference system • stated meas. uncertainty • stated homogeneity & stability • intended use
➢ performance controls (precision, consistency) of methods or labs (internal & external) ➢ method developments	**main applications**	➢ calibration ➢ trueness control ➢ full method validation ➢ all QA/QC measures

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Reference Materials for Laboratory Medicine

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Hormones in human serum

Cortisol (unspiked/spiked)	ERM AD 192/193
Cortisol (reference serum panel)	ERM AD 451
Progesterone (low/high)	ERM DA 347/BCR-348R
Thyroxine (T4)	IRMM-468
3,3',5-triiodothyronine (T3)	IRMM-469
Estradiol-17 beta (low/medium/high)	BCR 576/577/578

Electrolytes and trace elements in blood and serum

Human blood: Cd, Pb	BCR 634/635/636
Human serum: Al, Se, Zn	BCR 637/638/639

Organic molecules in human serum

Creatinine (low/medium/high)	BCR 573/574/575
Creatinine interfering substances	BCR 573i

Coagulation factors

Bovine thromboplastin	ERM AD 148
Rabbit thromboplastin	ERM AD 149

Proteins

Apolipoprotein A I	BCR 393
Thyroglobulin (Tg)	BCR 457
Glycated heamoglobin	**IRMM/IFCC 466**
Non-glycated heamoglobin	**IRMM/IFCC 467**
Alphafoetoprotein	BCR 486
Prostate specific antigen (PSA)	BCR 613

Proteins in human serum

12 proteins	**ERM-DA470k/IFCC**
(A2M, AAG, AAT, ALB, C3c, C4, HPT, IgA, IgG, IgM, TSF, TTR)	
CRP	**ERM-DA474/IFCC**
Cystatin C	**EMR-DA471/IFCC**

Enzymes

Gamma-glutamyltransferase	ERM AD 452
Lactate dehydrogenase 1	ERM AD 453
Alanine aminotransferase	ERM AD 454
Aspartate aminotransferase	**ERM-DA 457/IFCC**
Creatine kinase-2 (CK-MB)	ERM AD 455
Creatine kinase (CK-BB)	BCR 299
Pancreatic alpha-amylase	IRMM/IFCC-456
Prostatic acid phosphatase	BCR 410
Adenosine deaminase	BCR 647
Pancreatic lipase	BCR 693
Recombinant lipase	BCR 694

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Traceability in clinical chemistry

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Directive 98/79/EC on In Vitro Diagnostic Medical Devices requires traceability of values assigned to calibrators and controls through available reference measurement procedures and reference materials of higher order

EC mandated standard related to the IVD directive: ISO 17511; In vitro diagnostic medical devices - Metrological traceability of values assigned to calibrators and control materials

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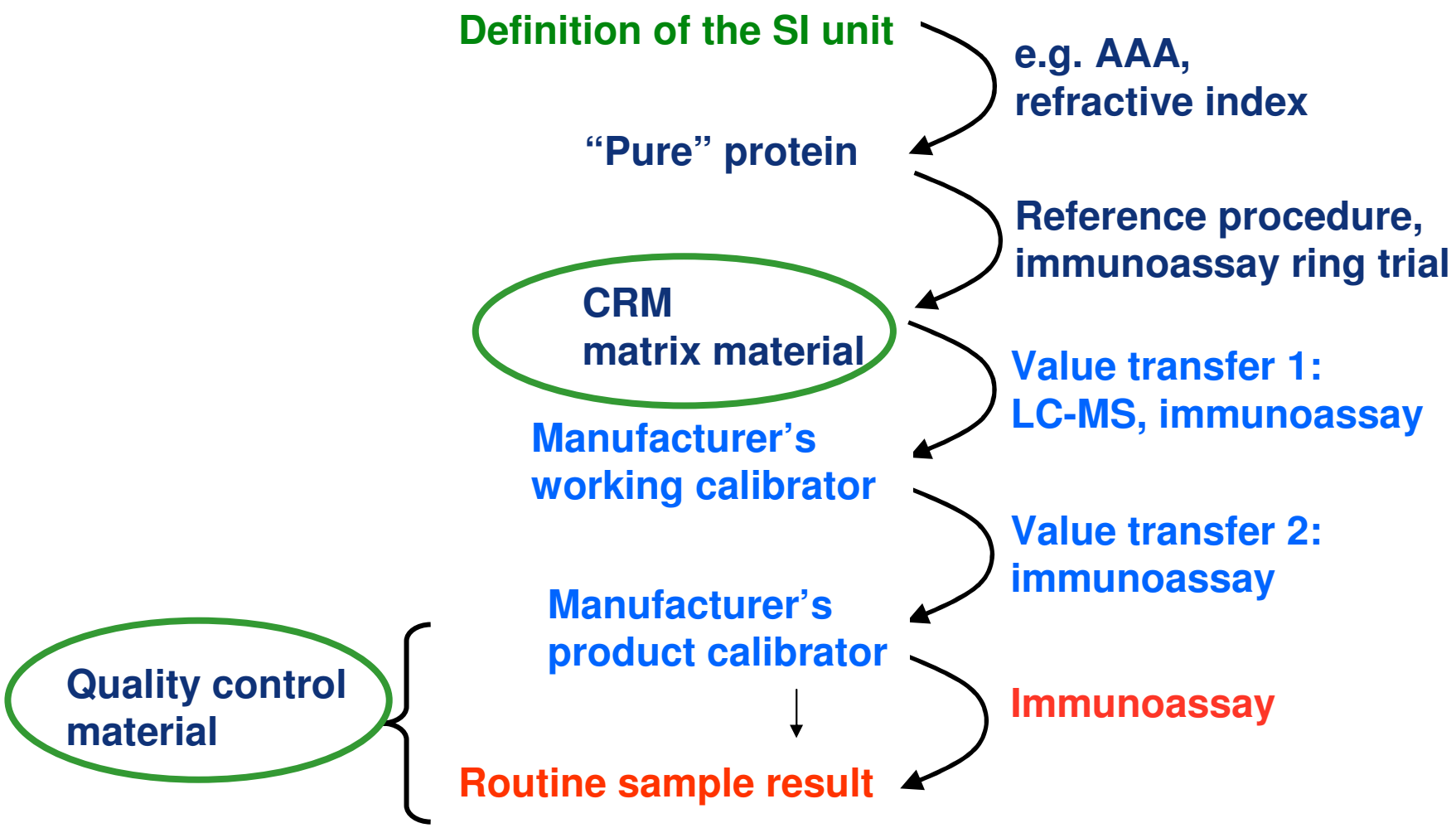
Traceability chain



