"New challenges for metrology in chemistry and biology"

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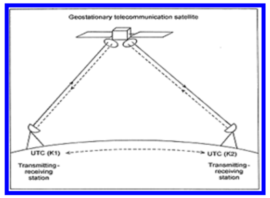
Director, BIPM

9th October 2013

CONGRÈS INTERNATIONAL DE MÉTROLOGIE
INTERNATIONAL CONGRESS OF METROLOGY
16th
7 | 8 | 9 | 10 | OCTOBER 2013
PARIS | FRANCE
measure | innovate | perform
COLLÈGE FRANÇAIS DE MÉTROLOGIE

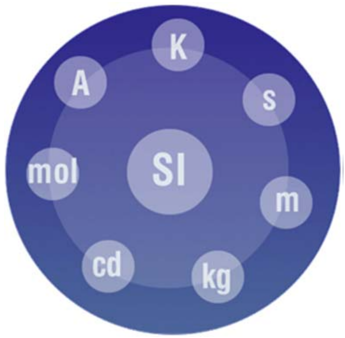
Bureau International des Poids et Mesures

- Established in 1875 by the Metre Convention
- Based in Paris and financed by 55 Member States and 37 Associate States/Economies.



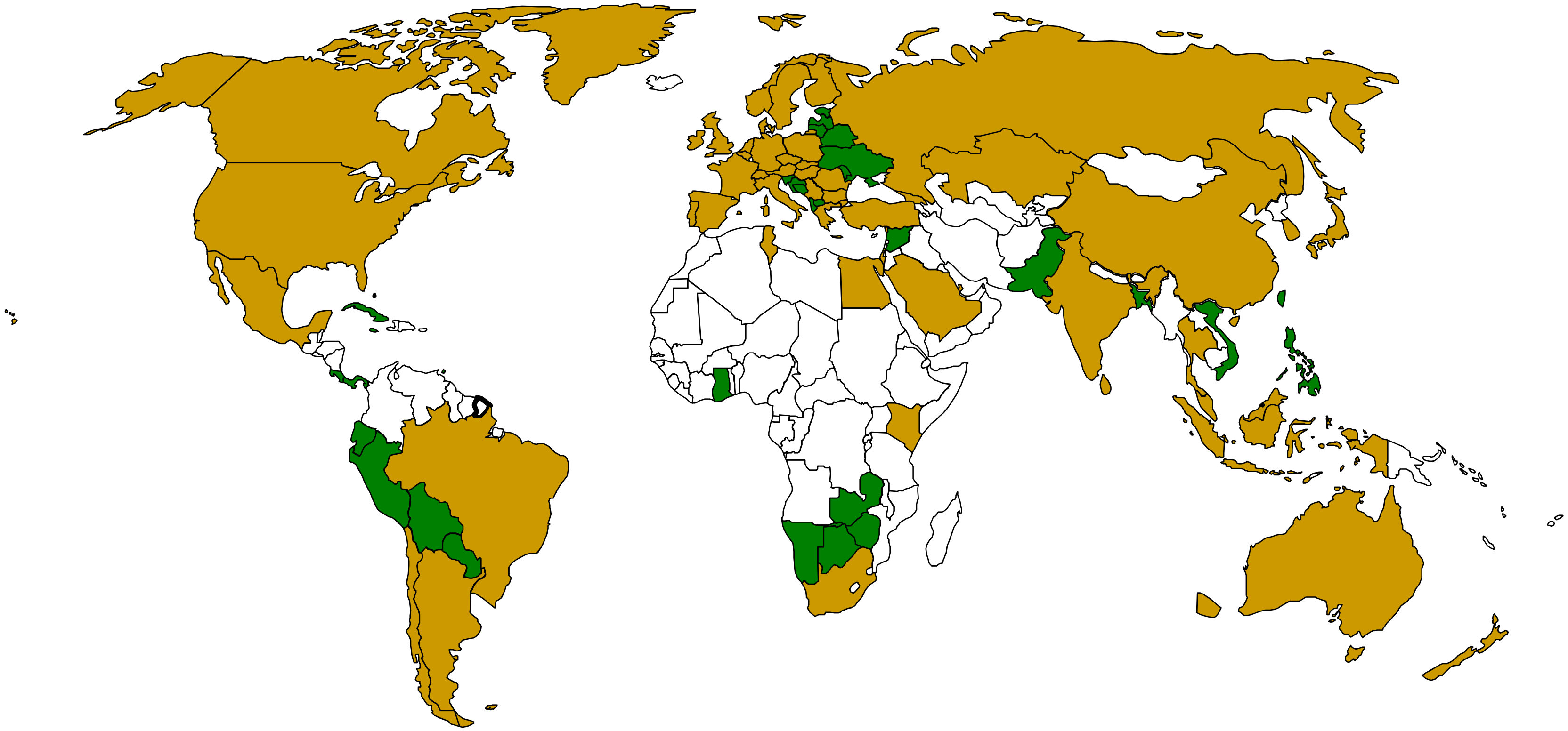
- **Our mission is to ensure and promote the global comparability of measurements.**
- This is achieved both through technical activities in our laboratories and through international coordination.

- Operate laboratories in: mass, time, electricity, ionizing radiation and chemistry.
- An international staff of around 75 with budget of approximately 12 million euros (for 2012).



Member States and Associates

Metre Convention
55 Member States & 37 Associates of the CGPM



- Member participating in the CIPM MRA
- Associate participating in the CIPM MRA

CIPM- MRA
92 NMIs and 146 Designated Institutes from
52 Member States & 36 Associates of the CGPM
& 4 international organizations

The International System of Units (SI)

Table 5. SI prefixes

Factor	Name	Symbol	Factor	Name	Symbol
10^{1}	deca	da	10^{-1}	deci	d
10^{2}	hecto	h	10^{-2}	centi	c
10^{3}	kilo	k	10^{-3}	milli	m
10^{6}	mega	M	10^{-6}	micro	µ
10^{9}	giga	G	10^{-9}	nano	n
10^{12}	tera	T	10^{-12}	pico	p
10^{15}	peta	P	10^{-15}	femto	f
10^{18}	exa	E	10^{-18}	atto	a
10^{21}	zetta	Z	10^{-21}	zepto	z
10^{24}	yotta	Y	10^{-24}	yocto	y

Prefixes

Base units

Table 1. SI base units

Base quantity		SI base unit	
Name	Symbol	Name	Symbol
length	*l, x, r*, etc.	metre	m
mass	*m*	kilogram	kg
time, duration	*t*	second	s
electric current	*I, i*	ampere	A
thermodynamic temperature	*T*	kelvin	K
amount of substance	*n*	mole	mol
luminous intensity	I_v	candela	cd

