Establishing metrological traceability for chemistry - from mono-elemental calibration solutions to metalo-proteins

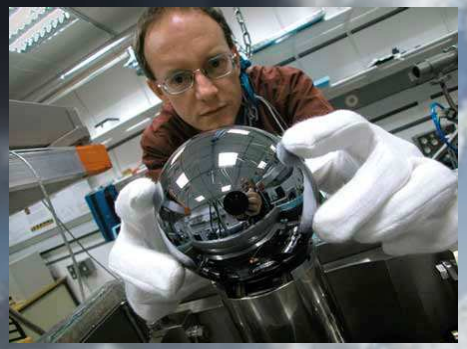


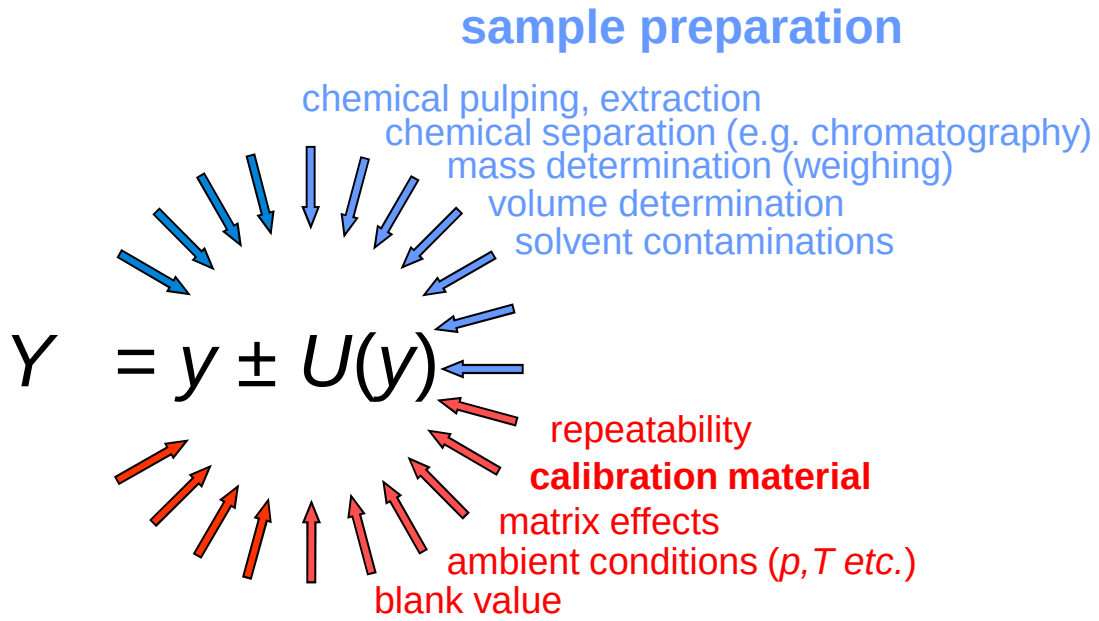
Foto: Okerlandarchiv

1. Traceability and measurement uncertainty for inorganic chemistry
2. Comparable measurements for water analysis in Europe
3. Species analysis for clinical markers

River Elbe between Hamburg and Cuxhaven © Vidicom / Christian Irrgang

Metrological Concept

A reliable measurement result is traceable and has a measurement uncertainty



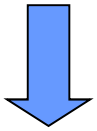
Reference: ISO Guide: *„Guide to the Expression of Uncertainty in Measurement"*

metrological traceability

property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty

International vocabulary of metrology (VIM), 2.41

$$Y = y \pm U(y)$$



International System of Units (SI)

why traceability?

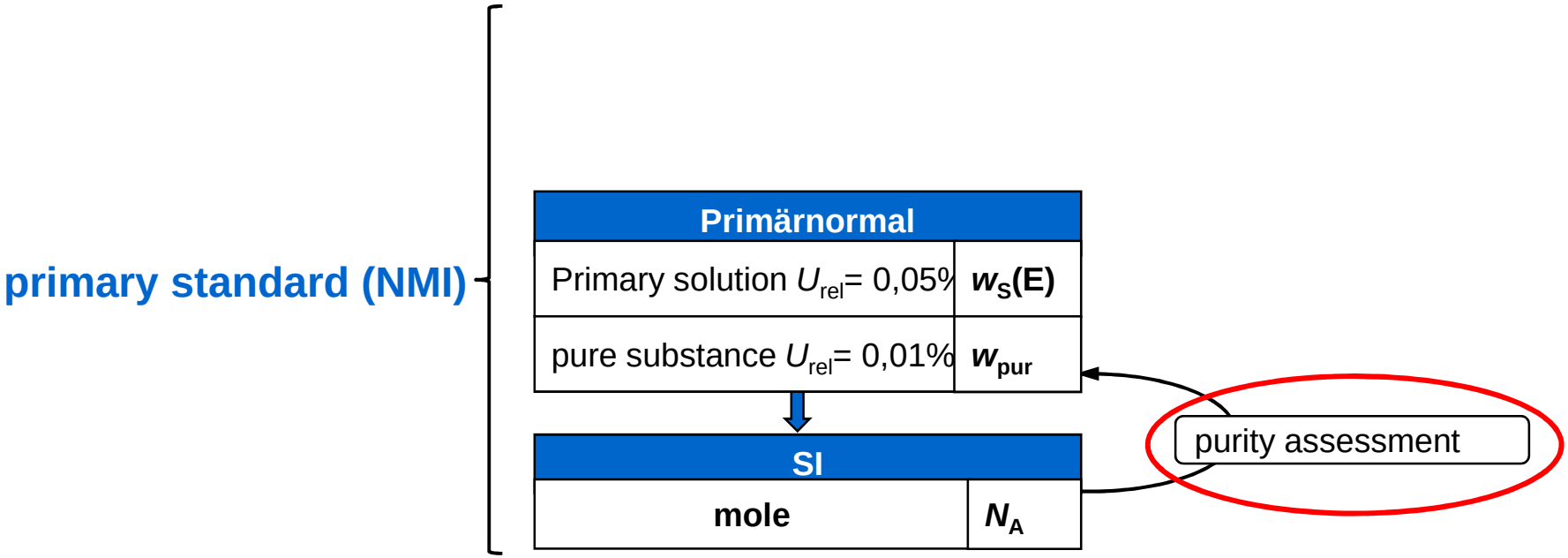
Comparison measurements with consensus value

- snapshot at a point in time
- only applicable to participants
- accuracy unknown

Traceability to the SI

- sustainable in space and time
- results internationally comparable
- Internationally accepted reference value

dissemination of elemental solution standards



Matschat, R. et. al., ACQUAL 10 (2006), S. 633-639

national standards for element analysis

BAM

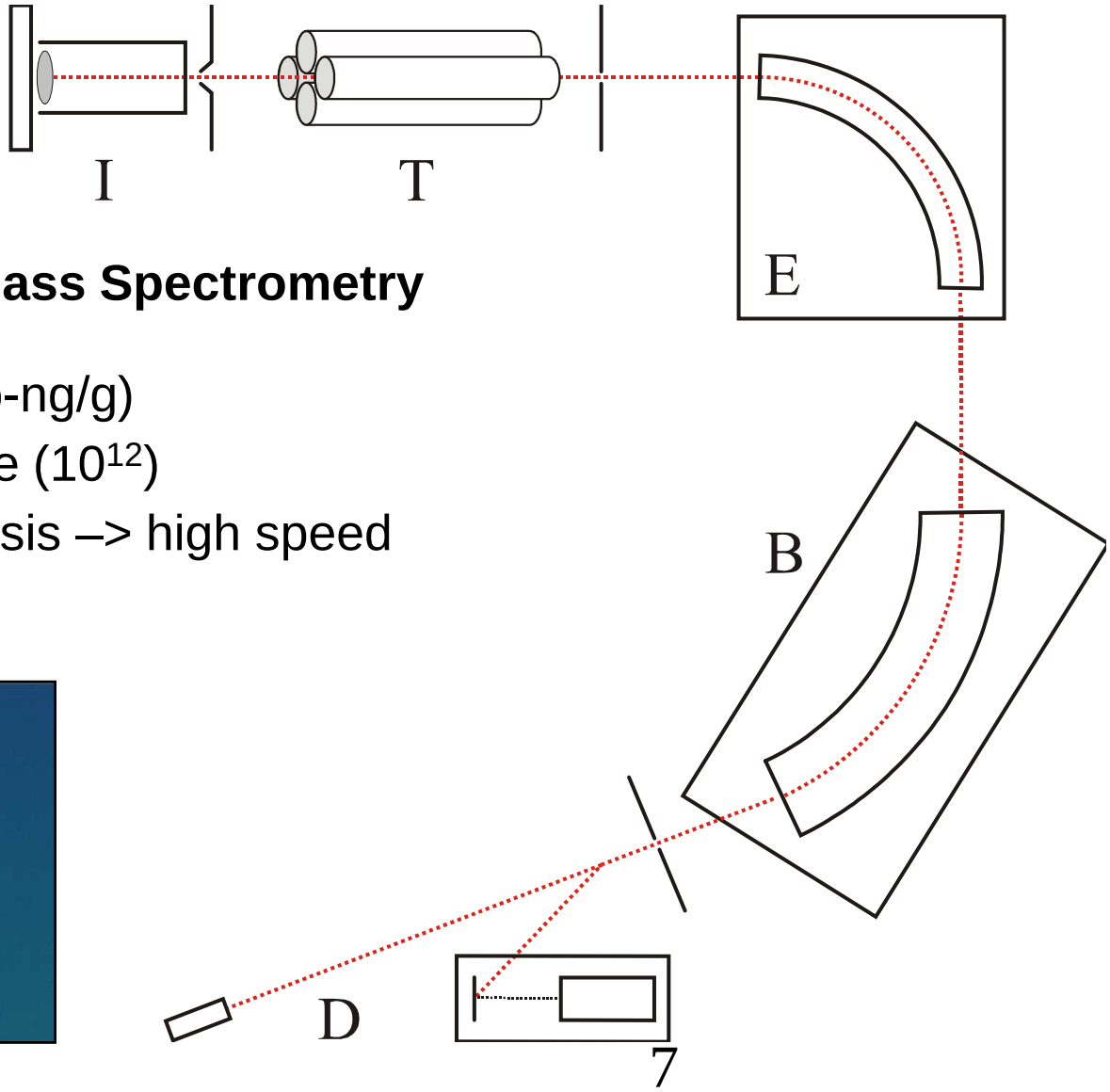
$$n = \frac{m \cdot w_{pur}}{M}$$

BAM-A-primary-Cu-1
LOT B27F17

	matrix	impurity	sum 'above'	um/2 'below'	not	under
	in %	in mg/kg	in mg/kg	in mg/kg	relevant	nvestigation
mass fraction	99,9968	32,33	22,38	9,95	(estimate)	
abs. uncertainty	0,0005	5,27	3,84	3,61		

H < 2,1																	He < 0,001
Li < 0,31	Be < 1,1											B < 3,2	C 0,04	N 0,2	O 1	F < 2	Ne < 0,001
Na 0,002	Mg < 0,05											Al < 0,07	Si < 0,002	P < 2	S 5,4	Cl < 0,6	Ar < 0,001
K < 0,002	Ca 0,1	Sc < 0,06	Ti < 0,32	V < 0,04	Cr 0,07	Mn 0,01	Fe < 5	Co < 0,11	Ni 1,64	Cu matrix	Zn 0,057	Ga < 0,11	Ge < 0,12	As 0,5	Se 0,22	Br < 0,014	Kr < 0,001
Rb < 0,05	Sr < 0,014	Y < 0,03	Zr < 0,015	Nb < 0,02	Mo < 0,06	Tc < 0,001	Ru < 0,03	Rh < 1,6	Pd < 0,014	Ag 11,3	Cd < 0,015	In < 0,05	Sn 0,14	Sb 1	Te < 0,22	I < 0,09	Xe < 0,001
Cs < 0,0057	Ba < 0,017	La < 0,002	Hf < 0,003	Ta < 0,003	W < 0,12	Re < 0,009	Os < 0,004	Ir < 0,007	Pt < 0,007	Au < 0,008	Hg < 0,03	Tl < 0,005	Pb 0,47	Bi 0,23	Po < 0,001	At < 0,001	Rn < 0,001
Fr < 0,001	Ra < 0,001	Ac < 0,001															
			Ce < 0,0057	Pr < 0,002	Nd < 0,21	Pm < 0,001	Sm < 0,007	Eu < 0,003	Gd < 0,001	Tb < 0,001	Dy < 0,001	Ho < 0,001	Er < 0,001	Tm < 0,001	Yb < 0,001	Lu < 0,002	
			Th < 0,02	Pa < 0,001	U < 0,001												

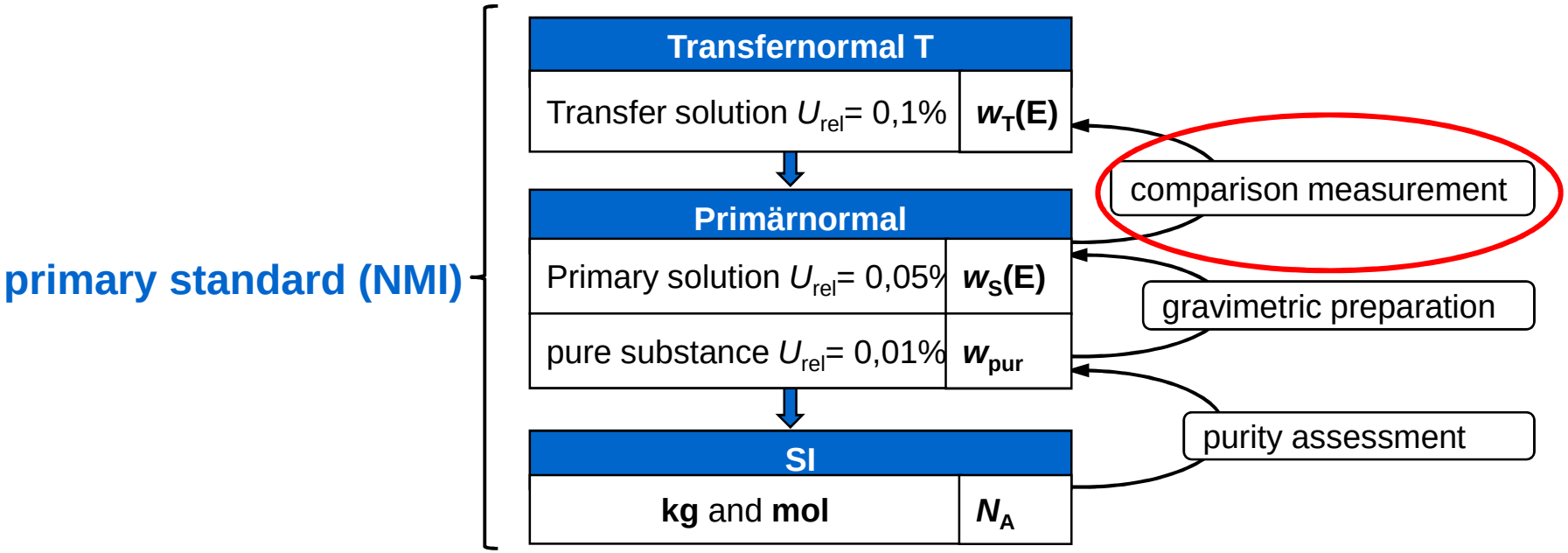
GD-MS – direct solid state analysis (purity assess)