Traceability hGH

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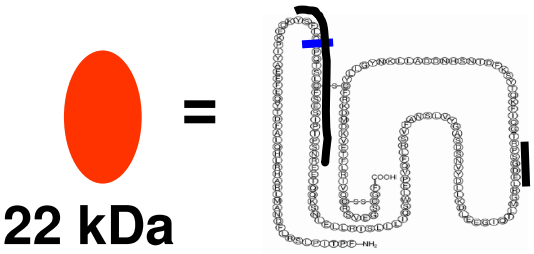
Definition of the SI unit

e.g. LC-MS



Recombinant protein

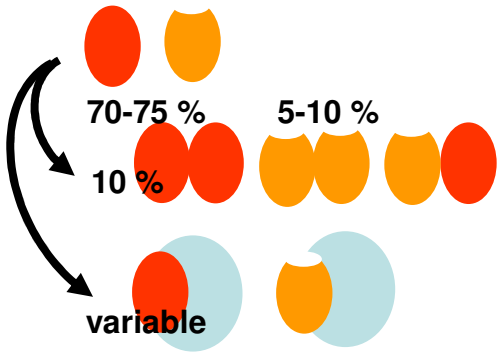
LC-MS

Manufacturer's working calibrator

polyclonal immunoassay

Manufacturer's product calibrator

Immunoassay



Routine sample result

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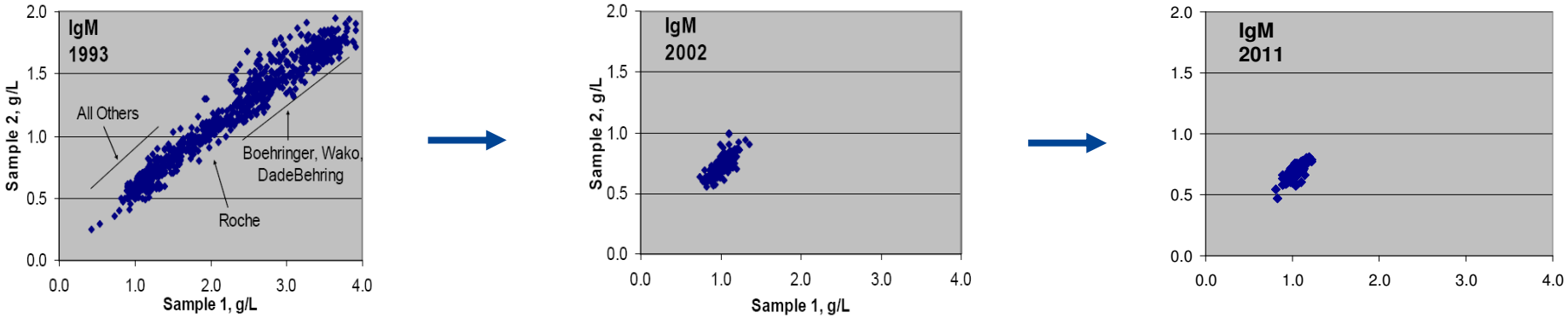
Traceability and comparability via reference materials

e.g. ERM-DA470k/IFCC

Certified for ALB, AAG, AAT, A2M, C3c, C4, HPT, IgA, IgG, IgM, TRF, TTR

Standardisation for IgM achieved by:



ERM-DA470

ERM-DA470k /IFCC

- **Collaboration between different partners (RM producer, IFCC, IVD manufacturers)**

- **Reference material that is fit for its purpose, and commutable (previous experience + 3 years of hard work by a large team)**

- **Development of value transfer protocols (Blirup-Jensen et al. 2001)**

Underlying assumption

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Being traceable **to a** common standard **or stated reference should ensure that** independently obtained measurement results will overlap **within their stated uncertainties and at a certain level of confidence with the true value and consequently with each other**

- provided measurement procedures applied in the traceability chain determine the **same measurand**
- if the comparison measurements do not introduce **unrecognised bias** (e.g. matrix effects, differential extraction etc.)
- if **all relevant uncertainty components** are included in the estimate of the combined uncertainties