Derived units

Table 3. Coherent derived units in the SI with special names and symbols

	SI coherent derived unit [(a)]			
Derived quantity	Name	Symbol	Expressed in terms of other SI units	Expressed in terms of SI base units
plane angle	radian [(b)]	rad	1 [(b)]	m/m
solid angle	steradian [(b)]	sr [(c)]	1 [(b)]	m^2/m^2
frequency	hertz [(d)]	Hz		s^{-1}
force	newton	N		$m\ kg\ s^{-2}$
pressure, stress	pascal	Pa	N/m^2	$m^{-1}\ kg\ s^{-2}$
energy, work, amount of heat	joule	J	N m	$m^2\ kg\ s^{-2}$
power, radiant flux	watt	W	J/s	$m^2\ kg\ s^{-3}$
electric charge, amount of electricity	coulomb	C		s A
electric potential difference, electromotive force	volt	V	W/A	$m^2\ kg\ s^{-3}\ A^{-1}$
capacitance	farad	F	C/V	$m^{-2}\ kg^{-1}\ s^4\ A^2$
electric resistance	ohm	Ω	V/A	$m^2\ kg\ s^{-3}\ A^{-2}$
electric conductance	siemens	S	A/V	$m^{-2}\ kg^{-1}\ s^3\ A^2$
magnetic flux	weber	Wb	V s	$m^2\ kg\ s^{-2}\ A^{-1}$
magnetic flux density	tesla	T	Wb/m^2	$kg\ s^{-2}\ A^{-1}$
inductance	henry	H	Wb/A	$m^2\ kg\ s^{-2}\ A^{-2}$
Celsius temperature	degree Celsius [(e)]	°C		K
luminous flux	lumen	lm	cd sr [(c)]	cd
illuminance	lux	lx	lm/m^2	$m^{-2}\ cd$
activity referred to a radionuclide [(f)]	becquerel [(d)]	Bq		s^{-1}
absorbed dose, specific energy (imparted), kerma	gray	Gy	J/kg	$m^2\ s^{-2}$
dose equivalent, ambient dose equivalent, directional dose equivalent, personal dose equivalent	sievert [(g)]	Sv	J/kg	$m^2\ s^{-2}$
catalytic activity	katal	kat		$s^{-1}\ mol$

The 8th edition of the SI Brochure is available from the BIPM website.

Why are we talking about chemistry and biology at Metrologie 2013?

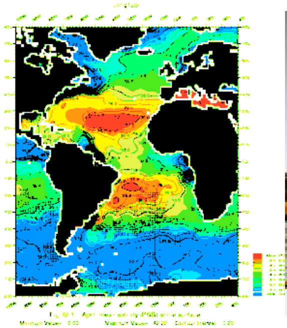
They are driven by and contribute to "grand challenges" of global importance:

- Food, water, air, climate, biodiversity, health …

They also contribute to very substantial industries with substantial growth and significant potential for innovation:

- Biotechnology, healthcare, pharmaceuticals …

(Physical measurements also contribute – but in a different way).

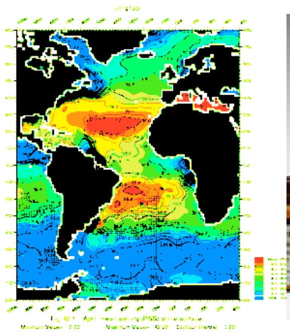



What are the key features of metrology in chemistry and biology?

Progress is strongly driven by advances in measurement technology

There is only very limited infrastructure in place worldwide

The scope of possible requirements is enormous

What are the key features of metrology in chemistry and biology?

Progress is strongly driven by advances in measurement technology

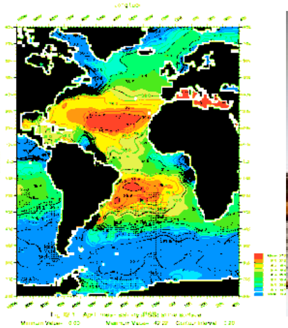
- Cavity Ring-down Spectroscopy (CRDS)
- Quantitative-NMR (qNMR)
- Digital Polymerase Chain Reaction (dPCR)

There is only very limited infrastructure in place worldwide

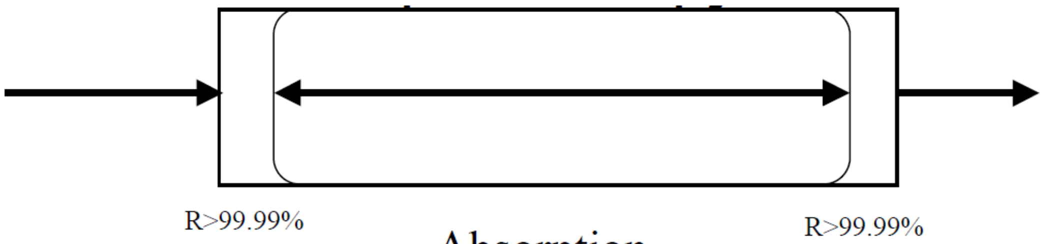
- Chains of traceability are short
- Dissemination is largely by distribution of (certified) reference materials

The scope of possible requirements is enormous

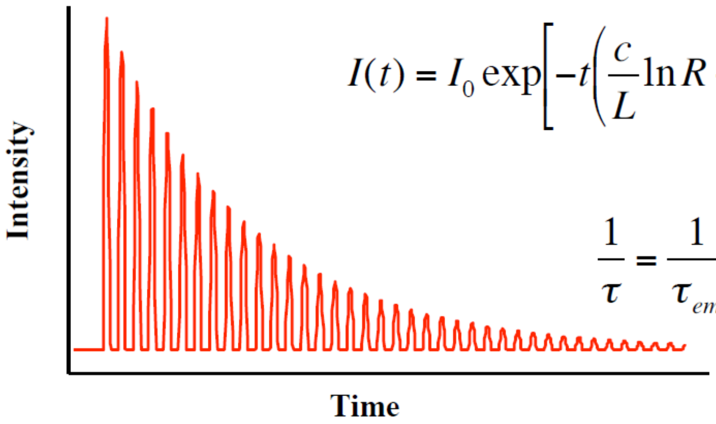
- A highly strategic approach is needed
- “How far does the light shine”