Glow-Discharge Mass Spectrometry

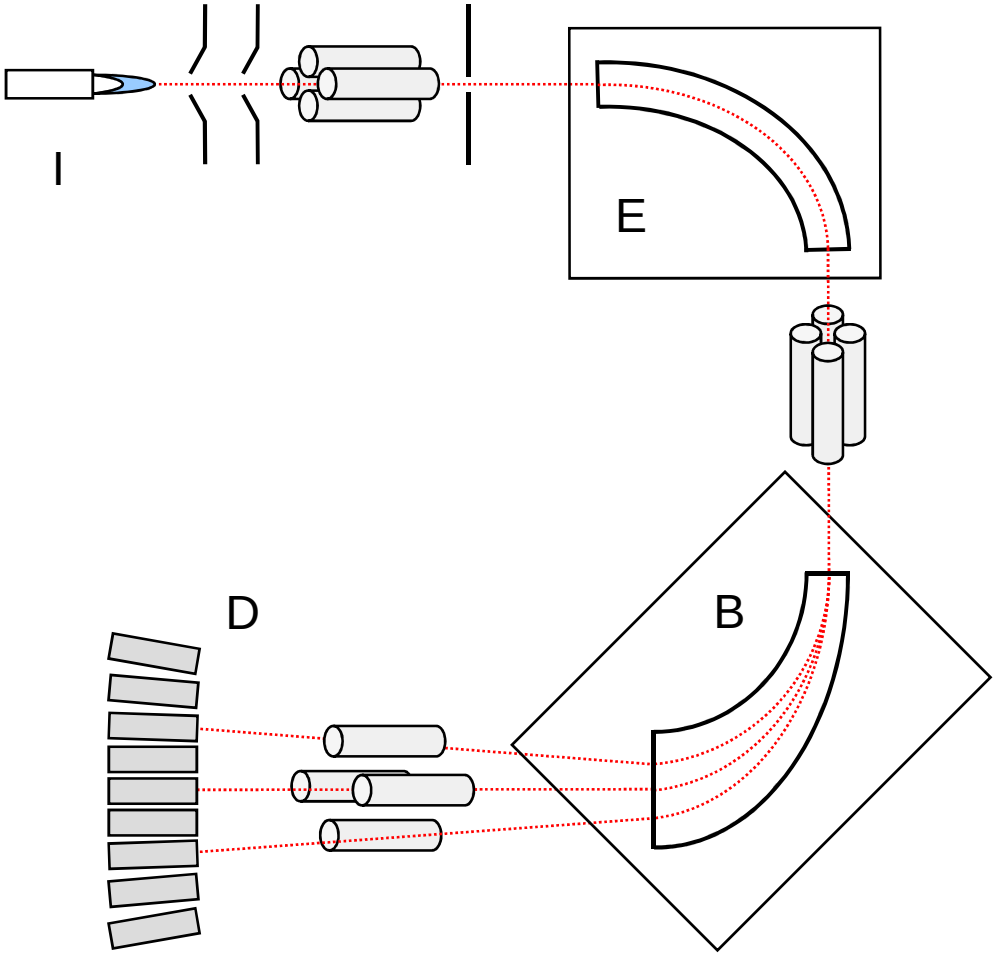
high sensitivity (sub-ng/g)
large dynamic range (10^{12})
multi-element analysis –> high speed
high precision

dissemination of elemental solution standards



Matschat, R. et. al., ACQUAL 10 (2006), S. 633-639

MC-ICP-MS (Matrix)



- ion source
  - plasma-ionisation: simple efficient, large energy range
  - discriminiation
- analyser
  - double focussing
- detector
  - simultaneous measurements
  - faraday-cups
  - high precision
  - medium sensitivity

element calibration solutions

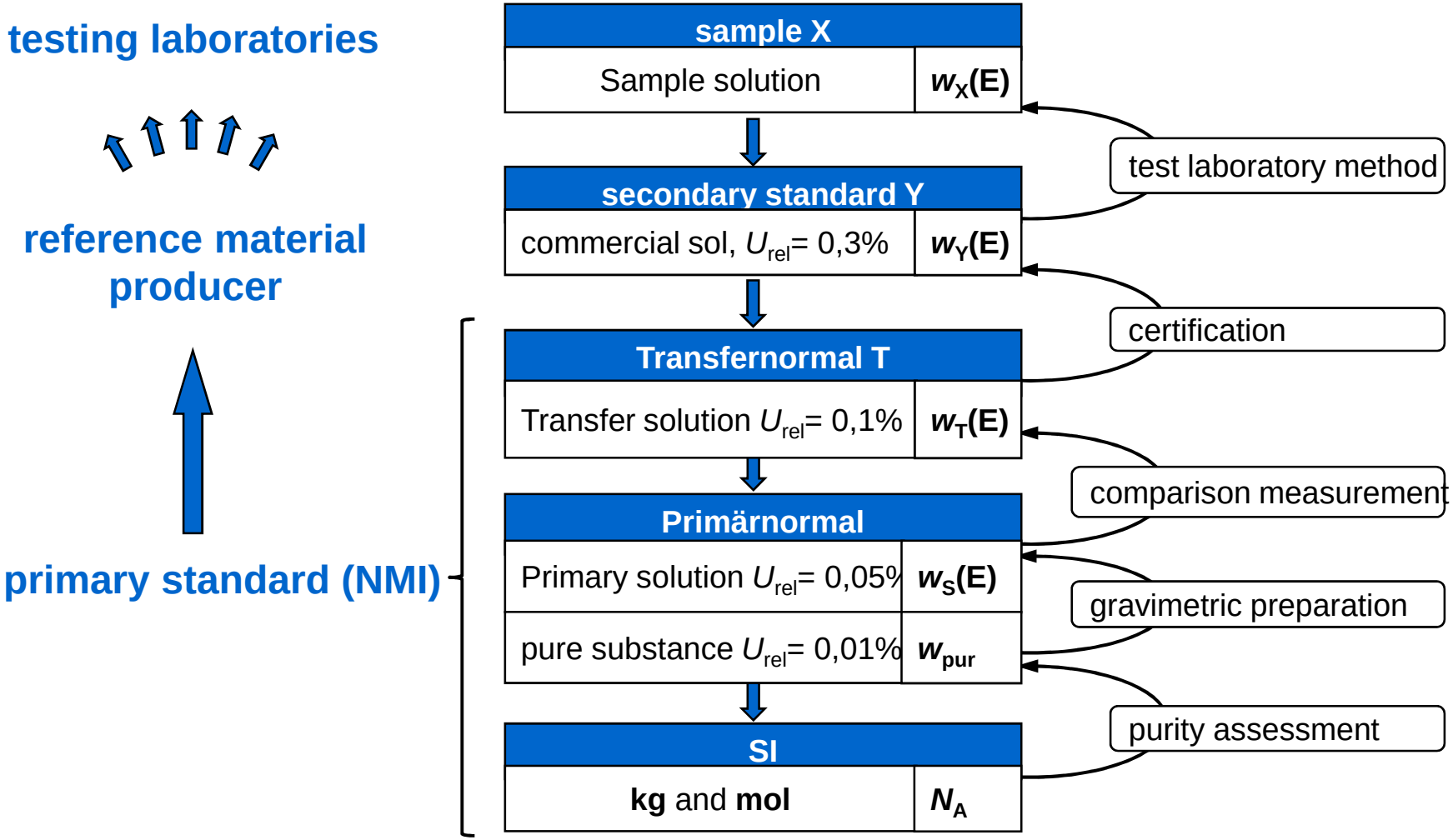
BAM/PTB/Merck-project – traceability system for element standard solutions

dissemination of elemental solution standards

testing laboratories

reference material producer

primary standard (NMI)



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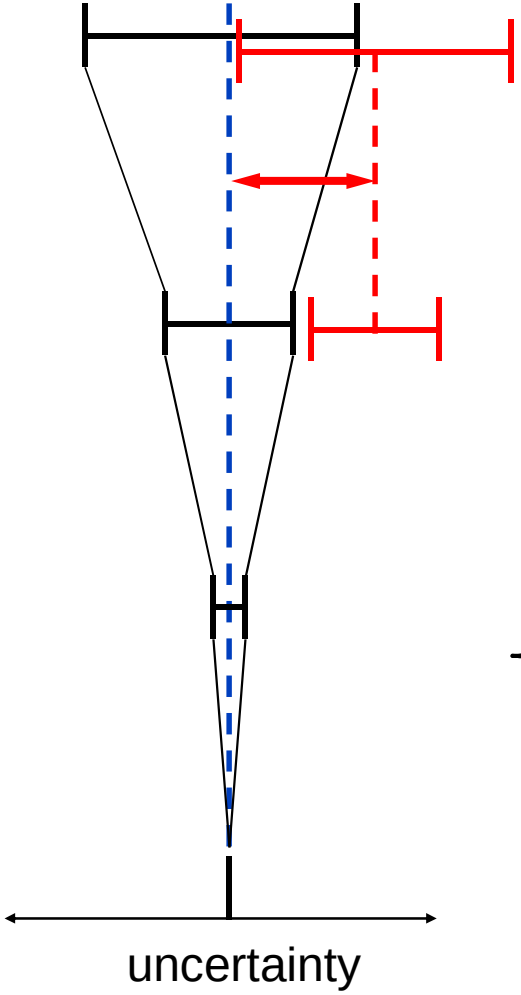
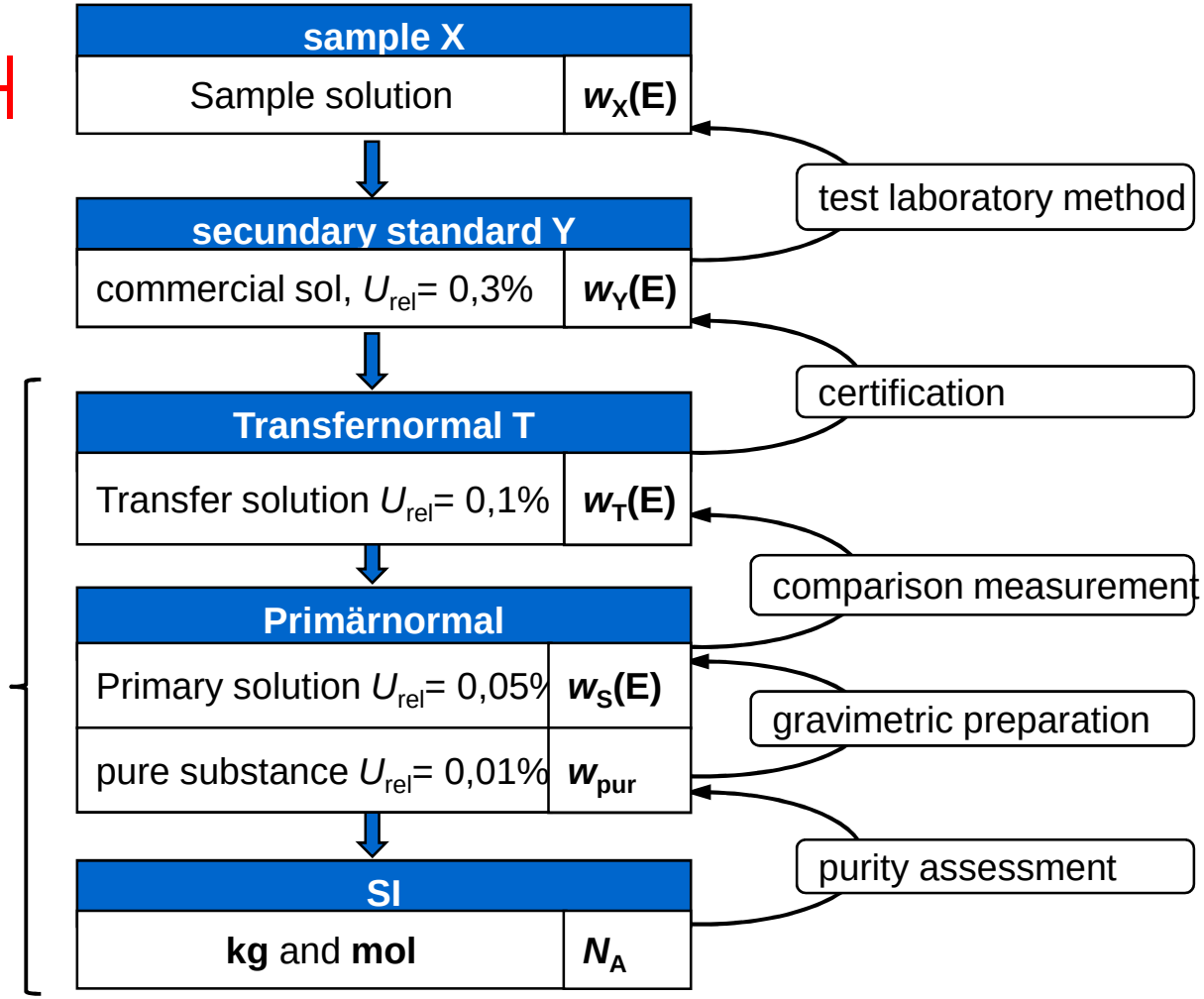
dissemination of elemental solution standards





Matschat, R. et. al., ACQUAL 10 (2006), S. 633-639

comparability in inorganic chemistry: water

Increasingly demanded as a quality criterion for measurement results in chemistry

EU Water Framework Directive 2000/60/EC (WFD)

Groundwater Directive 2006/118/EC

Drinking Water Directive 98/83/EC

Marine Strategy Framework Directive 2008/56/EC

In vitro Diagnostica 98/79/EG

ISO/IEC 17025

ISO/IEC 17043

Toxic elements in surface waters

EU Water Framework Directive 2000/60/EC (WFD)

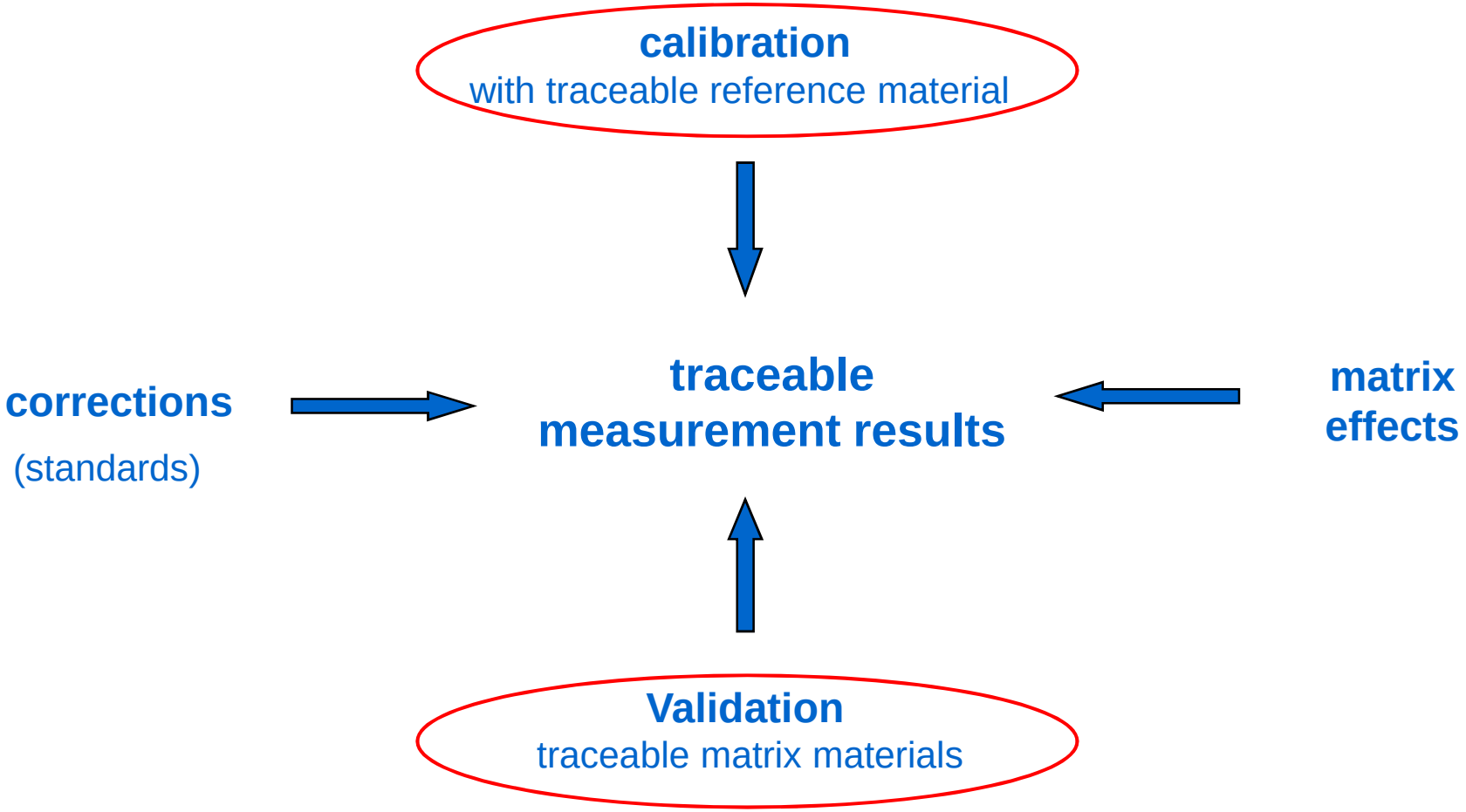
Groundwater Directive 2006/118/EC

Drinking Water Directive 98/83/EC

ISO/IEC 17025

ISO/IEC 17043

How to achieve traceability?