Commutability

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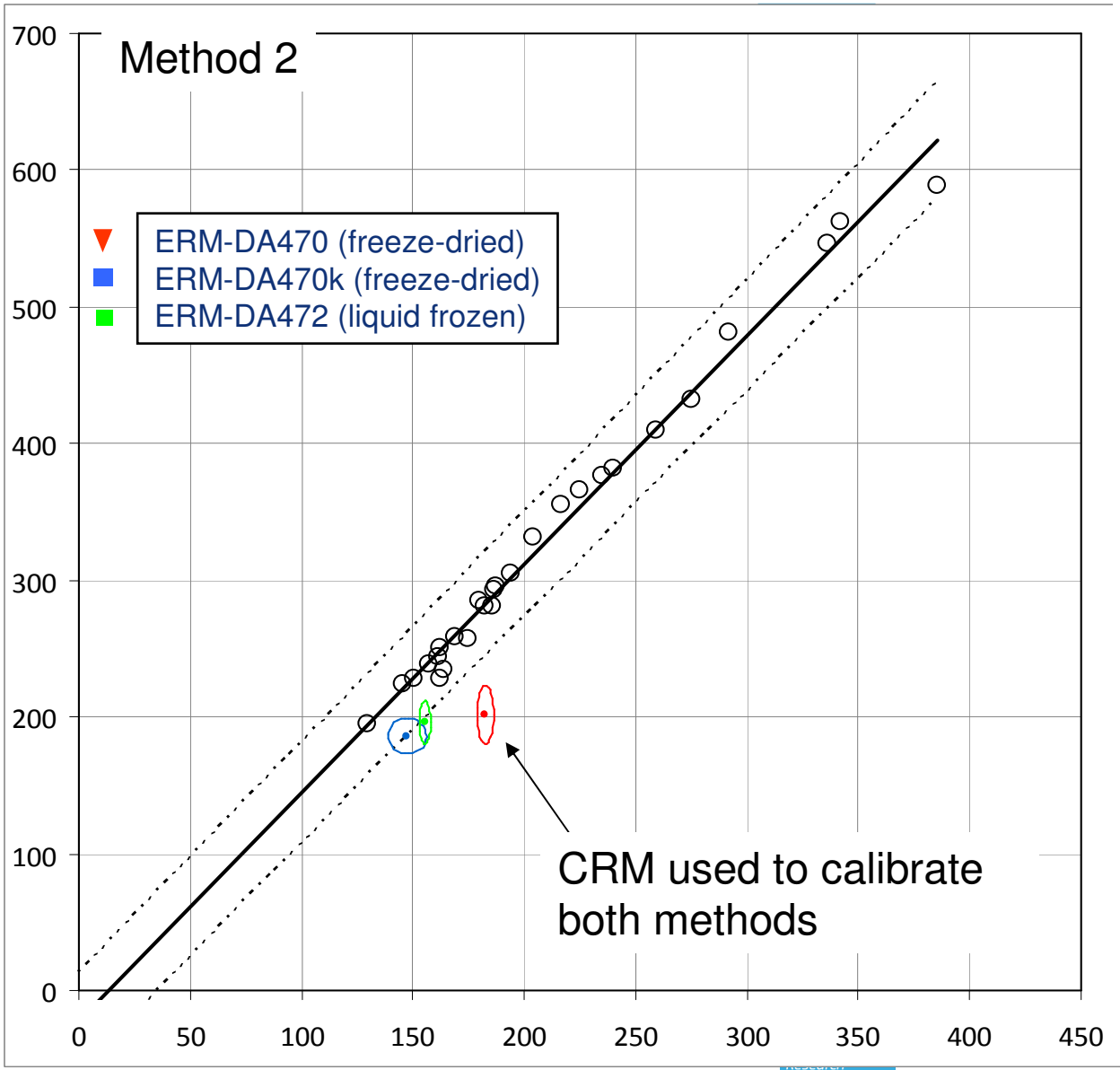
Wide spread belief:

Use of a common standard (eventually with an arbitrarily assigned unit) to calibrate different methods will improve comparability of measurement results

Only true under the condition that

- the methods to be compared **measure the same analyte** or different analytes but in a constant relationship in the samples to be analysed
- the common standard is **commutable**, i.e. behaves in the same way as patient samples for the methods used

Example: Commutability of CRMs for CER



Comparing the response of **(candidate) CRMs** with the response of **clinical samples**

ERM-DA470 is not commutable for this combination of methods

Requirements for a clinical CRM

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Traceability of values

To ensure the continuity of measurement results from assays calibrated against successive reference materials

Commutability: resemble patient samples

Homogeneity

The difference between the vials must be sufficiently small

Stability

The material must be stable over many years

Correct concentration range:

The relevant decision interval should be covered, e.g. for CRP a reference material at 1 mg/L is not useful

Low turbidity (for turbidimetry, nephelometry)

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Planning before CRM production

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- Evaluation of the comparability of measurement results obtained with different methods
- Definition of the measurand (e.g. Tau phosphorylated on position XXX)
- Concept for the traceability/ reference measurement system
- Reference method?
- Proposal for starting materials
  - e.g. CSF spiked with recombinant protein
- Feasibility studies on processing, stabilisation of the material.