New methods in chemistry - CRDS



Absorption



$$I(t) = I_0 \exp\left[-t\left(\frac{c}{L}\ln R + c \cdot \sigma(\lambda) \cdot N\right)\right]$$

$$L_{eff}=L/(1-R)$$

$$\frac{1}{\tau} = \frac{1}{\tau_{em}} + N \cdot \sigma \cdot c$$

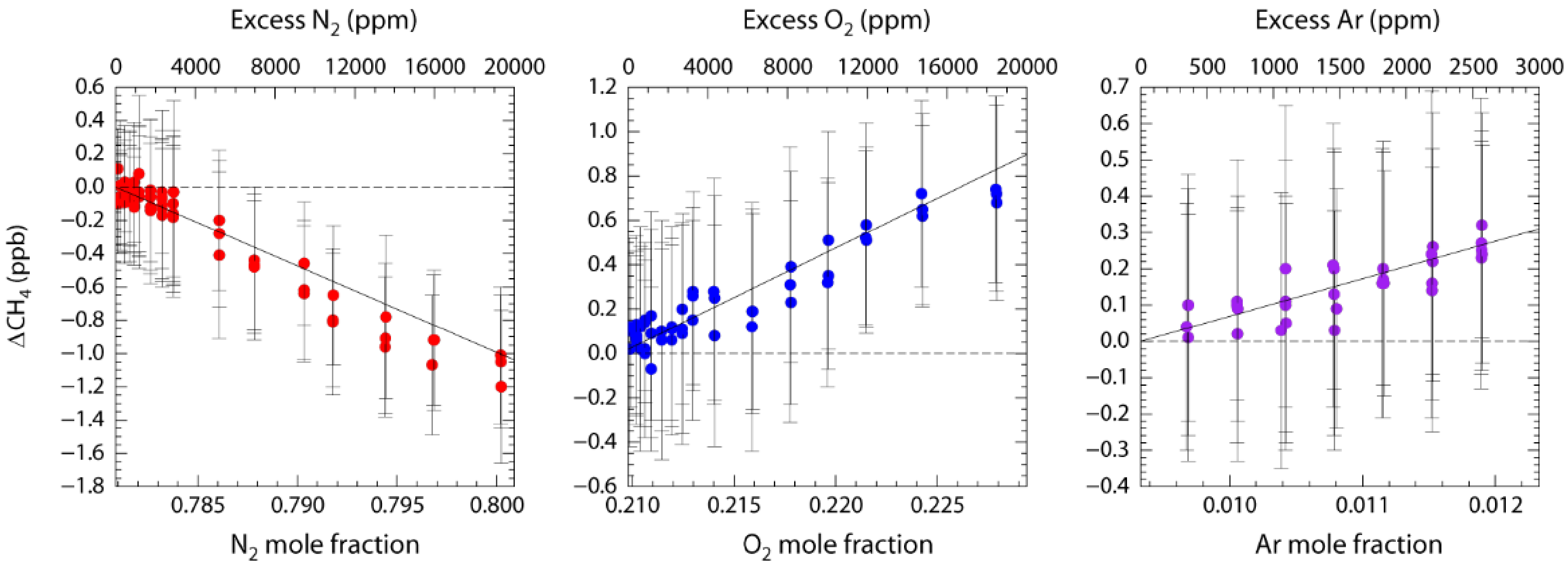
Where:
c : speed of light
L : length of cavity
R : mirror reflectivity
σ : absorption cross section
N : number density
L_{eff}: effective pathlength

- **Advantages**
  - Very long path length – very good sensitivity
  - The only measurand is a decay time (the intrinsic loss in the system can be measured independently).
  - If the absorption cross section is known, then the method is "calibration free"
  - Not true!

New methods in chemistry - CRDS

Although the measurement equation is correct, there are still corrections needed to CRDS Measurements

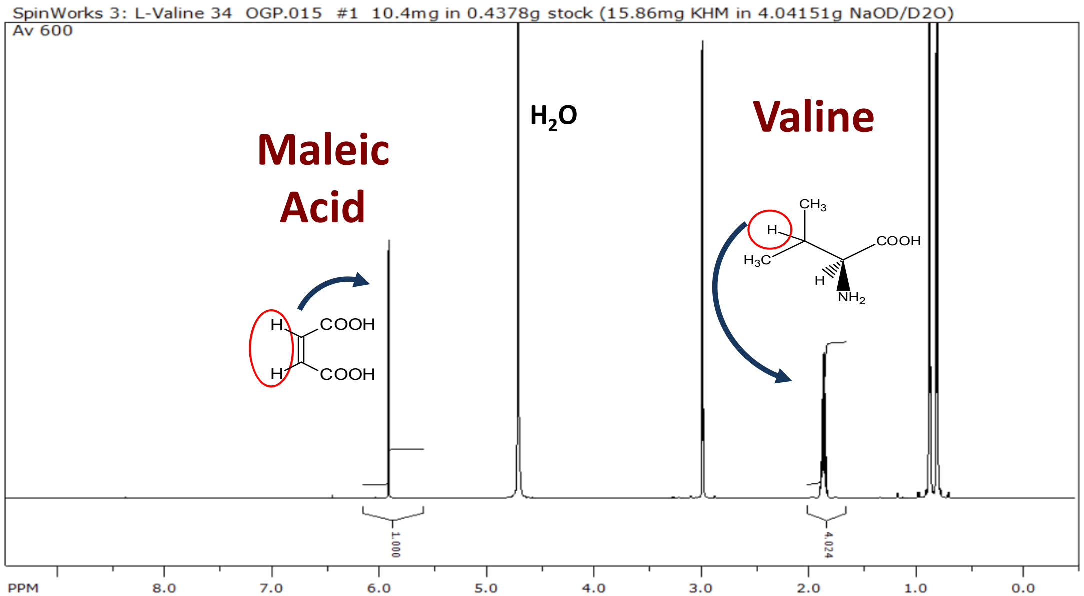
For example, **pressure broadening**:

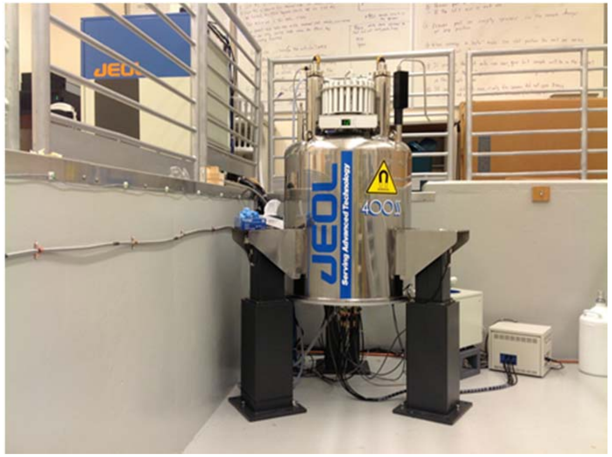


H. Nara; Tanimoto, H.; Tohjima, Y.; Mukai, H.; Nojiri, Y.; Katsumata, K.; Rella, C. W. "Effect of air composition (N2, O2, Ar, and H2O) on CO2 and CH4 measurement by wavelength-scanned cavity ring-down spectroscopy: calibration and measurement strategy".Atmos. Meas. Tech. 2012. 5. 2689-2701.