Water – global needs and problems

clean water is an indispensable prerequisite for life

- less than 1 % of all waters on Earth are suitable and available for human consumption.
- more than 1 200 million people have no access to clean drinking water
- more than 4500 children die worldwide every day because of diseases caused by water pollution

(EU, German Government)

Chemical status: Where do we come from ?

Status GDR 1990

- main rivers and many surface waters are heavily contaminated
- only 42% of the population has access to an adequate sewage system
- only 20% of water courses can be used for drinking water.

- life expectancy in certain areas is as much as ten years below the state average

(EU press release P/90/67 Date: 27/09/1990)

Water in the European Union

The water framework directive - a common legislation for at least 27 states and more than 500 million people

implementation of the directive under drastically different and changing conditions in different countries

EU Water Framework Directive 2000/60/EC (WFD)

Motivation

- 20 % of all surface waters in the European Union are heavily polluted.
- 65 % of the drinking water originate from ground water resources.
- 60 % of the European cities overuse their ground water resources.
- 50 % of the EU wetlands (and their biota) are endangered because of overused ground water
- The artificially watered areas in the EU increased by 20 % since 1985.

(e.g., European Environmental Agency, report 1995)

EU Water Framework Directive 2000/60/EC

Aims

- to establish a legal framework to protect and restore clean water across Europe and to ensure its long-term and sustainable use
- to expand the scope of water protection to all waters: surface waters, transitional waters, coastal waters and groundwater
- to achieve a "good status" for all waters
- to achieve a water management based on river basins
- to implement a "combined approach" of emission limit values and quality standards

WFD Quality standards: Chemical status

Good chemical status

- ... groundwater should broadly be that it should not be polluted at all (European Quality standards - EQS - level)

- compliance with all the quality standards for chemical substances at European level.

- prioritisation mechanism for hazardous chemicals: ensuring a minimum chemical quality, in relation to very toxic substances, everywhere in the Community

Chemical status: priority substances

selection criteria

- aquatic ecotoxicity and human toxicity via aquatic exposure routes
- Intrinsic hazard (toxic, persistent and liable to bio-accumulate)
- evidence from monitoring of widespread environmental contamination
- other proven factors indicating possible widespread environmental contamination (e.g., industrial production rates)

Decision 2455/2001/EC of the European Parliament and the Council

Chemical status: priority substances

Selected **33 priority substances** or groups of substances:
(WFD) 2000/60/EC & QA/QC Directive (2009/90/EC)

- 4 heavy metals
- 14 pesticides
- 15 organic compounds of industrial origin
  (e.g. flame retardants)

review every four years

priority substances: measurement requirements

Requirements of the QA/QC directive (2009/90/EC)
to assure comparable measurement results
(… pursuant to Directive 2000/60/EC)

- Methods have to be validated and documented in accordance to EN ISO /IEC17025
- Minimum performance criteria for the analytical method:

Limits of quantification	≤ 0.3 * EQS
Uncertainty	≤ 0.5 * EQS

- Monitoring laboratories have to participate in PT shemes
- organized by accredited or internationally recognized organisations
- evaluated according to ISO Guide 43-1 or ISO 13528 or equ.

EUROMET Project 924

A sustainable traceability system providing Europe-wide comparable measurement results in water monitoring under the WFD

organized by BAM, LNE, IRMM, PTB

Task:

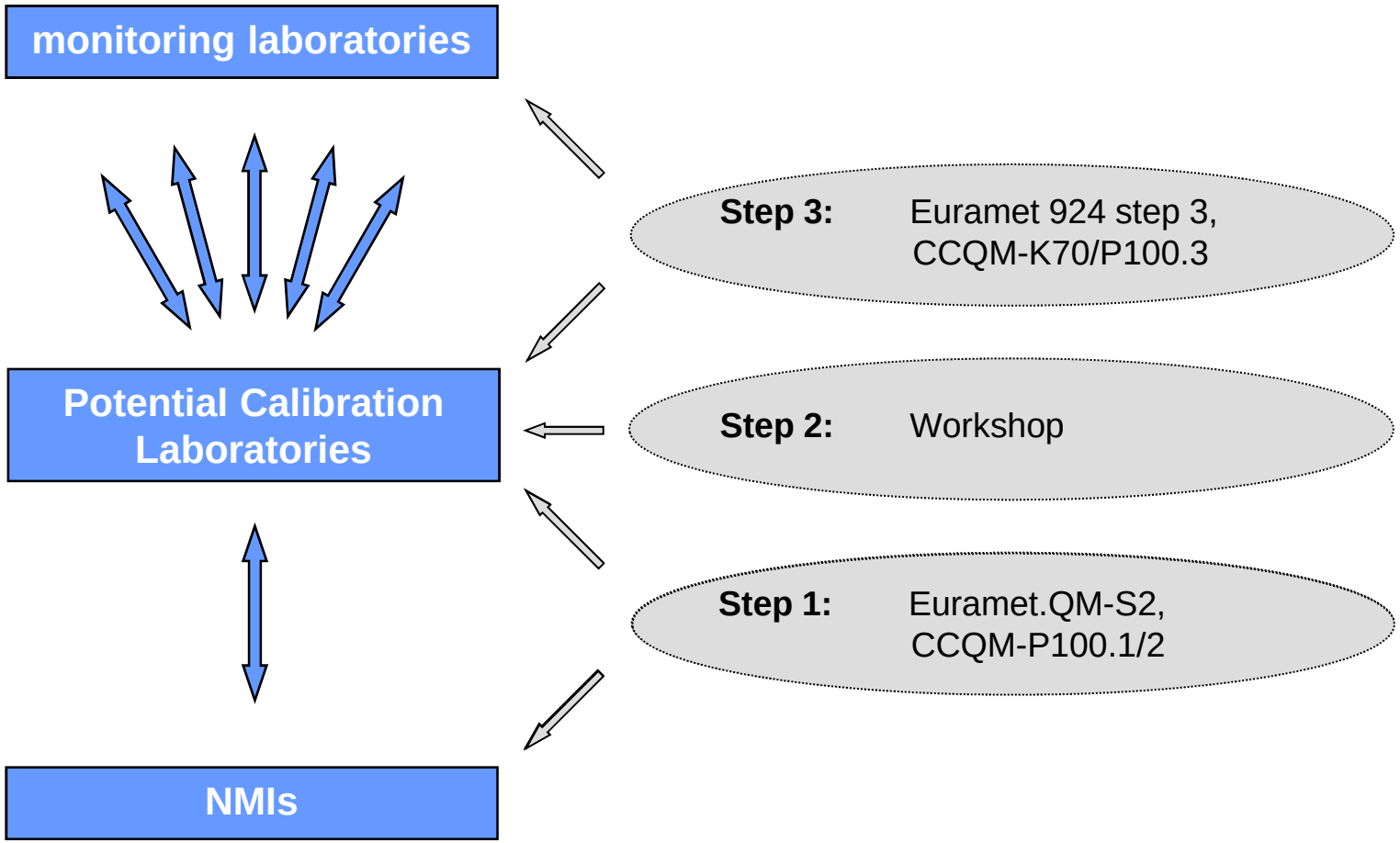
Comparison measurements for selected priority substances:
heavy metals (Ni, Pb, Cd, Hg)

Aims:

- provide reference points (National Standards, CMCs) of sufficient quality (suitable for EQS-requirements) to serve the WFD
- establish links to expert* and monitoring laboratories in Europe
  **pt-providers, potential calibration laboratories (PCL)*
- assess comparability and measurement uncertainties in the field
- validate methods of expert laboratories
- feasibility of a metrological dissemination system in Europe

Lake Pielinen, Finland

Dissemination Structure



Technical details (EURAMET 924, step 1)

- 2 samples:

  purified water and natural ground water

- Samples prepared and dispatched by PTB, LNE in cooperation with IWW

- Filtered

  purified water:

- Concentration ranges :

Hg	(30	-	70)	ng/l
Cd	(50	-	150)	ng/l
Ni	(10	-	30)	µg/l
Pb	(4	-	20)	µg/l

  EQS-levels!

- Gravimetric reference values available

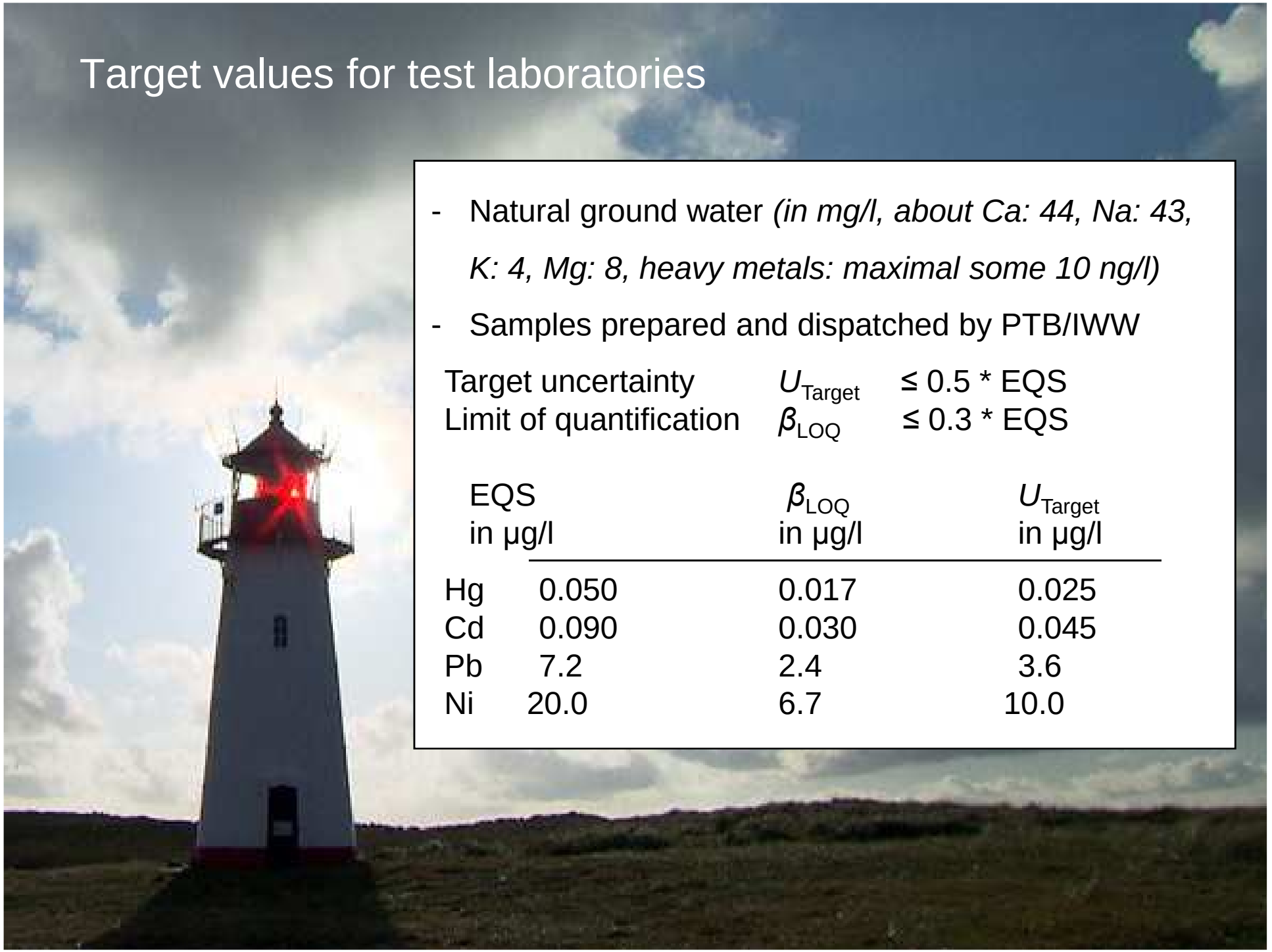
- Hg samples stabilized with BrCl according to EPA 1631

- Stability, homogeneity and Hg content of fresh (unspiked) water tested by PTB and IWW

EURAMET 924/Step 1: NMIs & Expert institutes

- NMIs:
  PTB (Ger), BAM (Ger), LNE (Fr), IRMM (EU)
  P100:
  NIST (USA), LGC (UK). NRC (Can)
- PT-Providers (PCL):
  8 Germany
  4 France
  1 Sweden (NMI)
  1 Norway
  1 Finland
  1 Romania (NMI)
  1 Bulgaria (NMI)
  1 Austria
  2 Portugal
  1 Italy
  1 Hungary
  2 Israel
  1 Chile (NMI)

Target values for test laboratories



- Natural ground water *(in mg/l, about Ca: 44, Na: 43, K: 4, Mg: 8, heavy metals: maximal some 10 ng/l)*
- Samples prepared and dispatched by PTB/IWW

Target uncertainty	U_{Target}	≤ 0.5 * EQS
Limit of quantification	β_{LOQ}	≤ 0.3 * EQS

	EQS in µg/l	β_{LOQ} in µg/l	U_{Target} in µg/l
Hg	0.050	0.017	0.025
Cd	0.090	0.030	0.045
Pb	7.2	2.4	3.6
Ni	20.0	6.7	10.0

Participants of Euramet 924 Step 3

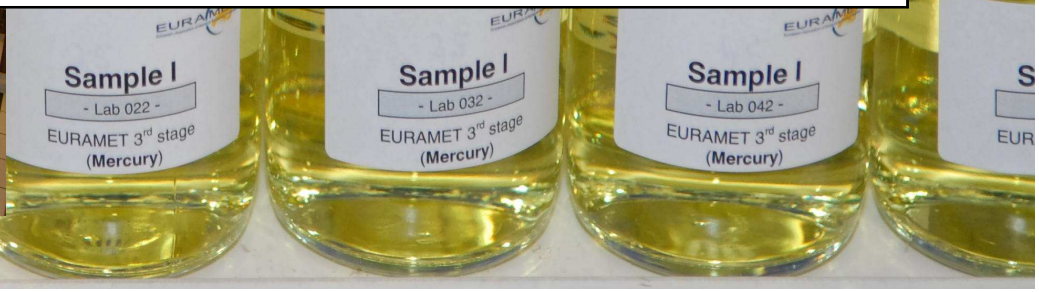
Germany	33
Israel	2
Hungary	12
Bosnia-Herzegovina	1
Portugal	4
Slovenia	1
Czech	1
Croatia	4
Bulgaria	6
Austria	11
Italia	9
Romania	1
Norway	1
Sweden	4
France	18
Spain	5
Finland	1
Σ Countries:17	Labs: 114

Euramet 924 Step 3:

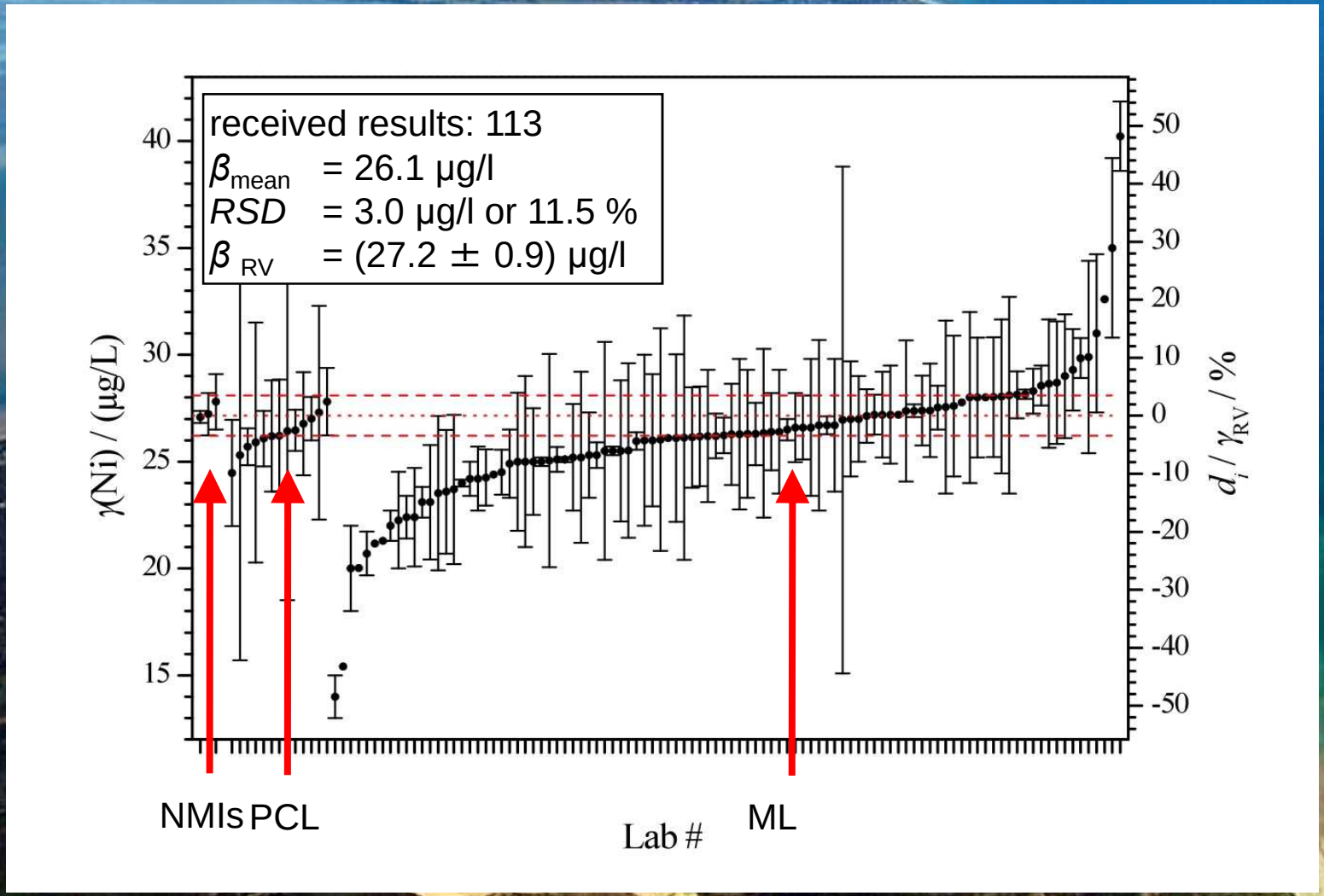
114 monitoring and expert labs from 17 countries

4 NMIs (BAM, LNE, PTB, NCM)

	registered	submitted results
Ni:	114	113 (99%)
Pb:	113	111 (98%)
Cd:	113	95 (84%)
Hg:	93	55 (59%)



Ni: Performance of monitoring labs



Island of Sylt, North Sea, Germany © Vidicom /Christian Irrgang

EUROMET 924: Performance of Test Laboratories

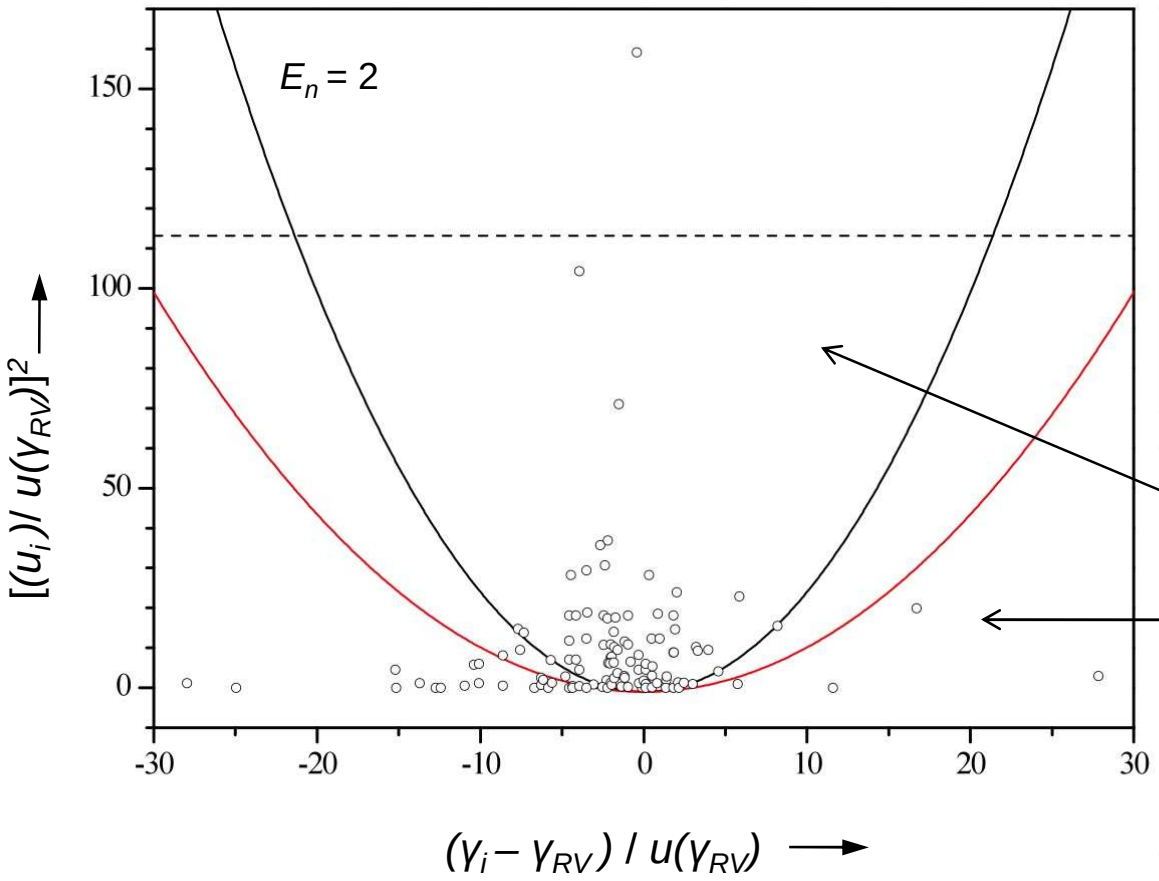
Evaluation criteria

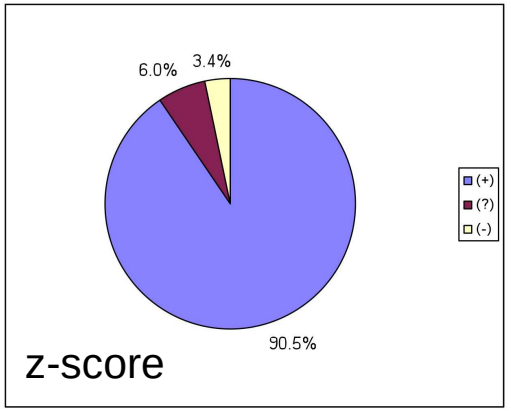
Result	Score
Satisfactory	$\lvert z \rvert \leq 2$
Questionable	$2 < \lvert z \rvert < 3$
Unsatisfactory	$\lvert z \rvert \geq 3$

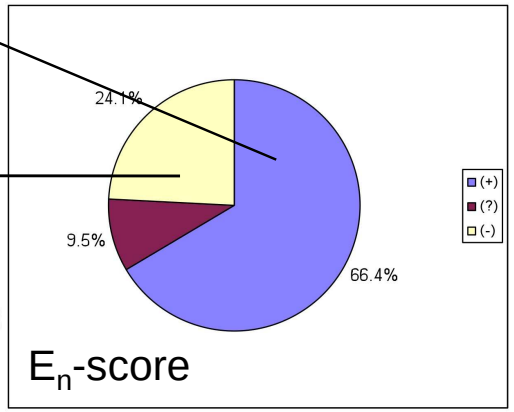
$$E_n = \frac{d_i}{u(d_i)} = \frac{\gamma_i - \gamma_{RV}}{\sqrt{u^2(\gamma_i) + u^2(\gamma_{RV})}} \qquad z = \frac{d_i}{s} = \frac{\gamma_i - \gamma_{RV}}{s}$$