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CRM planning

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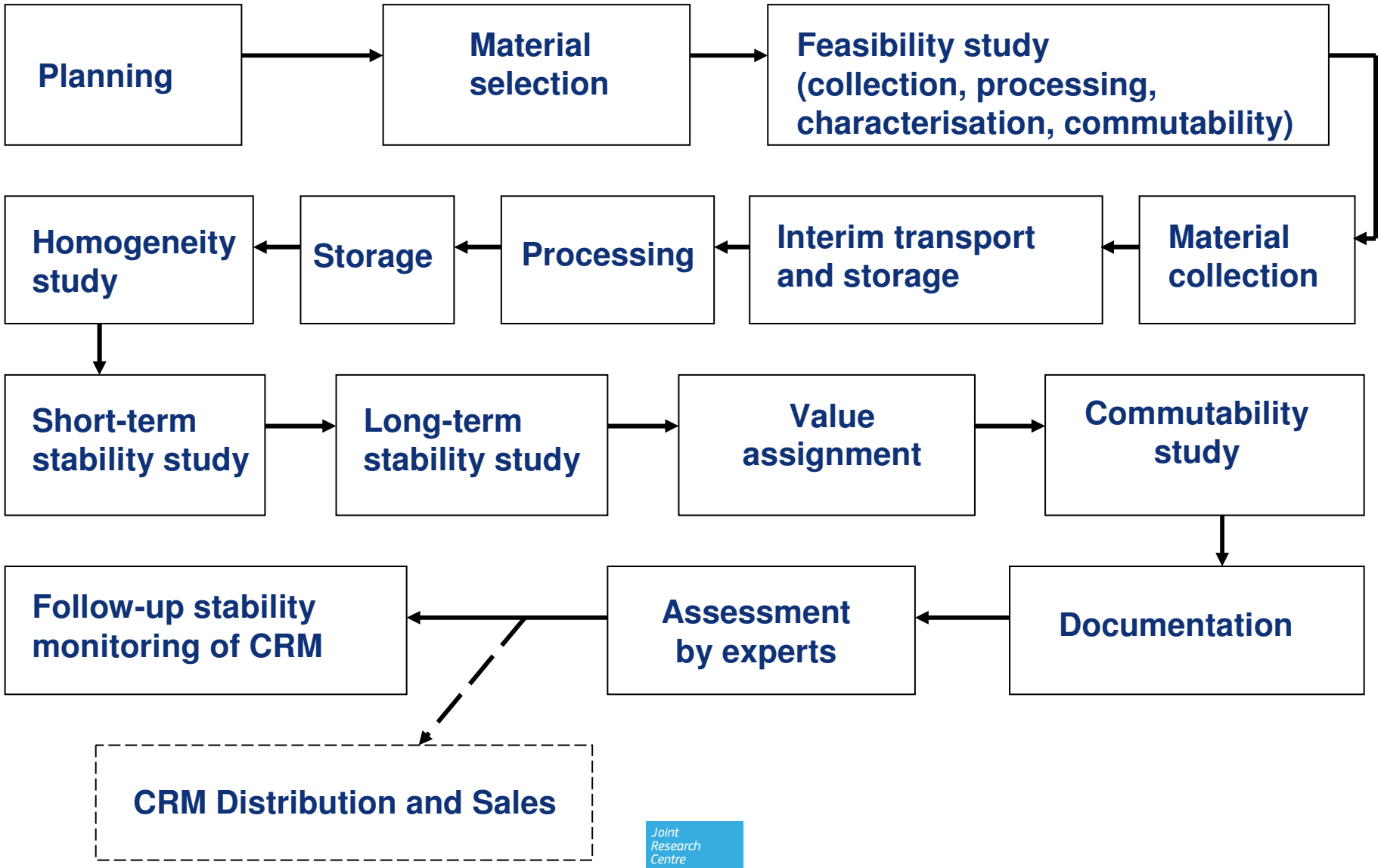

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Parameters of interest

In cerebrospinal fluid:

- Amyloid β 42 (Aβ42) healthy > 500 ng/L
  - Decrease in Alzheimer patients by about 50 %
- Total tau healthy < 300 ng/L
  - Increased in Alzheimer Disease (AD), Creutzfeld-Jacob (CJD), stroke by about 300 %
  - Reflects the intensity of neuronal degeration
  - 6 isoforms
- Phosphorylated tau (P-tau)
  - Increased in AD, not CJD and stroke
  - Many phosphorylation sites, Thr181 is main one
  - Reflects neurofibrillary tangles
- α-synuclein

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Methods

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All heterogenous immunoassays
Interlaboratory CVs 20-35 %

- Amyloid β 42 (Aβ42)
  - INNOTEST ELISA Novex
  - Luminex X-map with INNO-Bia AlsBio3
  - MesoScale Discovery (MSD), different antibodies
- Total tau
  - INNOTEST ELISA
  - Luminex X-map with INNO-Bia AlsBio3
- Phosphorylated tau (P-tau)
  - INNOTEST ELISA
  - Luminex X-map with INNO-Bia AlsBio3
- α-synuclein