Quantitative NMR (Q-NMR): An example – Valine

^1H NMR of CCQM-K55.c (Valine) [Internal standard: Maleic acid]





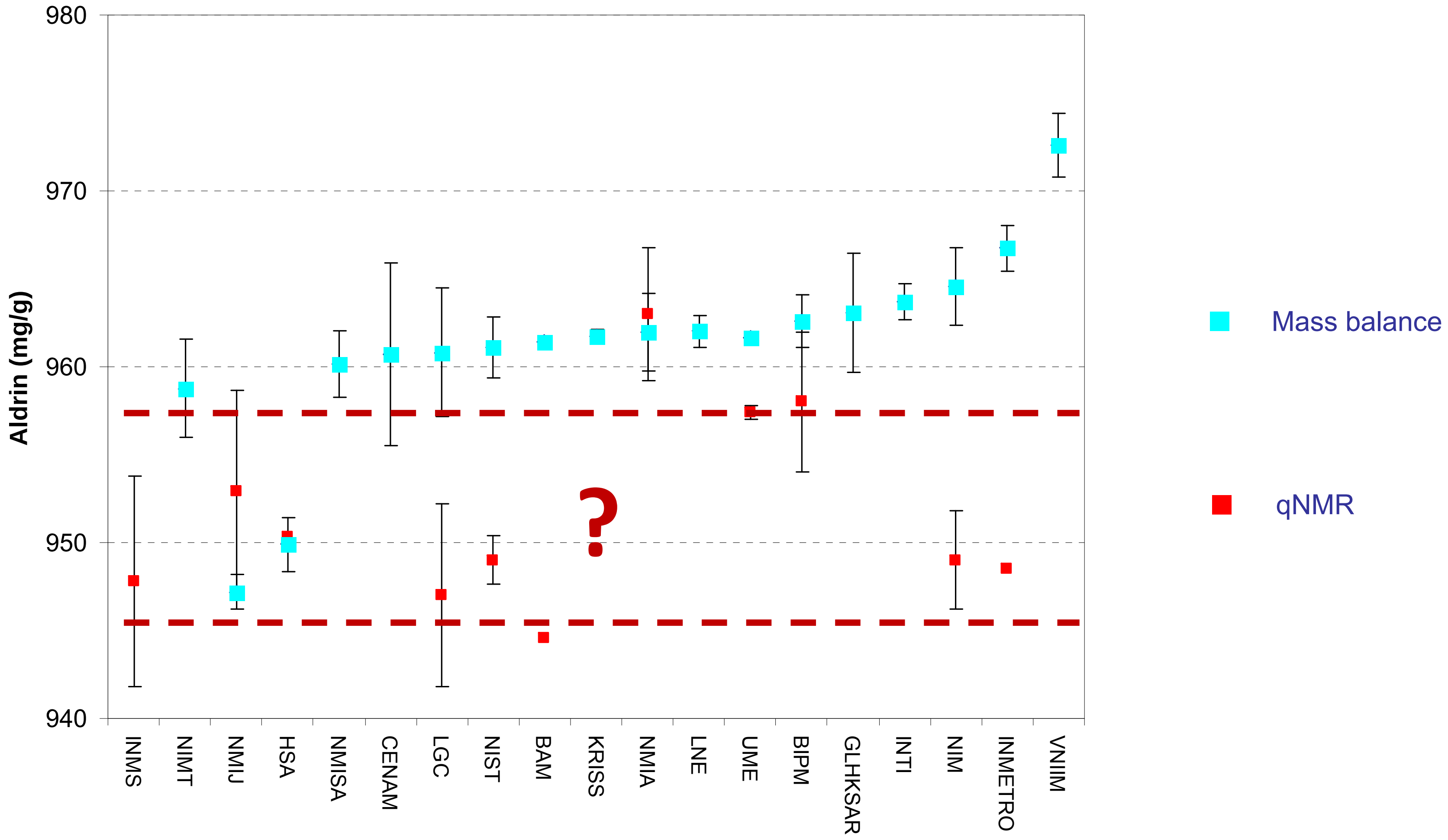
400 MHz NMR Spectrometer

$$P_{Valine} = \frac{I_{Valine}}{I_{Maleic\ Acid}} \cdot \frac{\rho_{Maleic\ Acid}}{\rho_{Valine}} \cdot \frac{M_{Valine}}{M_{Maleic\ Acid}} \cdot \frac{m_{Maleic\ Acid}}{m_{Valine}} \cdot P_{Maleic\ Acid}$$