$$s = 0.1 \cdot \gamma_{RV}$$

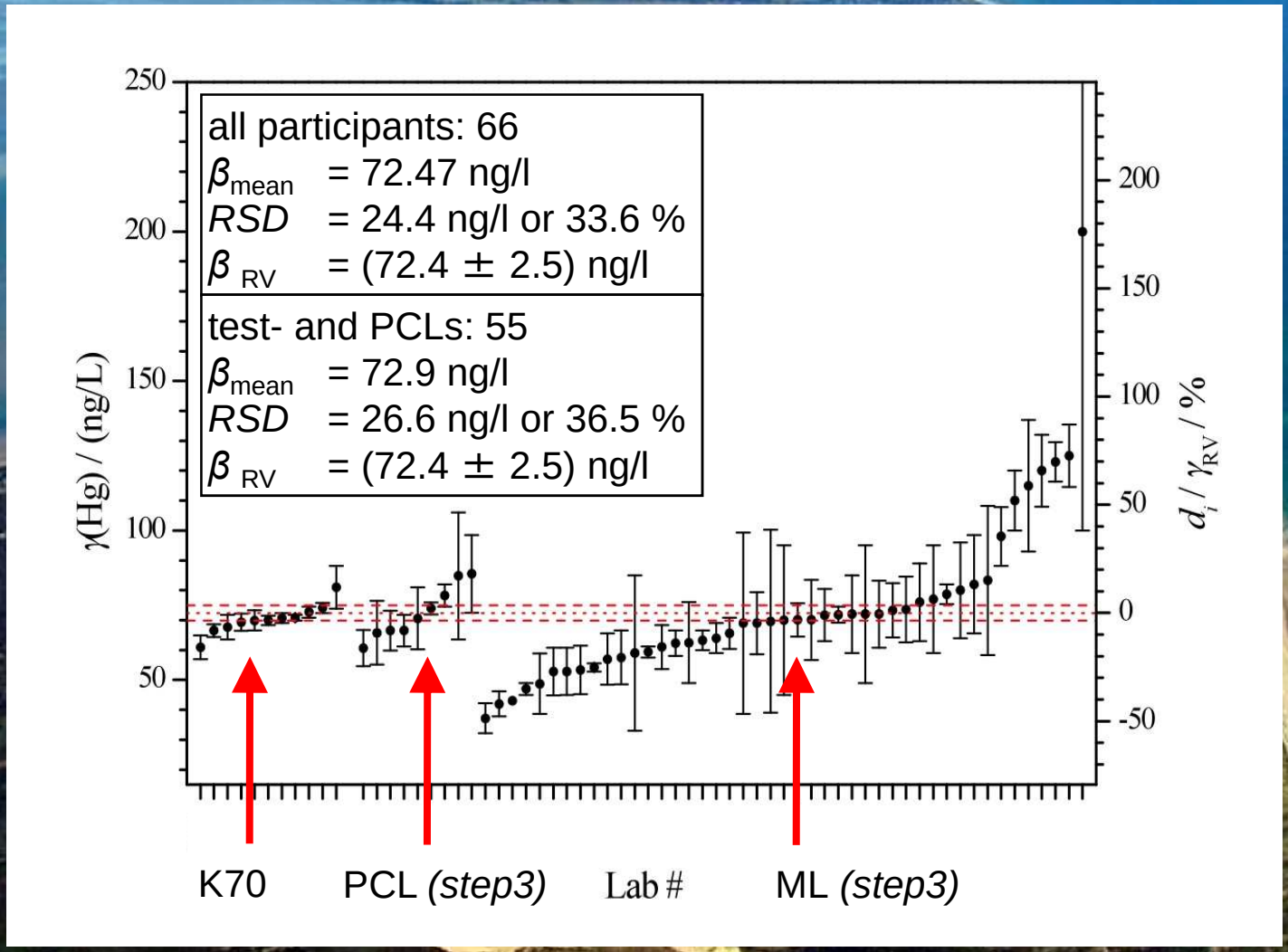
Ni: Performance of monitoring labs



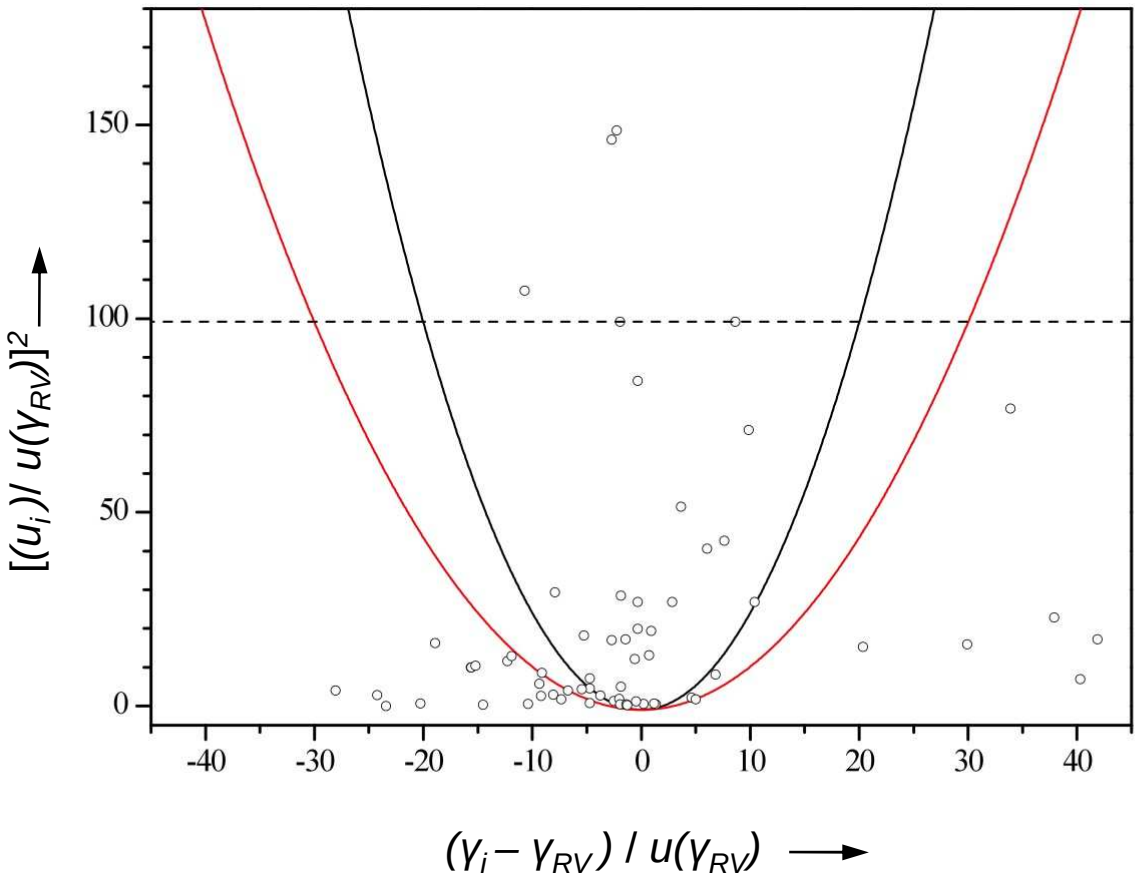


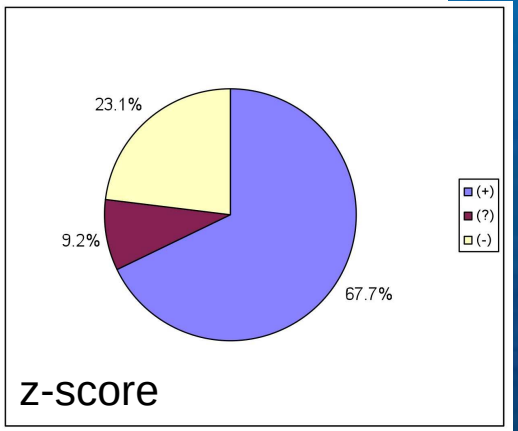


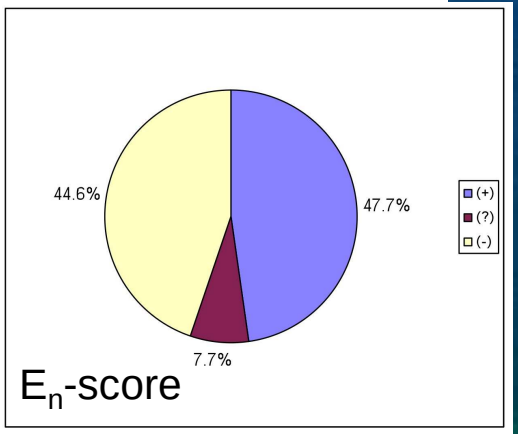
Hg : CCQM K70 & EUROMET 924 step3



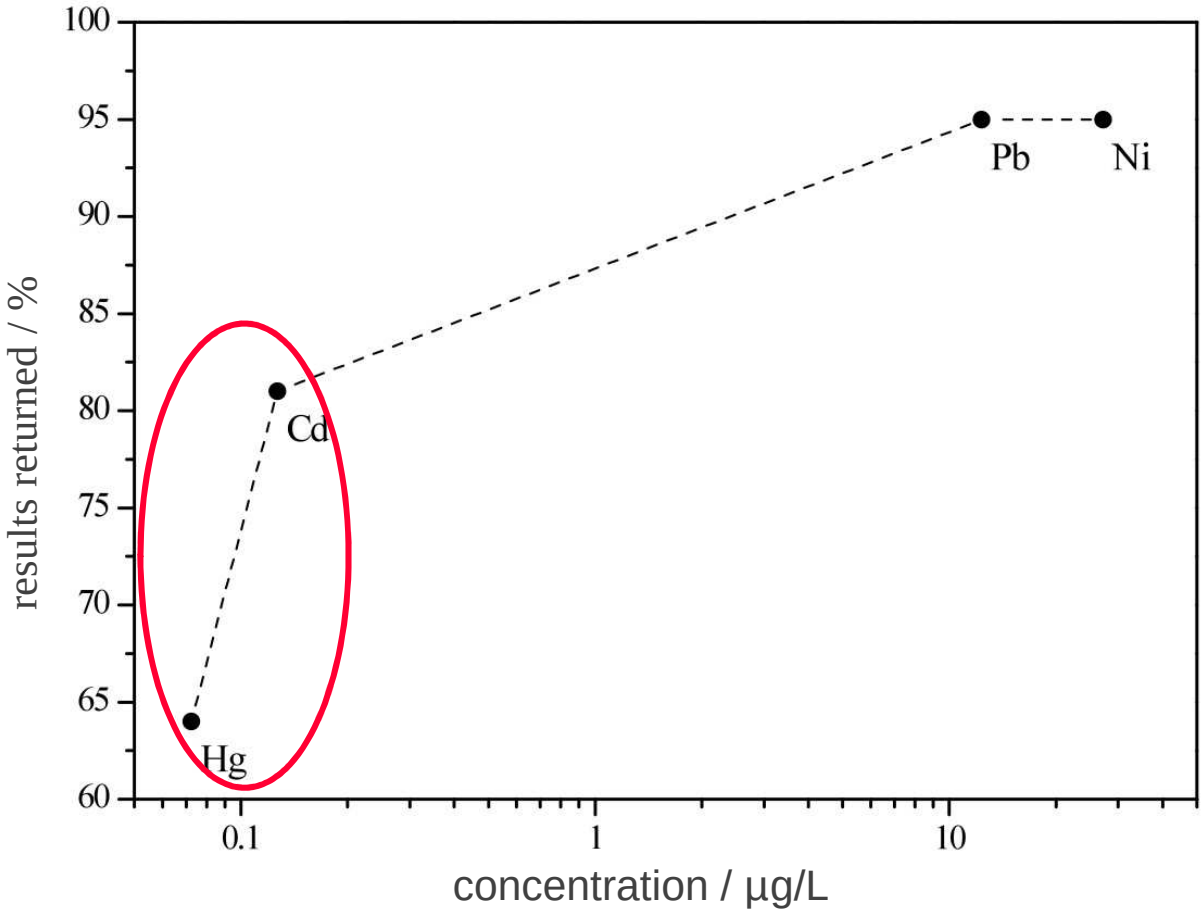
Hg: Performance of monitoring labs







EUROMET 924: subm. results - monitoring labs



EUROMET 924: measurement uncertainties

Comparison of standard deviations

E	$s / \bar{x}$		r
	%		1
	PCL	ML	
Cd	15	39	2.6
Ni	3.3	12.3	3.7
Pb	2.9	23.3	8.0
Hg	12	38	3.2

PCL: PT-providers *ML: monitoring labs* *ratio*

Conclusions EUROMET 924

- A three level system for traceability of chemical measurements under the EU water framework directive was established an tested for the relevant heavy metals
- The feasibility of a system ensuring traceable results is demonstrated - at least for the heavy metals
- but data must be carefully evaluated and improvements are necessary especially when low concentration levels are required (e.g., Hg)

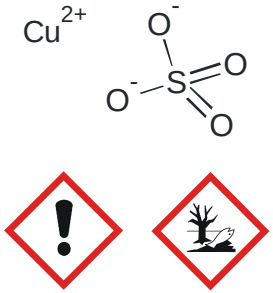
Metalloproteins in blood serum

In vitro Diagnostica 98/79/EG

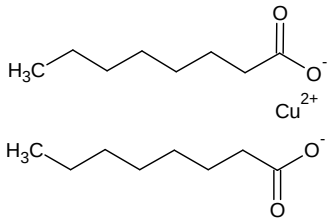
ISO/IEC 17025

ISO/IEC 17043

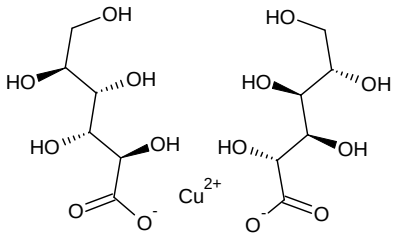
why elemental analysis is not enough: example clinical chemistry



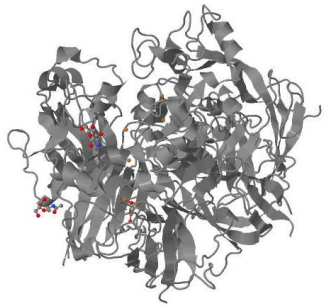
copper sulfate
highly water soluble



copper octanoate
fungicide in vintage



copper gluconate
food supplement



ceruloplasmin
Cu storage protein

decreasing toxicity

LD_{50} (Oral Ratte)
482 mg/kg

LD_{50} (Oral Ratte)
2000 mg/kg

no toxic effects found

Freely based on Paracelsus:
Not only the dose but also the compound makes an element toxic!

...and makes the compound interesting for clinical investigation.

clinical importance of metalspecies

as drugs

- cyanocobalamin (vitamin B12)
- cis-platin (cancer therapy)
- sodium aurothiomalate (rheumatoid athritis treatment)
- lithium carbonate (depression treatment)
- iron sulfate (anemia treatment)
- gadolinium complexes (MRT diagnostic)

etc.

as biomarkers

- haemoglobin (anaemia, thalassemia, diabetes control)
- transferrin (acute phase protein, alcoholism, liver damage)
- superoxide dismutase (acute phase protein, amyotrophic lateral sclerosis)
- ceruloplasmin (Wilson‘ disease, Menke‘s disease)
- ferritin (anaemia, thalassemia, haematochromatosis)
- C-reactive protein (chronic inflammation, atherosclerosis, cardiovascular diseases)

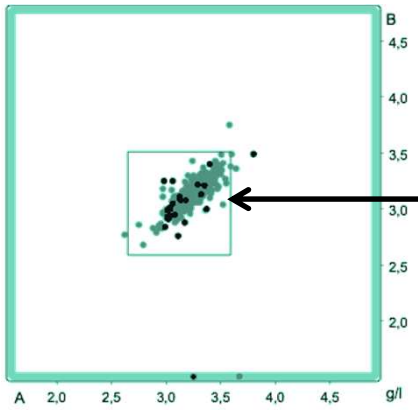
why reference methods for metalloproteins?

Captain, I found an exciting new life form with interesting new proteins!

Captain, I don‘t understand! I reanalysed the samples here at the Enterprise and the results are completely different. That‘s not logical!

because...

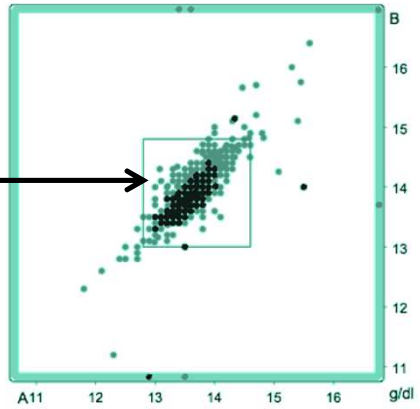
IG3/14
Transferrin
Kit 28



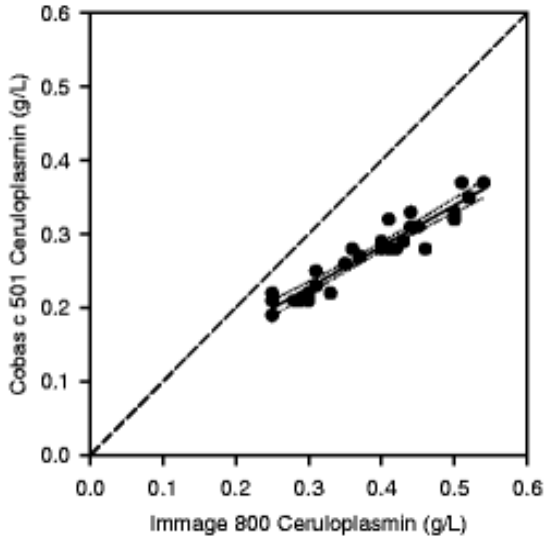
15 %

HA3/14
Haemoglobin
Kit 70

6 %



Reference institute for bioanalytics
http://www.dgkl-rfb.de/



Example: Ceruloplasmin (CER)

- Cu storage protein
- marker for Wilson‘s disease - a genetic defect in 1:30 000 to 1:300 000 persons
- if untreated ⇒ liver failure and neurological defects
- methods have to distinguish between functional and non-functional protein forms

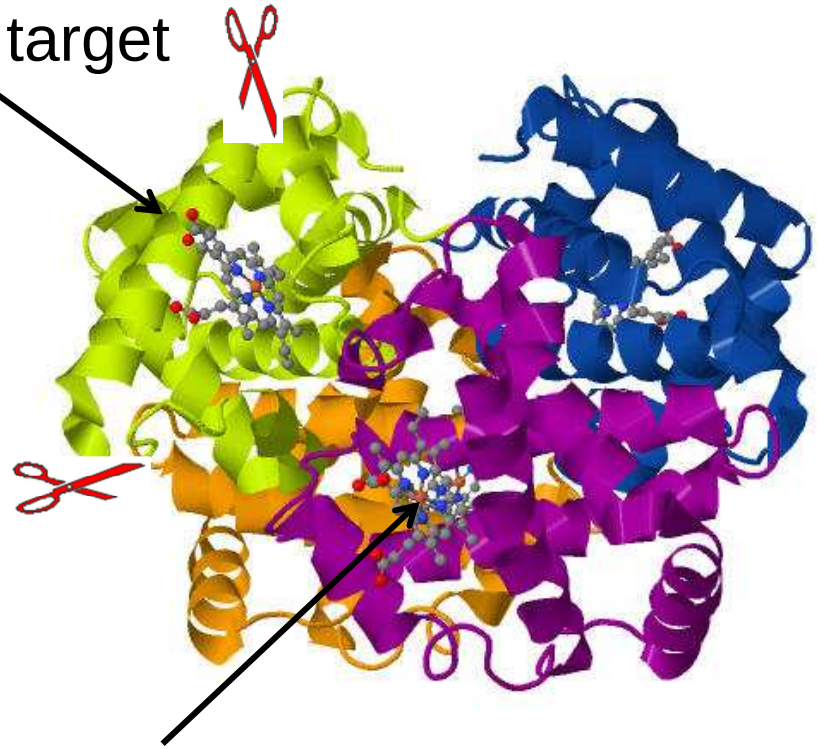
[1]Infusino I. et al., Anal Bioanal Chem (2010) 397:521-525

challenges in protein quantification

- definition of analyte
- clinical samples have often complex matrices (blood, tissue, urine etc.)
- ensuring the stability of the analyte until analysis (e.g. proteolysis sensitive proteins such as Cp)
- ensuring complete separation from other compounds containing the same metal
- often no species specific spike material available
  $\Rightarrow$ has to be produced and characterised in-house
- often no adequate reference material available
  $\Rightarrow$ purchase of pure protein and characterisation in-house