1 Comparability of measurement results

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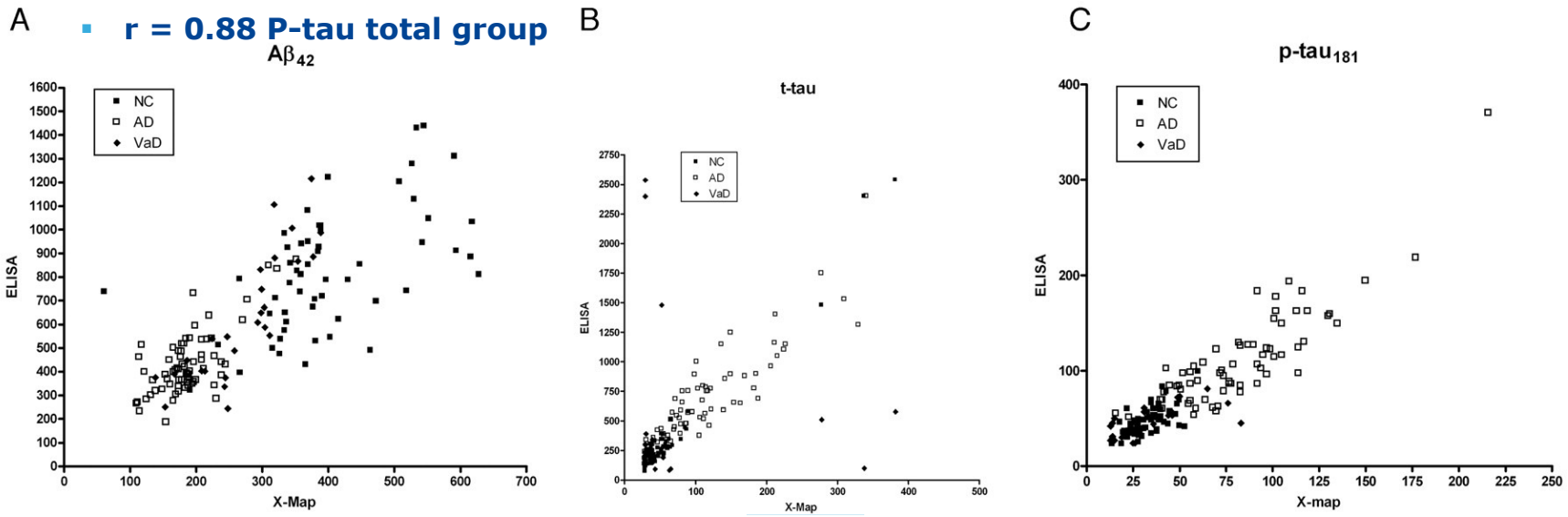
- Variation between laboratories: Aliquots of pooled CSF (Mattsson et al. 2011)
- Innotest ELISA, Luminex xMAP, Mesoscale: between lab CV 13-36 %
- Difference in absolute values (Mattsson et al. 2011) :
- Factor 2-10 difference
- Correllation: 150 Individual clinical samples (Reijn et al. 2007)
- Innotest (ELISA), Luminex (xMAP): Between method CVs about 30 %
- Correllation between ELISA and xMAP:
  - r = 0.87 Aβ42 total group
  - r = 0.93 t-tau total group
  - r = 0.88 P-tau total group



Feasibility studies

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Pilot batches can be produced to:

- Test the stability of the material
- Verify the commutability of the material
- Compare different formats of the material (e.g. liquid frozen and lyophilised)

⇨ Decision of format of the material

- Perform a feasibility studies for the value assignment with all the laboratories involved

Format of the material ?

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- Intended use: **Quality control or calibration?**
- Target analytes:
  - **Amyloid β 42 (Aβ42)**
  - **Total tau**
  - **Phosphorylated tau (P-tau)**
- Target concentration of analytes: **For calibration the concentration should be at the high end of the measurement interval, e.g. 500 ng/L**
- Nature of the matrix: **natural if commutable material**
  CSF collected from neurosurgery patients with ventricular drains or hydrocephalus patients?
- Nature of the protein: **native, isoform mix or recombinant**
- Definitation of the measurand: **e.g. Aβ42 peptide sequence, either method defined or structurally defined**
- Intended traceability statement: **traceable to the SI**

Certification measurements

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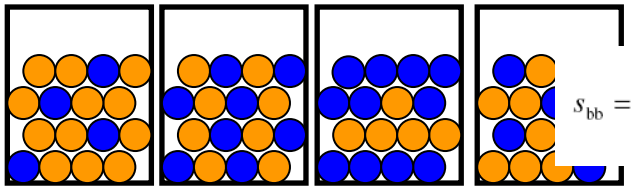
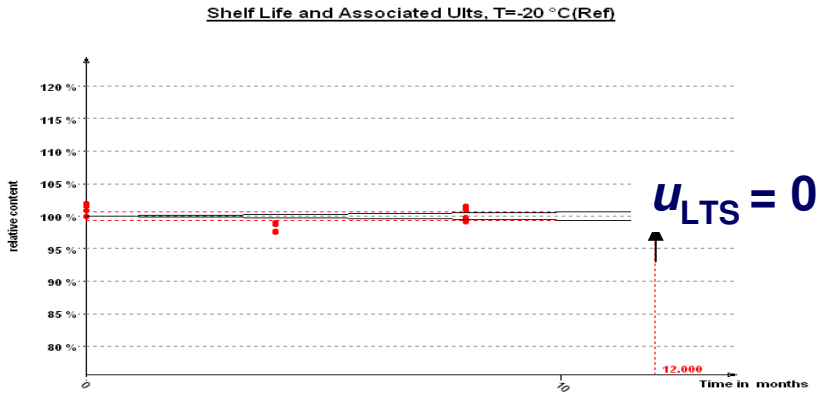
- **Homogeneity**



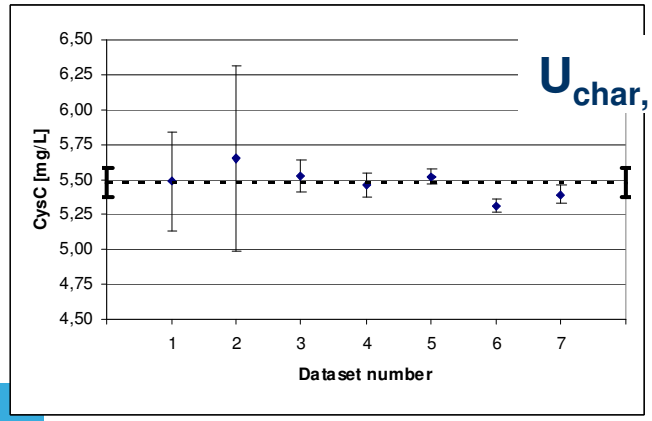
$$s_{bb} = \sqrt{\frac{MS_{bb} - MS_{wb}}{n}}$$