Purities = Signal areas . No's of protons . Molar masses . Sample masses

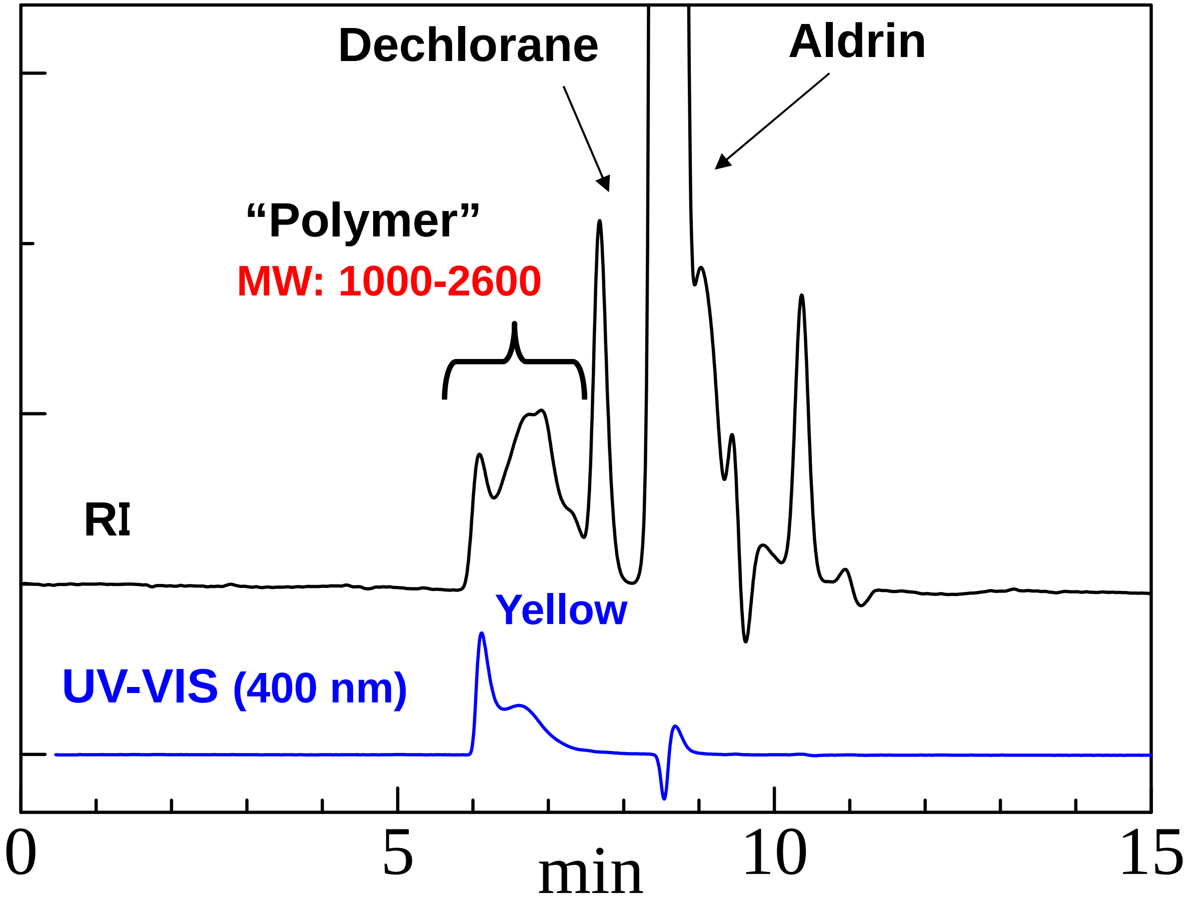
CCQM-K55.b: Purity of aldrin

Mass balance versus qNMR results

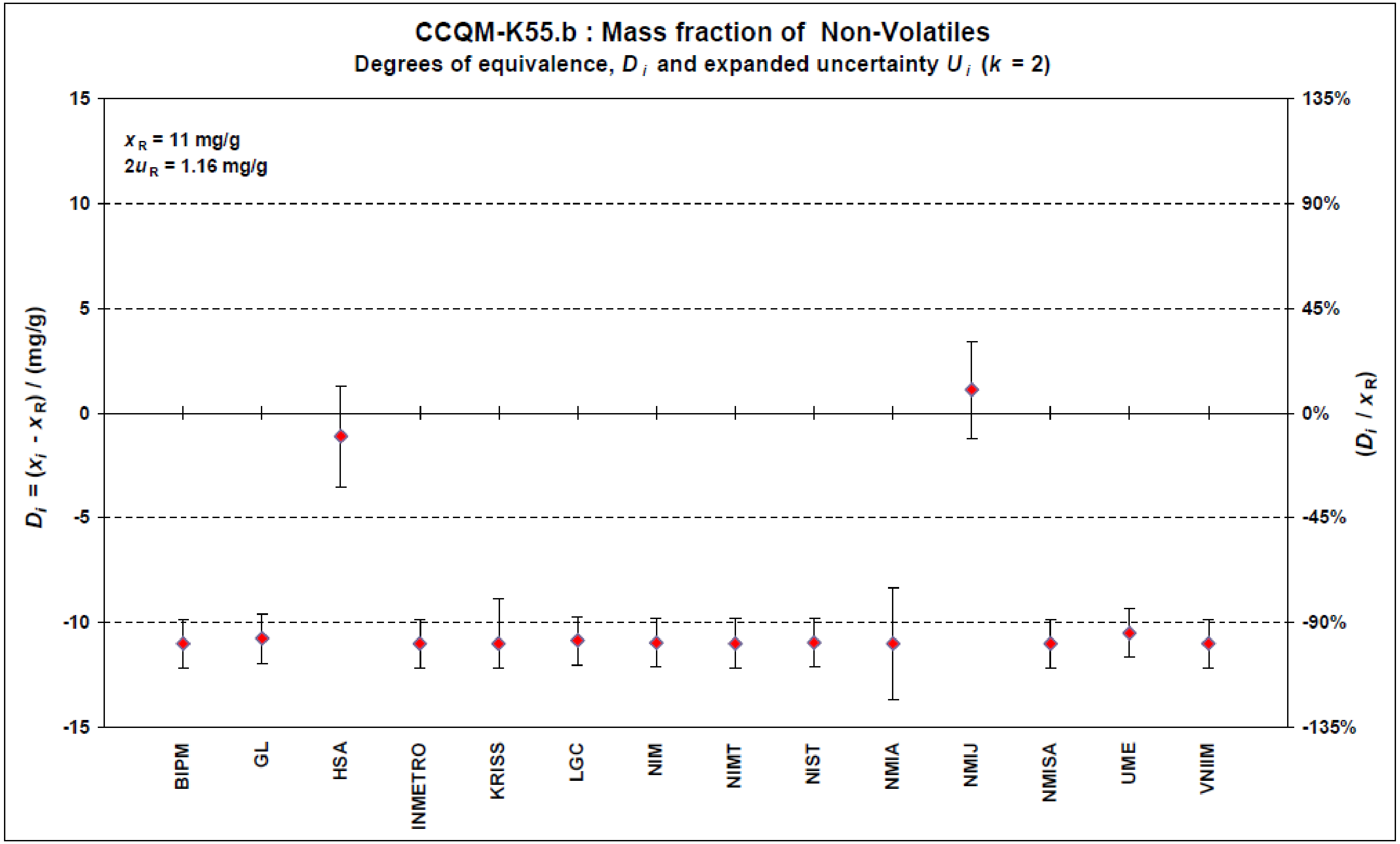


CCQM-K55.b: Purity of aldrin

Gel Permeation Chromatogram (CCQM-K55.b Sample)



CCQM-K55.b: Aldrin Purity
The importance of assay methods



New methods in biology - d-PCR

- The Polymerase Chain Reaction (PCR) method is used to quantify nucleic acids by amplifying a nucleic acid molecule with the enzyme DNA polymerase.
- Conventional PCR is based on the theory that amplification is exponential
  - An advantage and a disadvantage
  - Enormously sensitive, but it is difficult to quantify such large amplifications.

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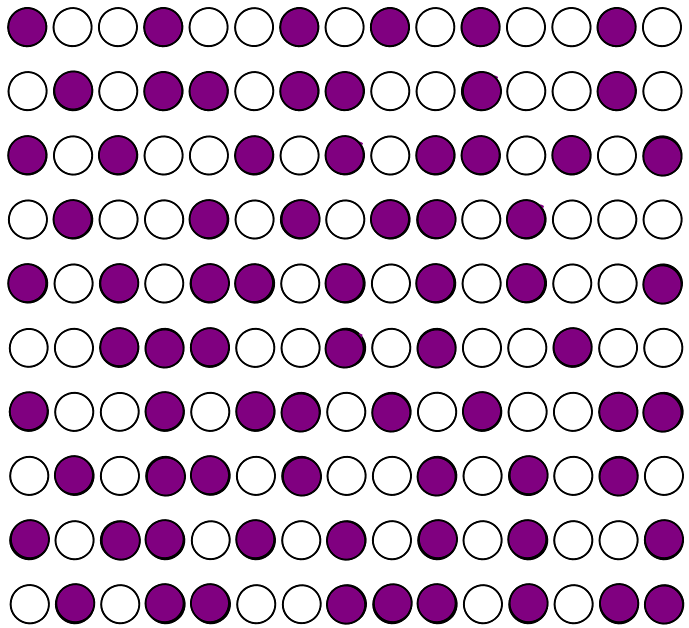

- For d-PCR the sample is separated into a large number of partitions with similar probabilities that one or zero nucleic acid molecules are present
- The reaction is carried out in each partition individually.
- Hence – it is calibration free!
- Not really.