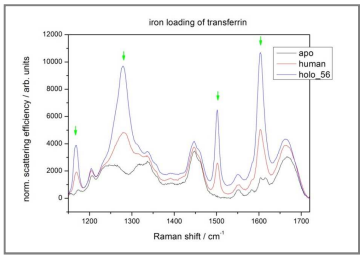
multimodal approach for protein quantification

organic MS
peptide target

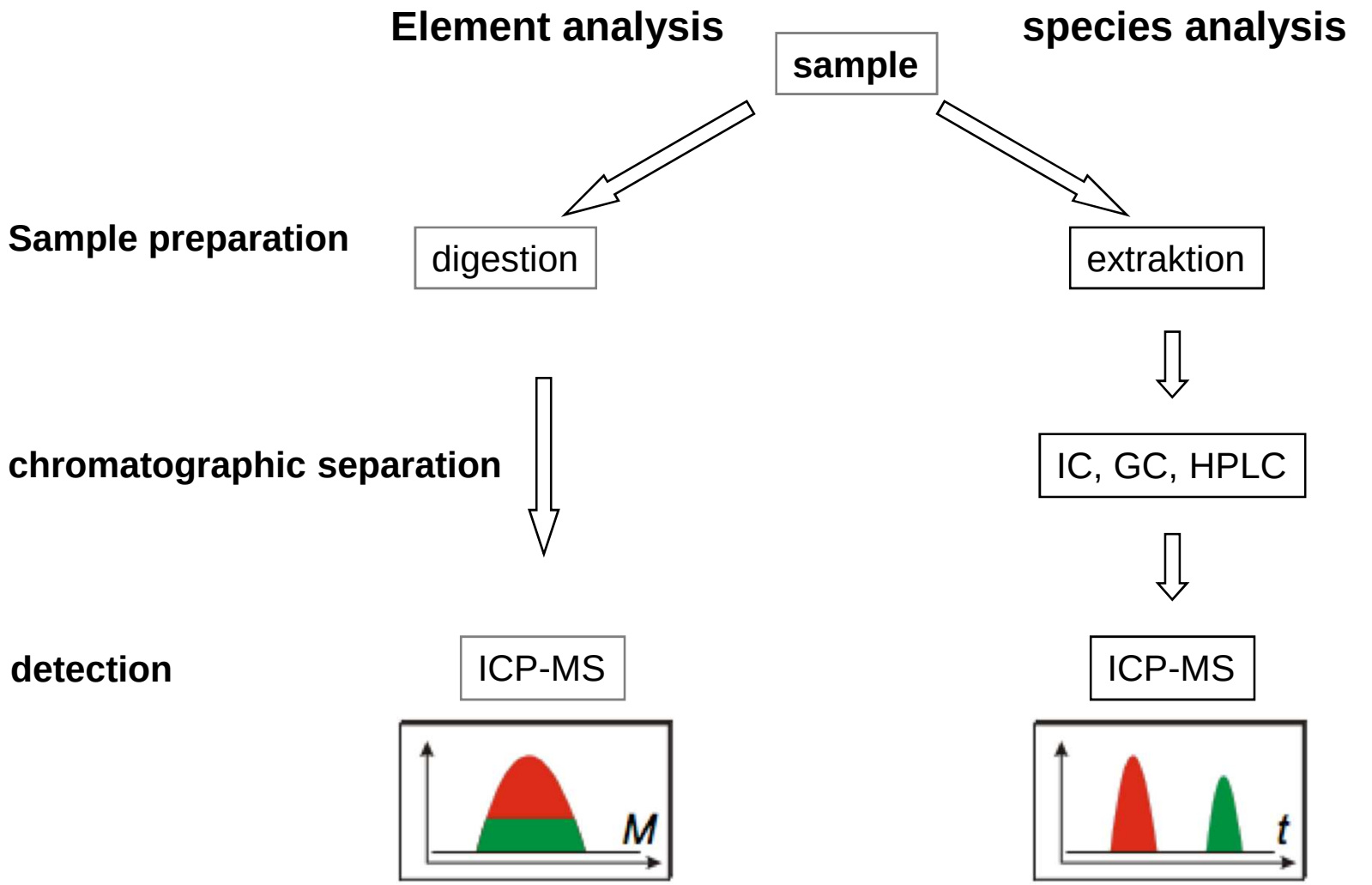


ICP-MS
determination of elements

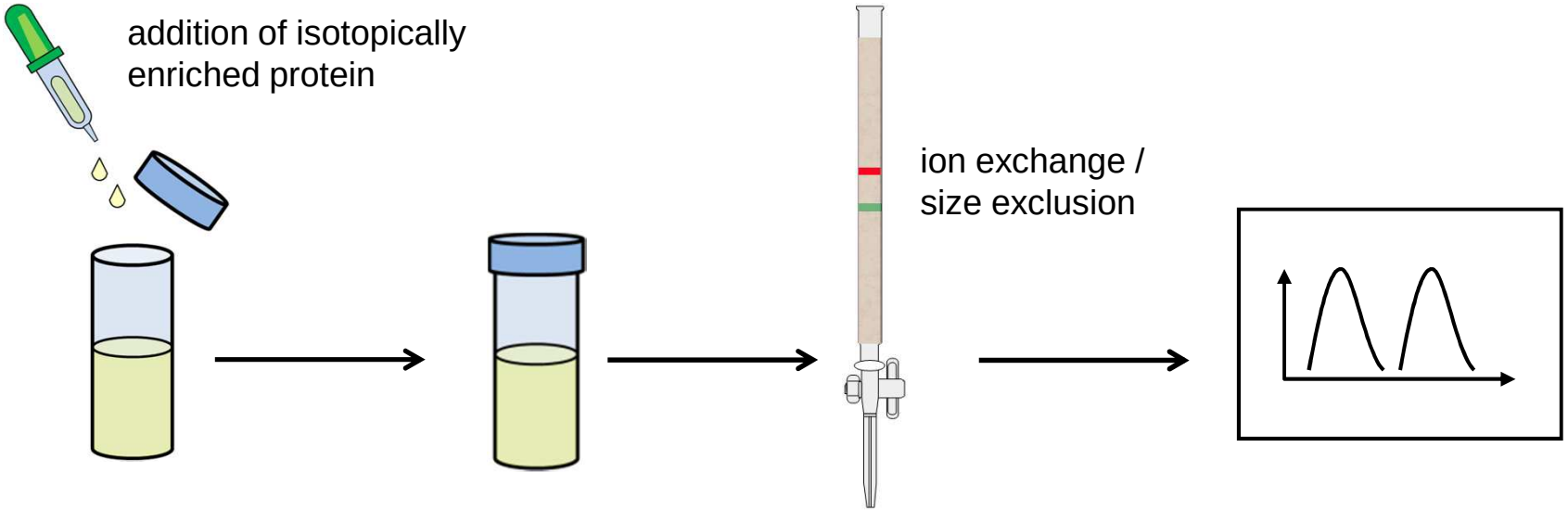
RAMAN



species analysis for quantification



quantification of proteins using ID



sample with natural isotopic composition

homogenisation

separation

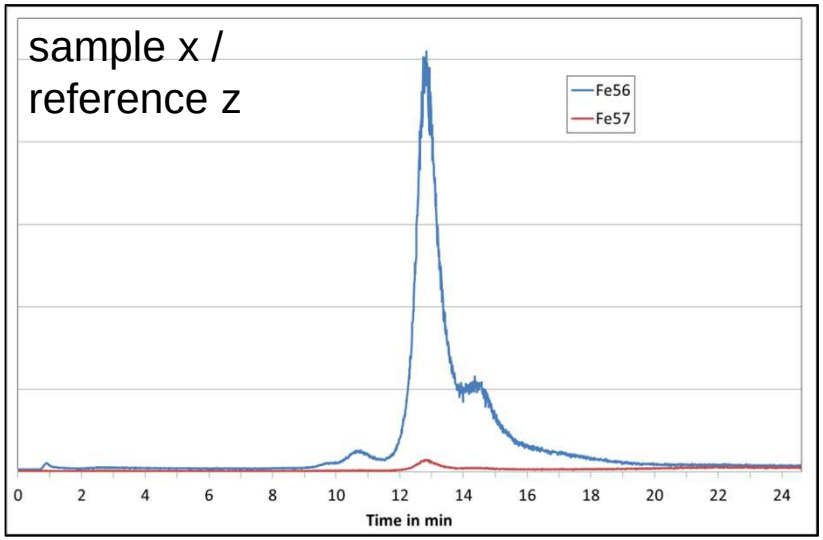
detection with

ICP-MS

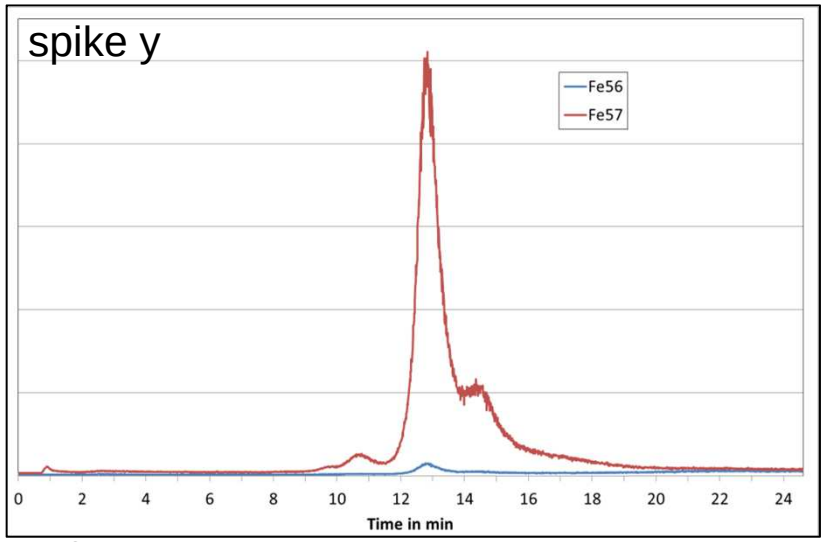
MS/MS

Raman

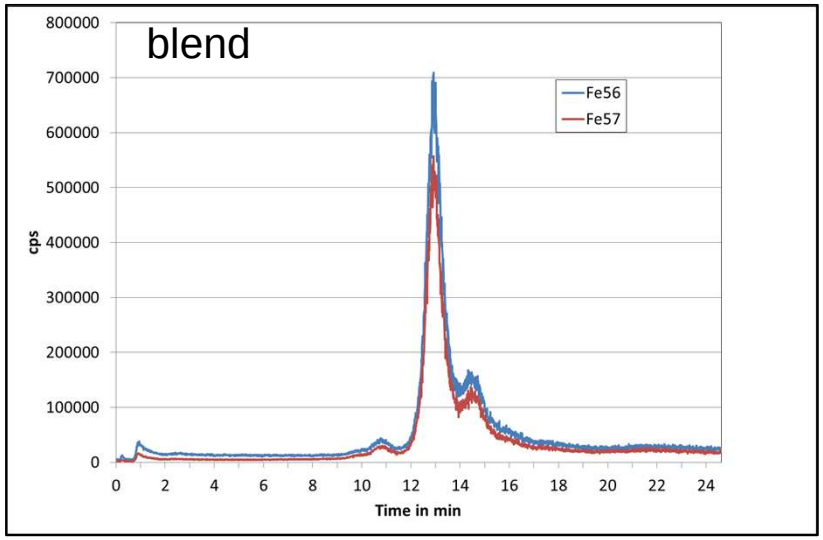
isotope dilution approach



+



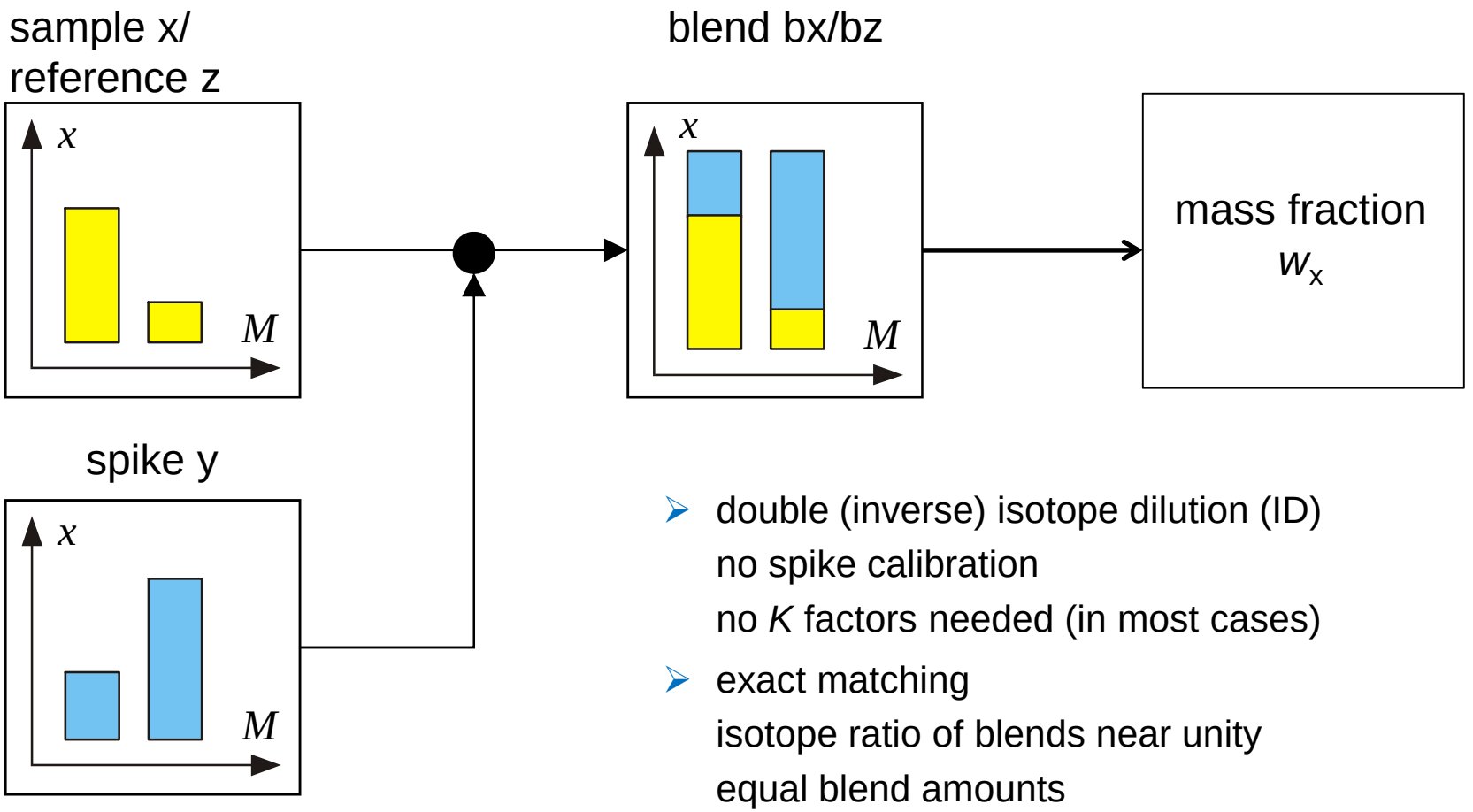
→



↓

$$w_x = w_z \cdot \frac{m_{xy}}{m_x} \cdot \frac{m_z}{m_{zy}} \cdot \frac{R_y - R_{bx}}{R_{bx} - R_x} \cdot \frac{R_{bz} - R_z}{R_y - R_{bz}}$$

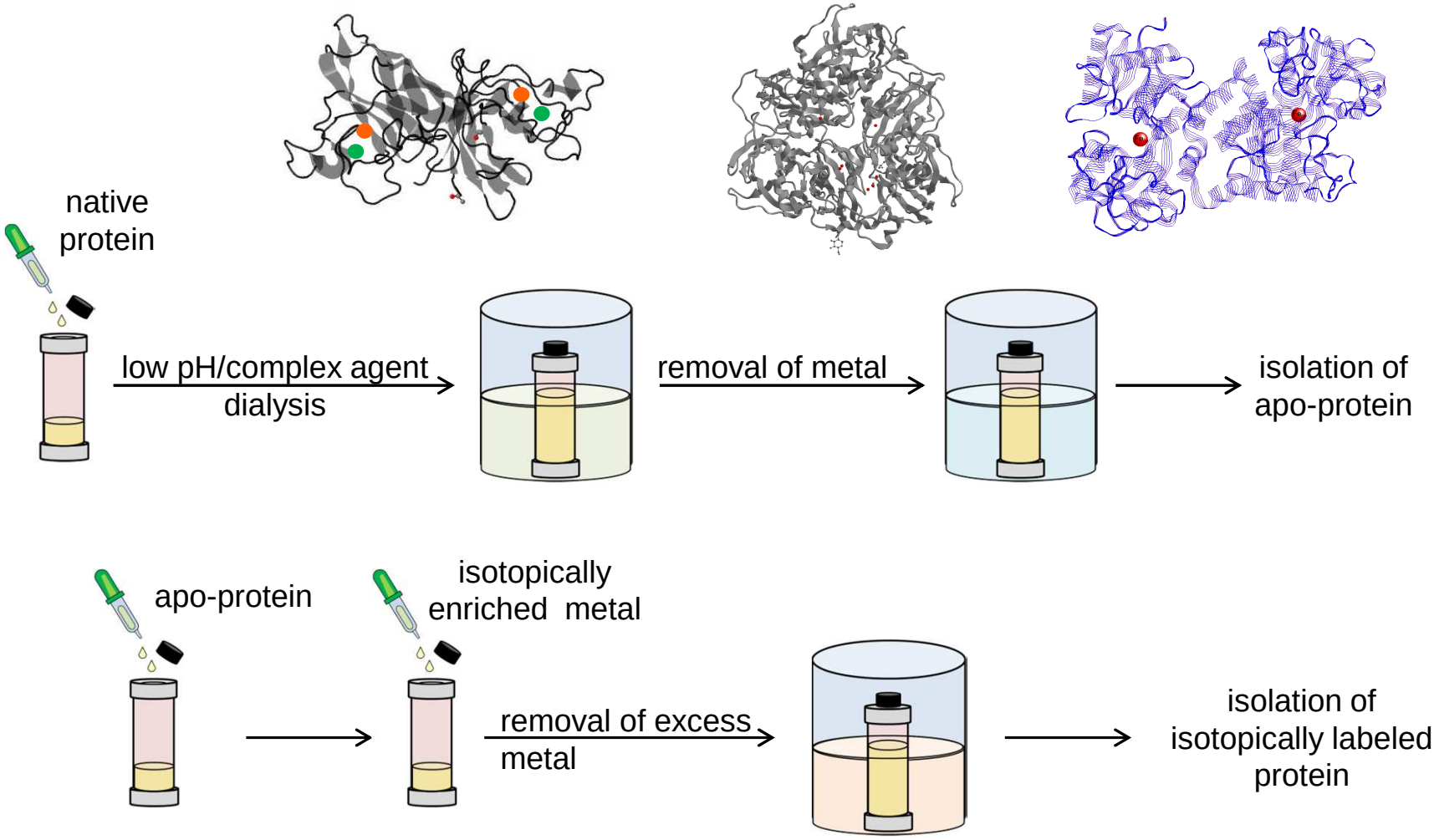
isotope dilution approach



- double (inverse) isotope dilution (ID)
  no spike calibration
  no *K* factors needed (in most cases)
- exact matching
  isotope ratio of blends near unity
  equal blend amounts
  no dead time, back ground, … correction
  both blends share the same “fate”