- **Stability**



u_{LTS} = 0.73 %

- **Characterisation/ value assignment**



$U_{char, rel}$ = 0.74 %

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Method performance

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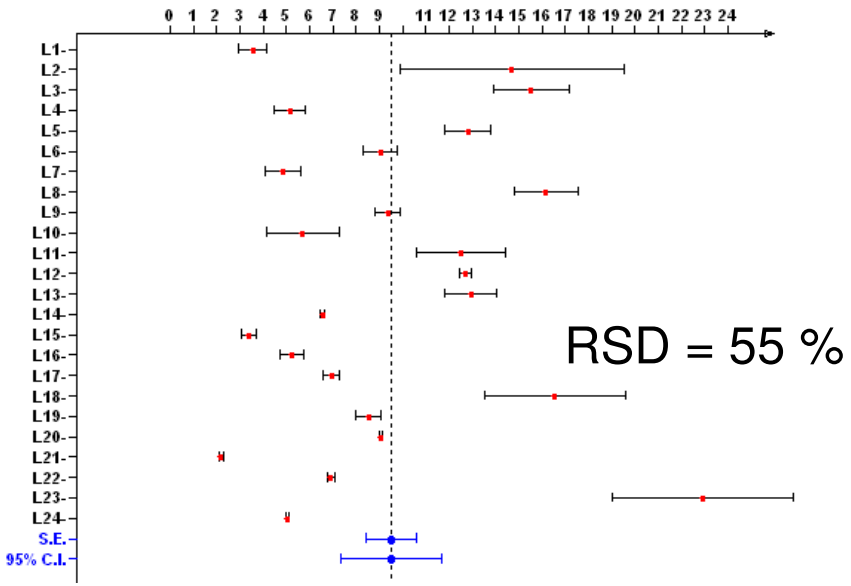
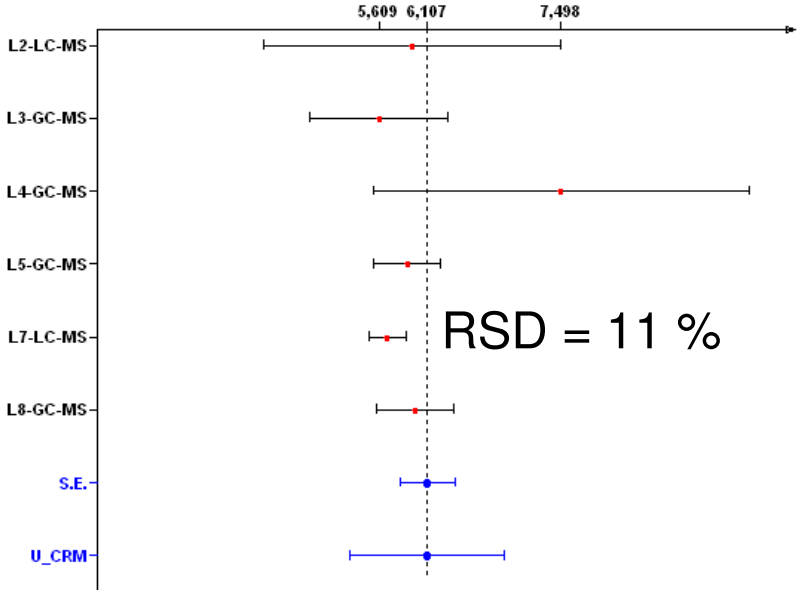
In house: Biochem/ Biotech laboratories

External: validated suppliers / collaborators

- Selection criteria (# for homogeneity/stability and characterisation)
- Organisation of feasibility studies, training



PT study



CRM characterisation

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value assignment

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Different approaches possible:

- Arbitrary units (IU) – normally not our approach
- By reference method (not yet available for Alzheimers' proteins)
- By ring trial:
  - At least 6 independent datasets
  - Requires a valid calibrant: e.g. pure protein solution value assigned by amino acid analysis and dry mass determination spiked into a backgound matrix.