The objectives of metrology

Measurements that are stable

- Long-term trends can be used for decision making

Measurements that are comparable

- Results from different laboratories can be brought together

Measurements that are coherent

- Results for different compounds and from different methods can be brought together

The objectives of metrology

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Measurements that are coherent

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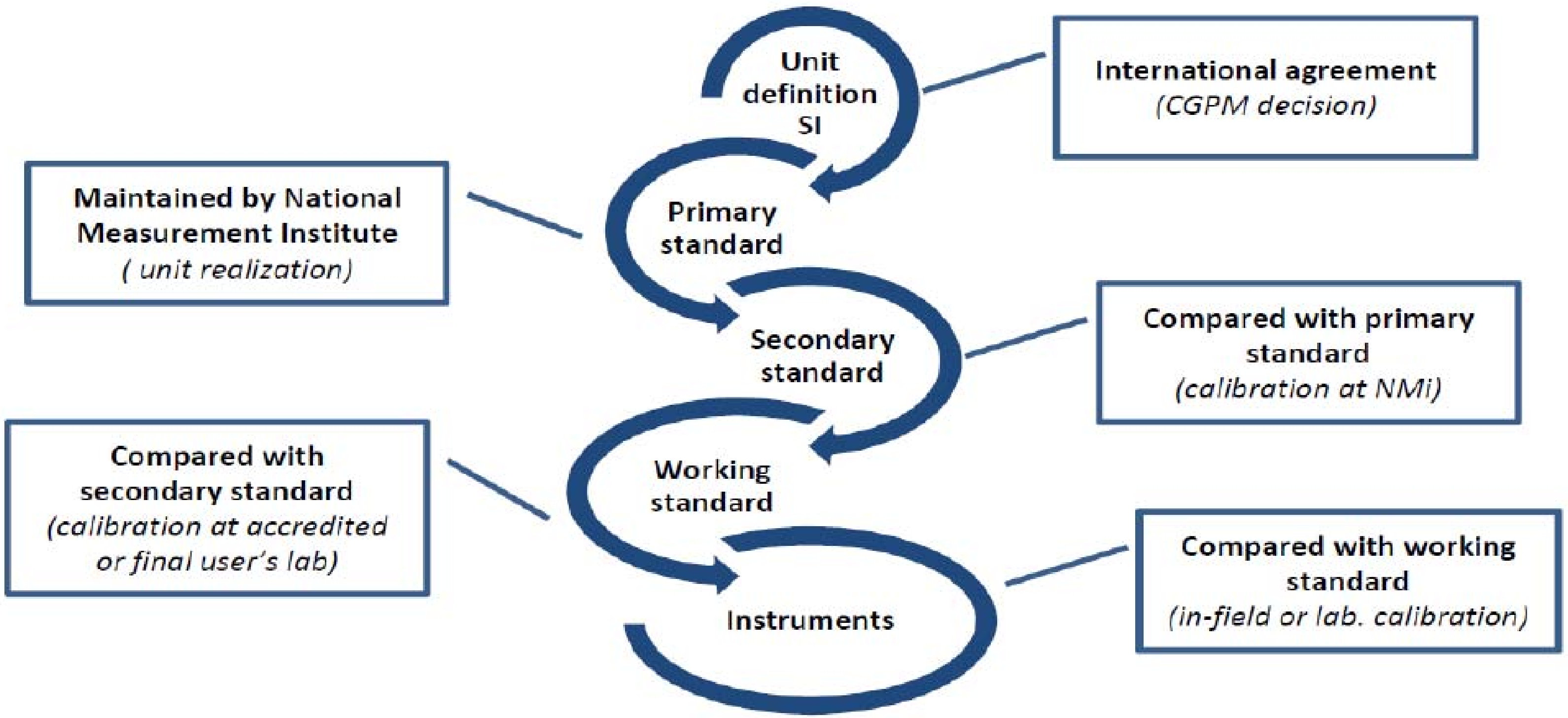
These are achieved through providing the infrastructure to support traceable measurement results (and uncertainties).