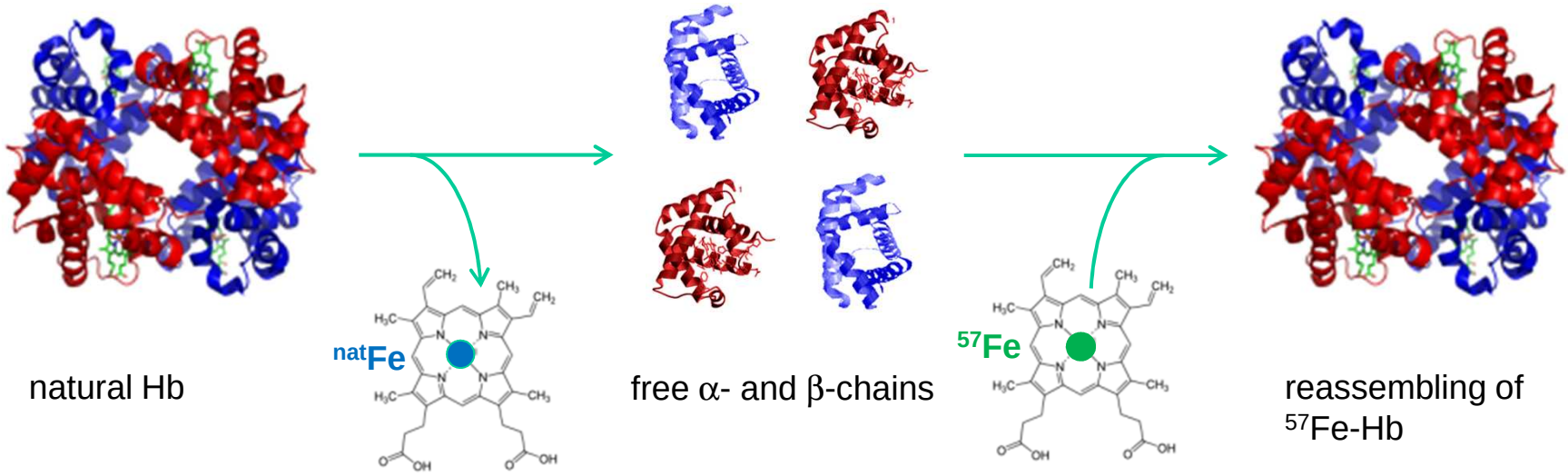
species specific spike production

metals easily exchangeable such as Cu in SOD or Cp or Fe in Tf



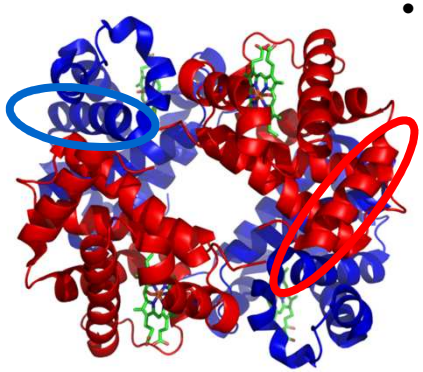
species specific spike production

proteins with metals more strongly bound such as Fe in Hb



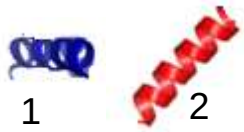
Thalassemia: Hb quantification via char. peptides

- measurand of clinical importance: percentage of HbA_2 in blood
- IDMS for determination of HbA_2 and total Hb in reference materials

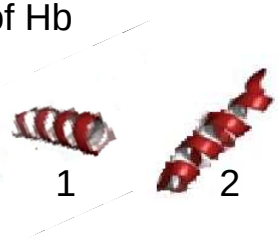


HbA_0/A_2 ($\alpha_2\beta_2$ / $\alpha_2\delta_2$)

proteolysis (trypsin)

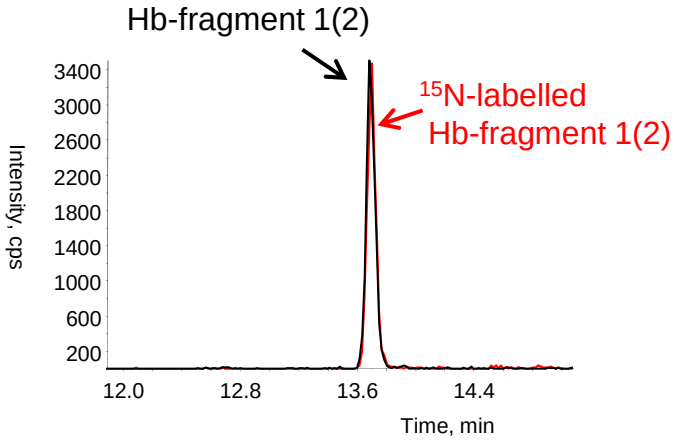


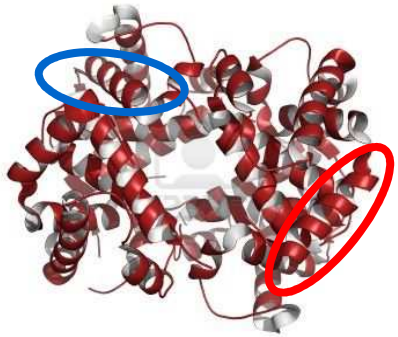
proteolytic fragments of Hb



^{15}N -labelled proteolytic fragments of Hb

LC/MS-MS





U-^{15}N-HbA_0/A_2 (internal standards)
- added to sample in defined amounts

result 1 for HbA_0 or HbA_2
by quantifying Hb-fragment 1 of β- or δ-globin

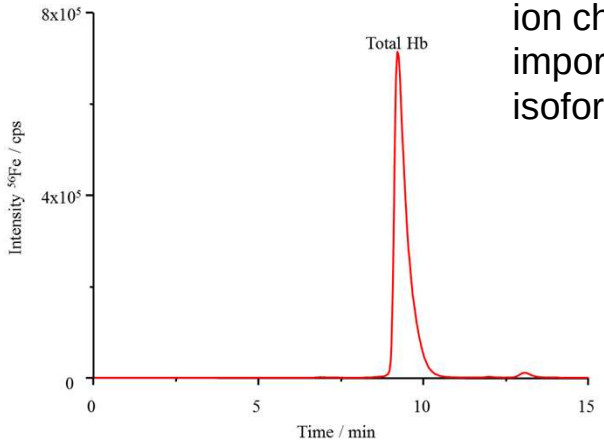
result 2 for „total Hb“
by quantifying Hb-fragment 2 of α-globin

percentage of HbA_2=[HbA_2]*100/[total Hb]

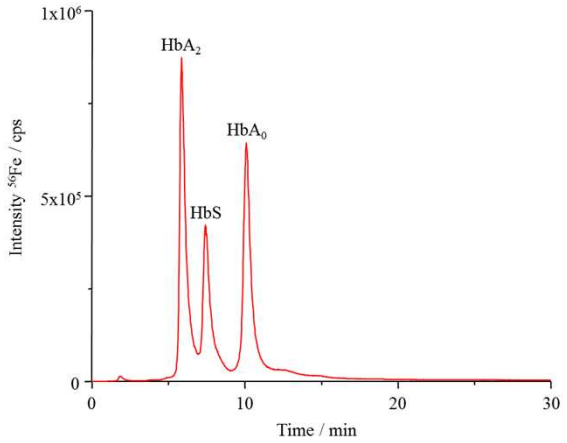
advantages of quantification via metal

- less interferences ⇒ less sample preparation necessary
- use of isotope dilution approach possible
- sum parameters possible for structural variations (e.g. 750 known variants of Hb)
- different clinical questions can be answered by different separation/detection conditions

size exclusion chromatography: total Hb



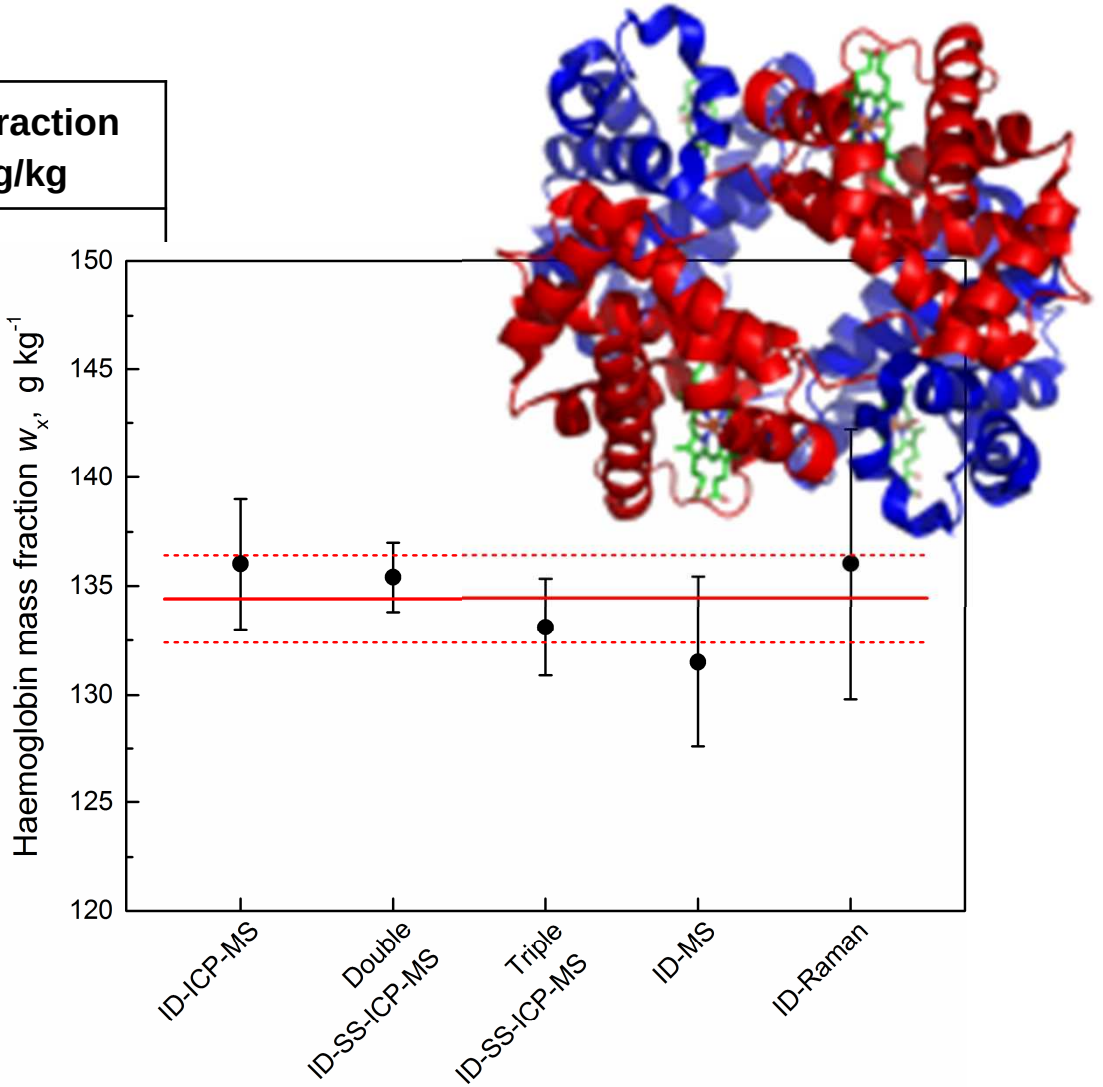
ion chromatography: important Hb isoforms



However: structural confirmation of compound in the peak necessary by other means

comparison of Hb_{tot} results

Method	Mass fraction *w* / g/kg
IDMS	131.5
triple SS-ICP-IDMS	133.1
double SS-ICP-IDMS	135.4
ID-ICP-MS	136.0
ID-Raman	136.0



...with reference methods

Captain, I‘ve found a solution: Reference measurement procedures!

Although using different techniques, all tricorder give now a comparable result!

thank you...

...for your interest !

...and to

BAM, LNE, IRMM

R. Matschat, H. Scharf, J. Birkhahn,
G. Labarraque, P. Fisicaro, C. Quetel

IWW Mühlheim/Ruhr, Uni Stuttgart

(PT-providers water analysis)

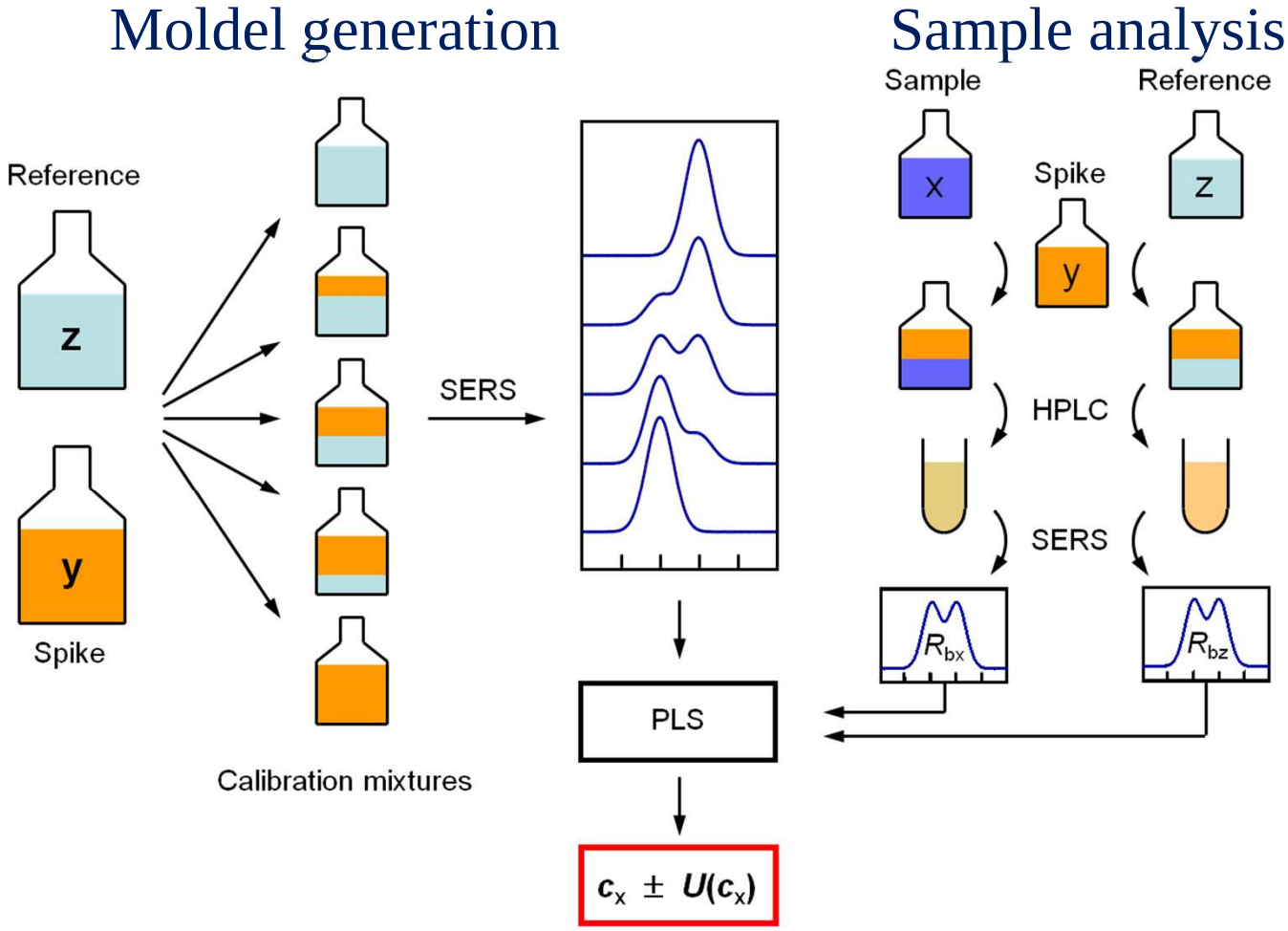
U. Borchers, D. Schwesig, M. Koch

and my colleagues in PTB

in particular: O. Rienitz, C. Swart, J. Noo

cranes flying to Africa in autumn, North Sea © Vidicom / Christian Irrgang ...and Vidicom-Television for photos