dispatch conditions

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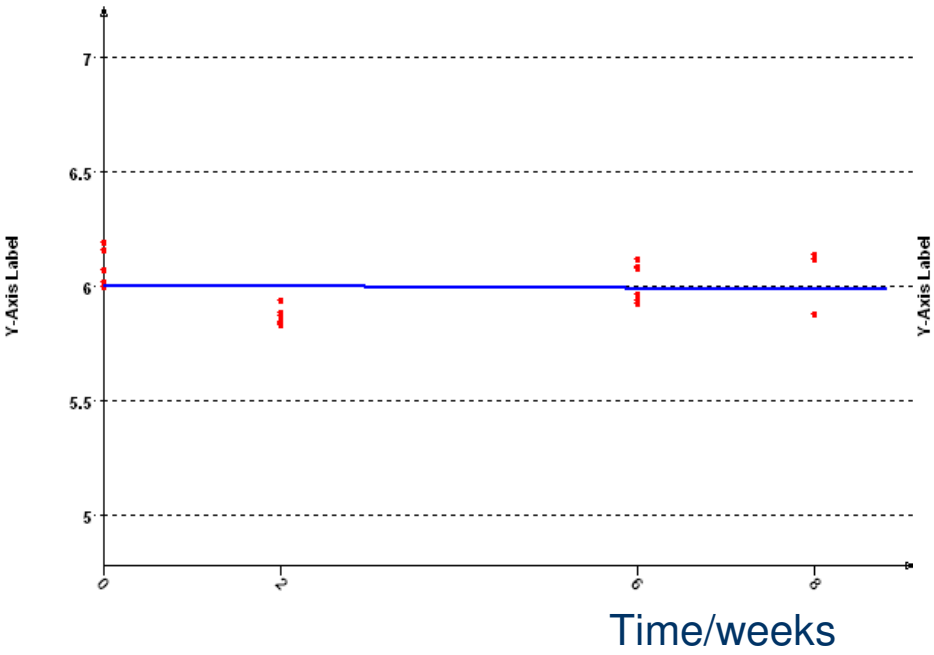
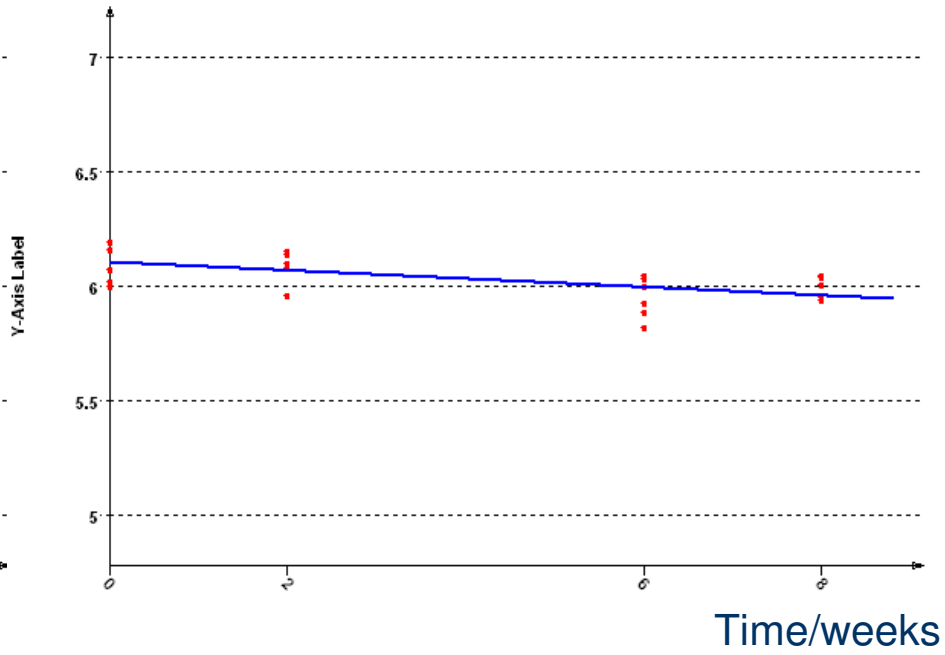
- stability during dispatch (up to 60 °C)
- relevant temperatures tested (-20, +4, 18, 40, 60)

4 °C



60 °C



- Significant slope?
- Uncertainty negligible?

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Shelf life

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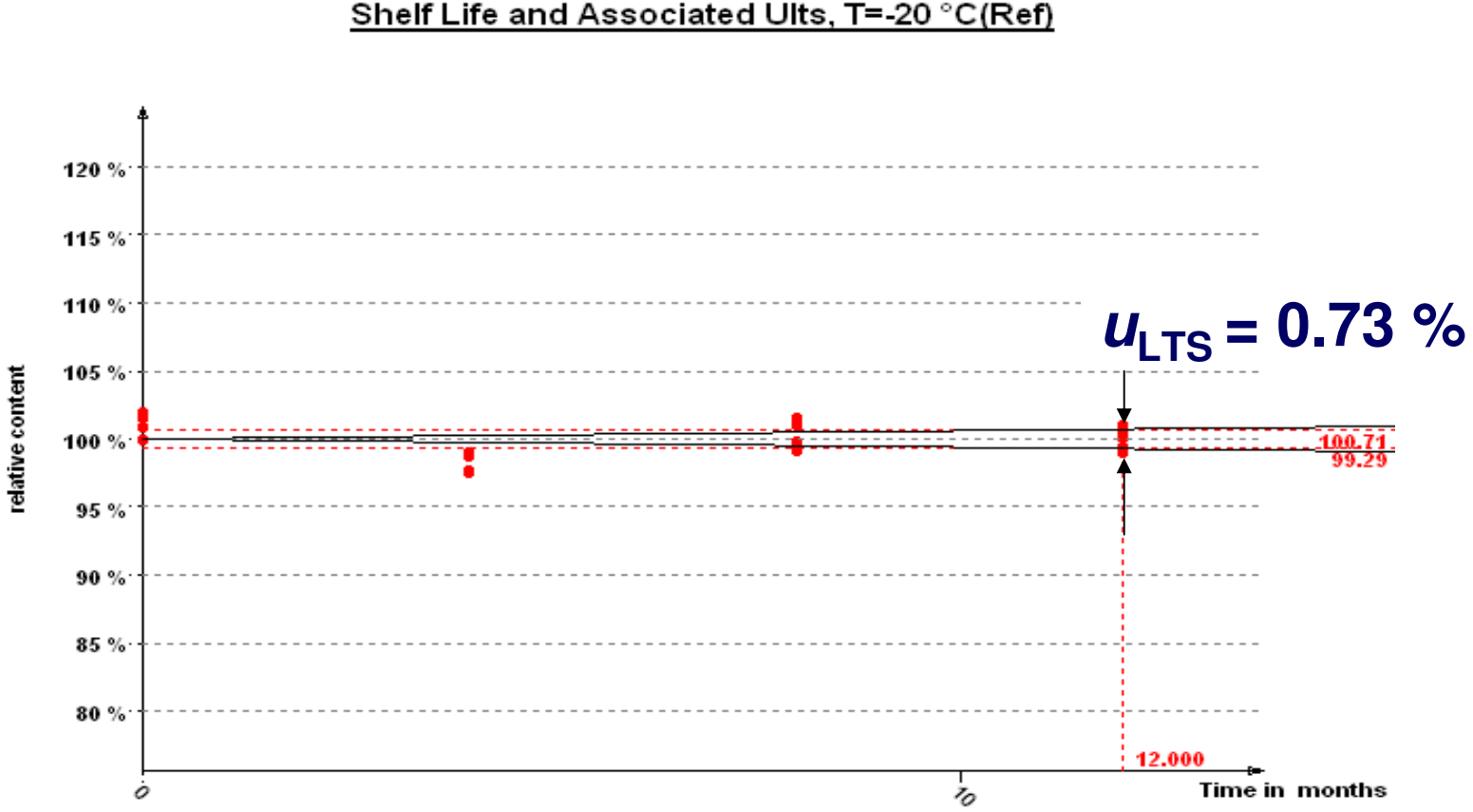