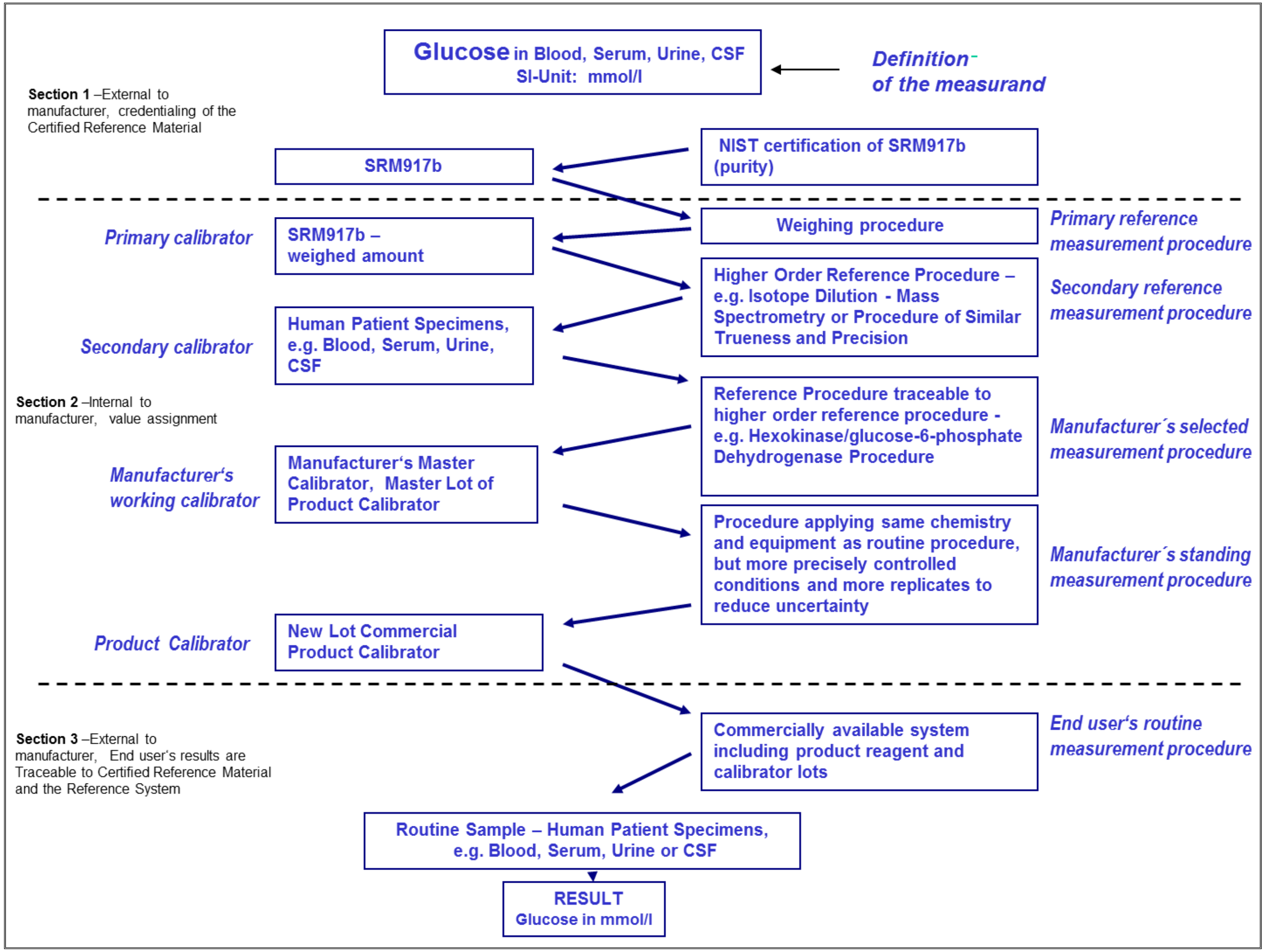
Is this possible in chemistry and biology?

The traceability "chain"



Slide courtesy
Dr S Davidson, NPL, UK

Traceability Chain for the Measurement of Glucose in Body Fluids



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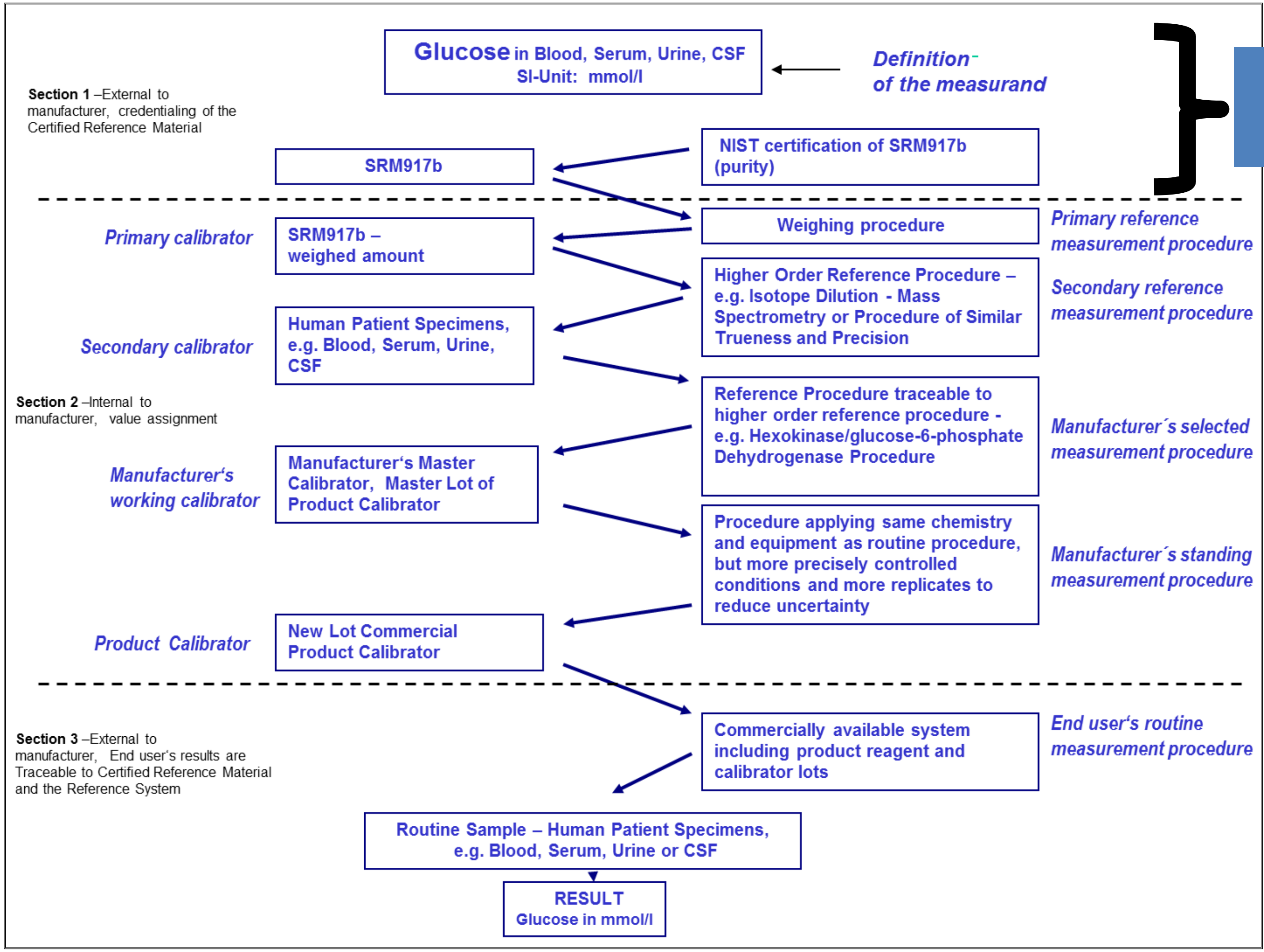