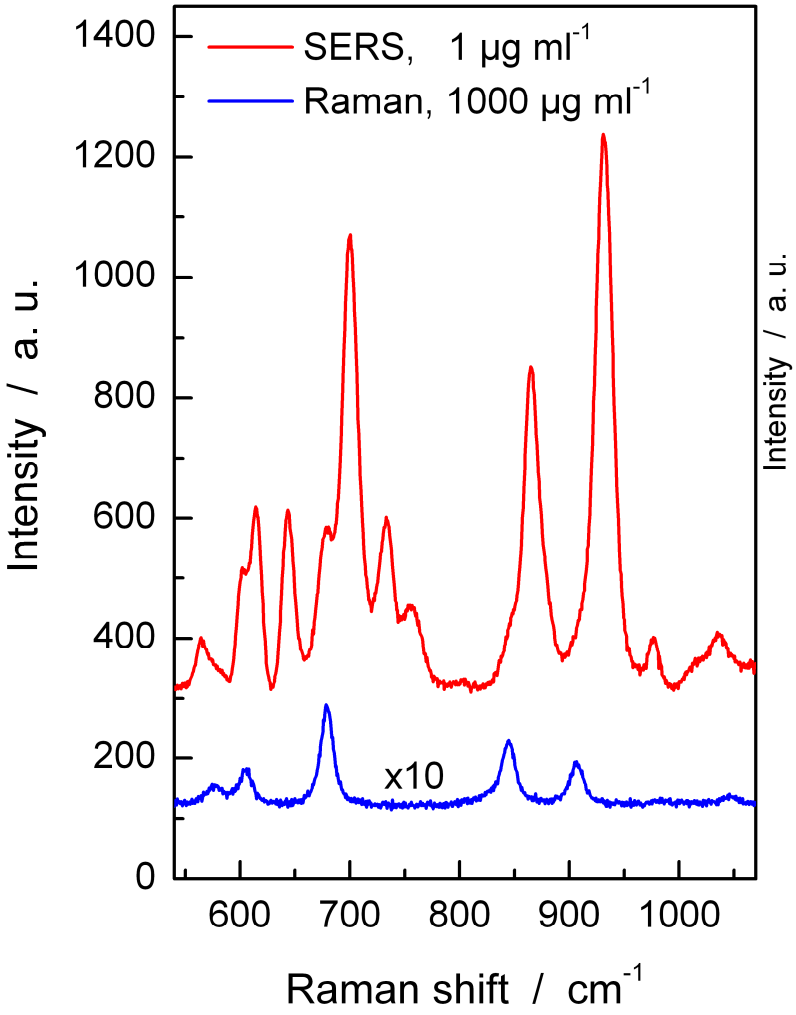
double IDSERS Raman: principle



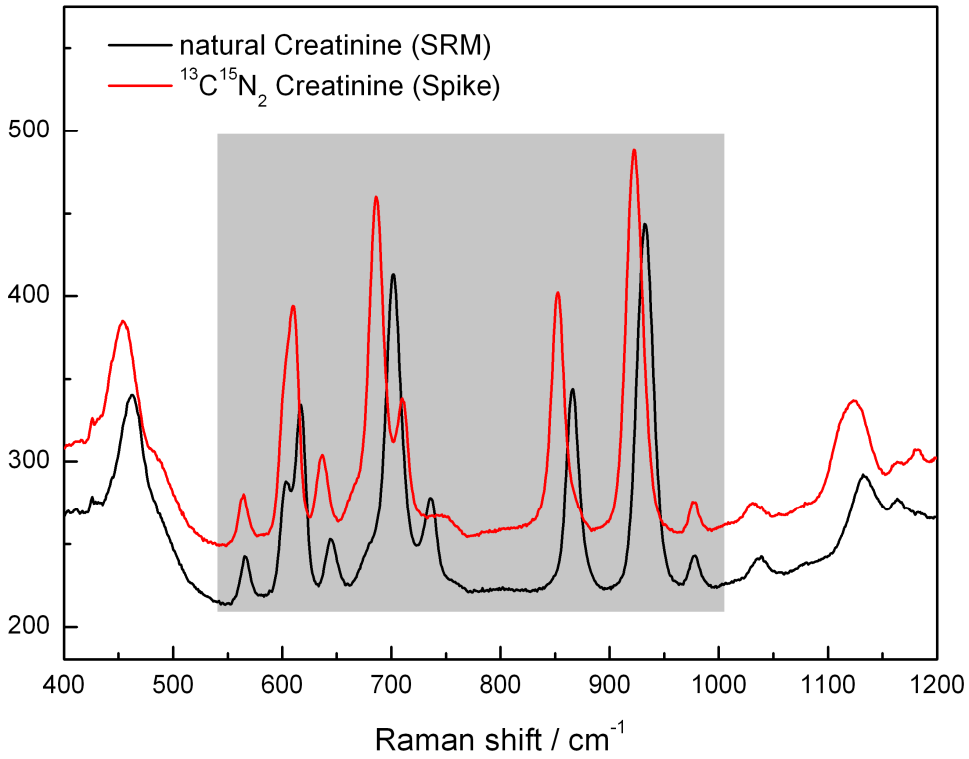
S. Zakel, O.Rienitz, B.Güttler, R. Stosch, Analyst **2011**, 136, 3956-3961

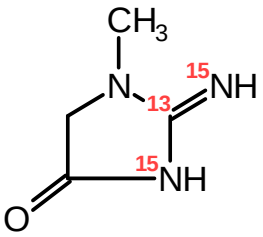
R. Stosch, A. Henrion, D. Schiel, B. Güttler, Anal. Chem. **2005**, 77, 7386-7392

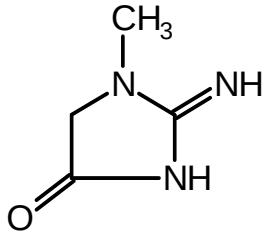
ID-SERS Raman: Signal enhancement / Isotopic shift



SERS detection limit: <0.1 µg ml^{-1}







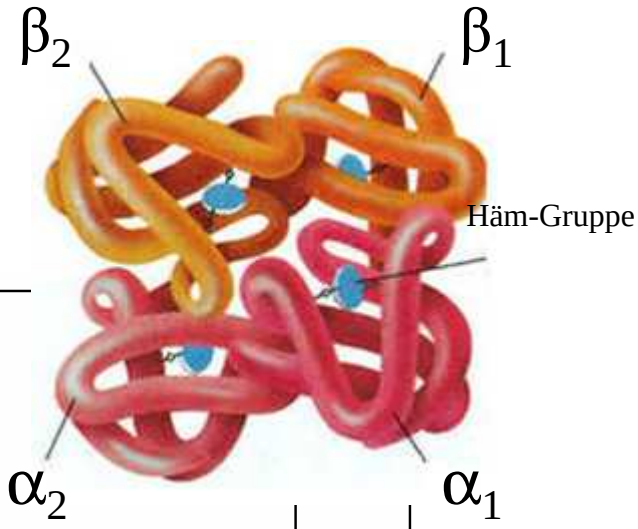
Analyte

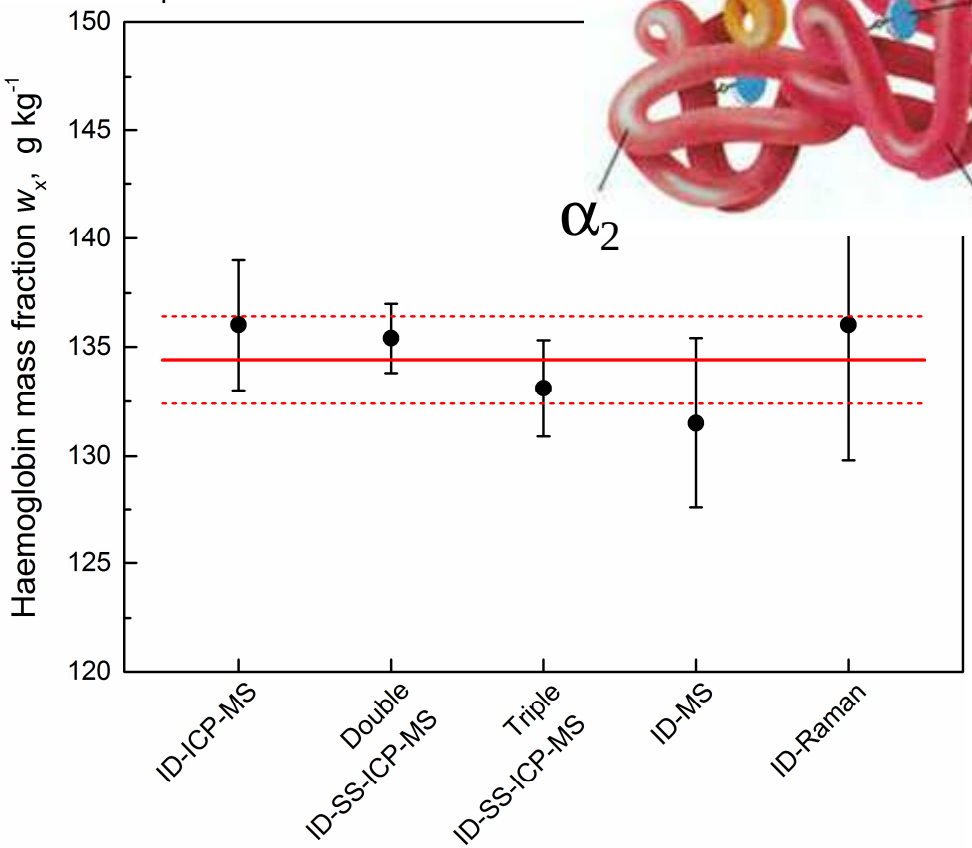
113 g/mol

Isotopic shift: up to 26 cm^{-1}

comparison of Hb_{tot} results

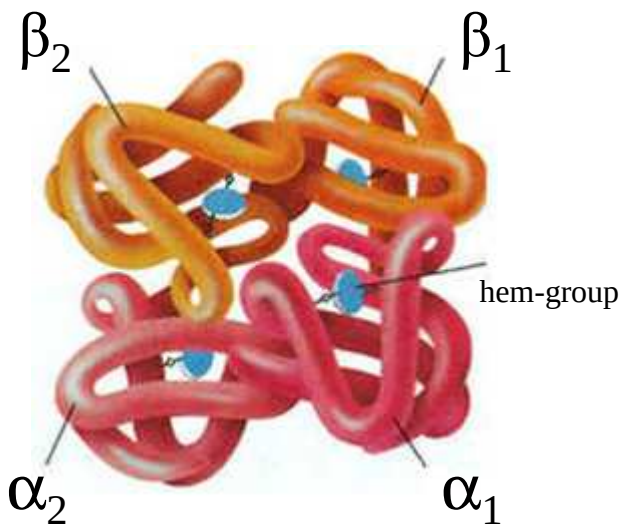
Method	Mass fraction *w* / g/kg
IDMS	131.5
triple SS-ICP-IDMS	133.1
double SS-ICP-IDMS	135.4
ID-ICP-MS	136.0
ID-Raman	136.0





Haemoglobin (Hb) variants in blood (adults)

Hb A0 ($\alpha_2\beta_2$): 95-98%
Hb A2 ($\alpha_2\delta_2$): 2-3% ⟶ fraction of A2:
Hb F ($\alpha_2\gamma_2$): 0.8-2%
4-8% β-thalassemia,
3.5-4% boarderline cases



haemoglobin A0

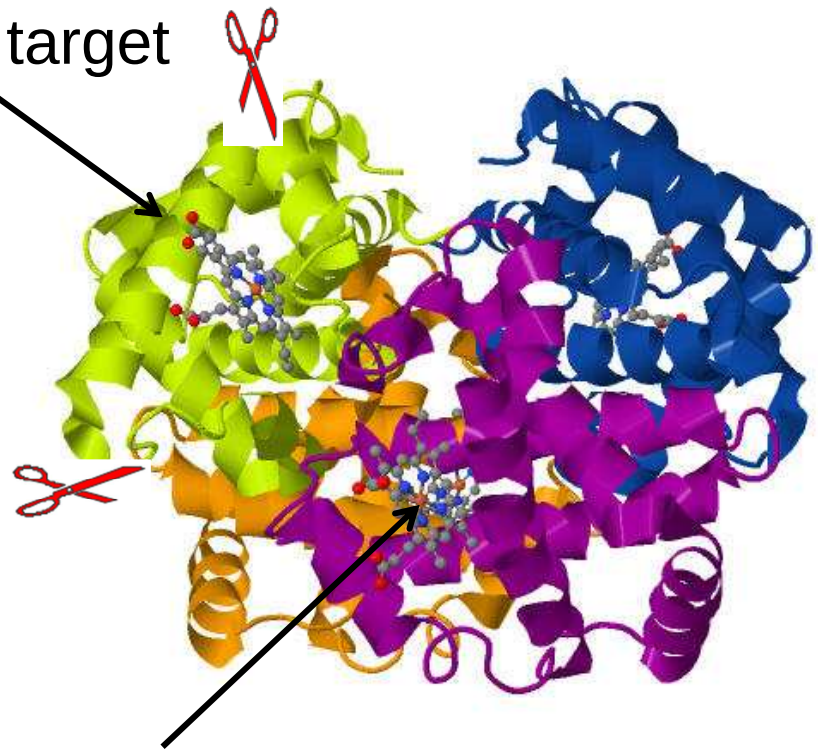
globin:	**measurand:**
α–globin	total haemoglobin
β–globin	haemoglobin A0
δ–globin	haemoglobin A2

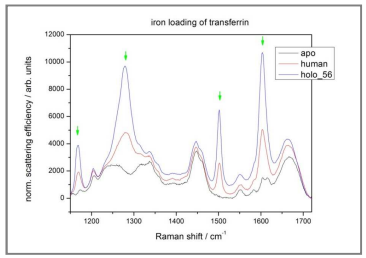
methods for haemoglobin A0/2 from Hb-fragments (β/δ-Globin)

cooperationpartner: P. Kaiser (INSTANT e.V., Düsseldorf),
Prof. A. Mosca (Centre for Traceability in Laboratory Medicine, Univ. Mailand ,Italien)

multimodal approach for protein quantification

organic MS
peptide target





RAMAN

ICP-MS
determination of elements