Stability monitoring over entire shelflife of material (may be up to 20-30 years)

Documentation

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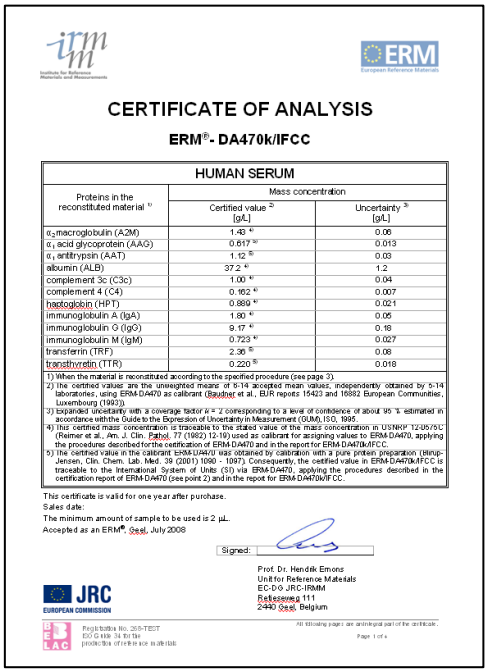

CERTIFICATE OF ANALYSIS

ERM®- DA470k/IFCC

HUMAN SERUM		
Proteins in the reconstituted material [1]	Mass concentration	
	Certified value [2] [g/L]	Uncertainty [3] [g/L]
α_2-macroglobulin (A2M)	1.48 [4]	0.06
α_1 acid glycoprotein (AAG)	0.617 [4]	0.013
α_1 antitrypsin (AAT)	1.12 [5]	0.03
albumin (ALB)	37.2 [4]	1.2
complement 3c (C3c)	1.00 [4]	0.04
complement 4 (C4)	0.162 [4]	0.007
haptoglobin (HPT)	0.889 [4]	0.021
immunoglobulin A (IgA)	1.80 [4]	0.05
immunoglobulin G (IgG)	9.17 [4]	0.18
immunoglobulin M (IgM)	0.723 [4]	0.027
transferrin (TRF)	2.36 [5]	0.08
transthyretin (TTR)	0.220 [5]	0.018

1) When the material is reconstituted according to the specified procedure (see page 3).
2) The certified values are the unweighted means of 9-14 accepted mean values, independently obtained by 9-14 laboratories, using ERM-DA470 as calibrant (Baudner et al., EUR reports 15423 and 16882 European Communities, Luxembourg (1993)).
3) Expanded uncertainty with a coverage factor $k = 2$ corresponding to a level of confidence of about 95 % estimated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM), ISO, 1995.
4) This certified mass concentration is traceable to the stated value of the mass concentration in USNKP 12-0575C (Reimer et al., Am. J. Clin. Pathol. 77 (1982) 12-19) used as calibrant for assigning values to ERM-DA470, applying the procedure described for the certification of ERM-DA470 and in the report for ERM-DA470k/IFCC.
5) The certified value in the calibrant ERM-DA470 was obtained by calibration with a pure protein preparation (Hirup-Jensen, Clin. Chem. Lab. Med. 39 (2001) 1090 - 1097). Consequently, the certified value in ERM-DA470k/IFCC is traceable to the International System of Units (SI) via ERM-DA470, applying the procedures described in the certification report of ERM-DA470 (see point 2) and in the repot for ERM-DA470k/IFCC.

This certificate is valid for one year after purchase.
Sales date:
The minimum amount of sample to be used is 2 µL.
Accepted as an ERM®, Geel, July 2008

Signed:

Prof. Dr. Hendrik Emons
Unit for Reference Materials
EC-D-9 JRC-IRMM
Retieseweg 111
2440 Geel, Belgium

JRC EUROPEAN COMMISSION

Registration No. 265-TEST
ISO Guide 34 for the production of reference materials

All following pages are an integral part of the certificate.

Page 1 of 4

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CERTIFICATION REPORT

Certification of proteins in the human serum

Certified Reference Material ERM®- DA470k/IFCC

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material **certificate** **Full report**

Examples: http://irmm.jrc.ec.europa.eu/Pages/rmcatalogue.aspx

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Thanks!

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