The work of the NMIs

Joint Committee on Traceability in Laboratory Medicine - JCTLM

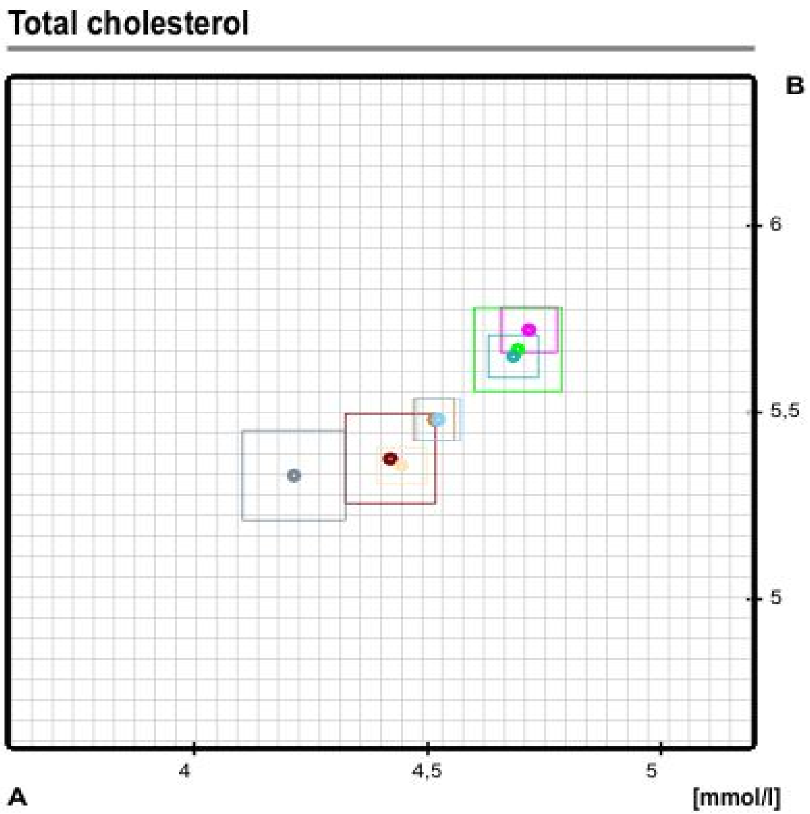
Principal promotors

- International Federation of Clkinical Chemistry (IFCC)
- International Laboratory Accreditation Coopoeration (ILAC)
- CIPM/BIPM

Supported by

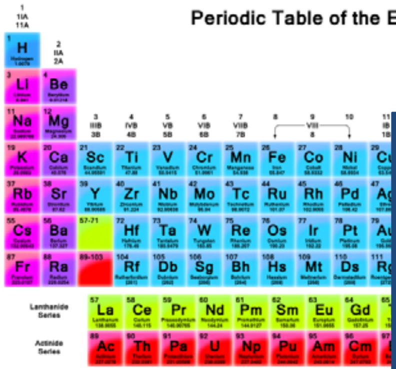
- WHO
- Regulators (FDA, EC, Japan)
- CRM producers (NIST, IRMM, a.o.)
- Reference laboratories (CDC, DGKS, etc.)
- PT and QA organisations (CAP, EQA, etc)
- Written Standards (NCCLS, JCCLS, ISO)
- IVD industry (ADVAMED, EDMA, JARC)
- Other stakeholders

Results of inter-laboratory comparison on two samples carried out for the JCTLM



By courtesy of
RELA-IFCC DGKL-RFB
EQAS 2010

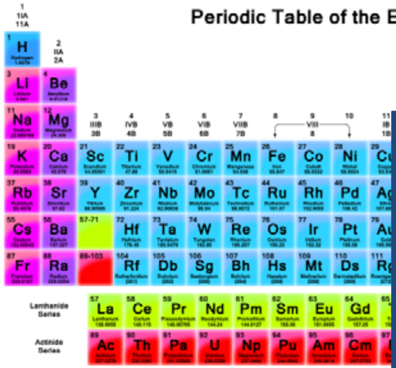
How far does the "light" shine ?



The " Chemical Measurement Universe"

- All possible chemical compounds
- Concentrations from ppt to "pure"
- All likely matrices (from minerals to shell fish
- Other possible compounds present

How far does the “light” shine ?



The “ Chemical Measurement Universe”

- All possible chemical compounds
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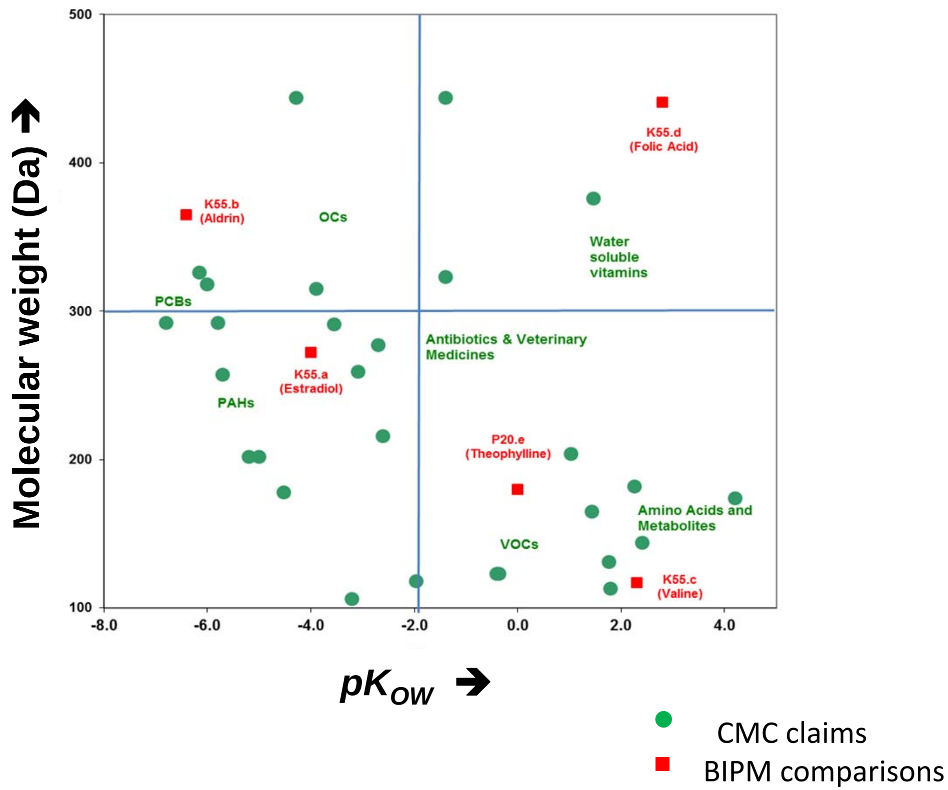
- **How far does the light shine?**
  - *“If we test the capability to measure substance X in matrix Y, how many other substances and matrices can we deduce performance about*?”
  - Invented because of the need to limit the number of CMCs in chemistry.
  - It is now of much more general importance
- Complex and well-supported traceability chains will only be possible in some areas – mainly where regulation applies.

How far the light shines

The analysis of organic compounds is an essential part of many different fields of analysis, including environmental, food, clinical, pharmaceutical, drugs of abuse, and forensics.

BIPM Organic Comparison Program 2007-15



<u>BUT</u>

the “universe” of possible organic compounds is enormous.

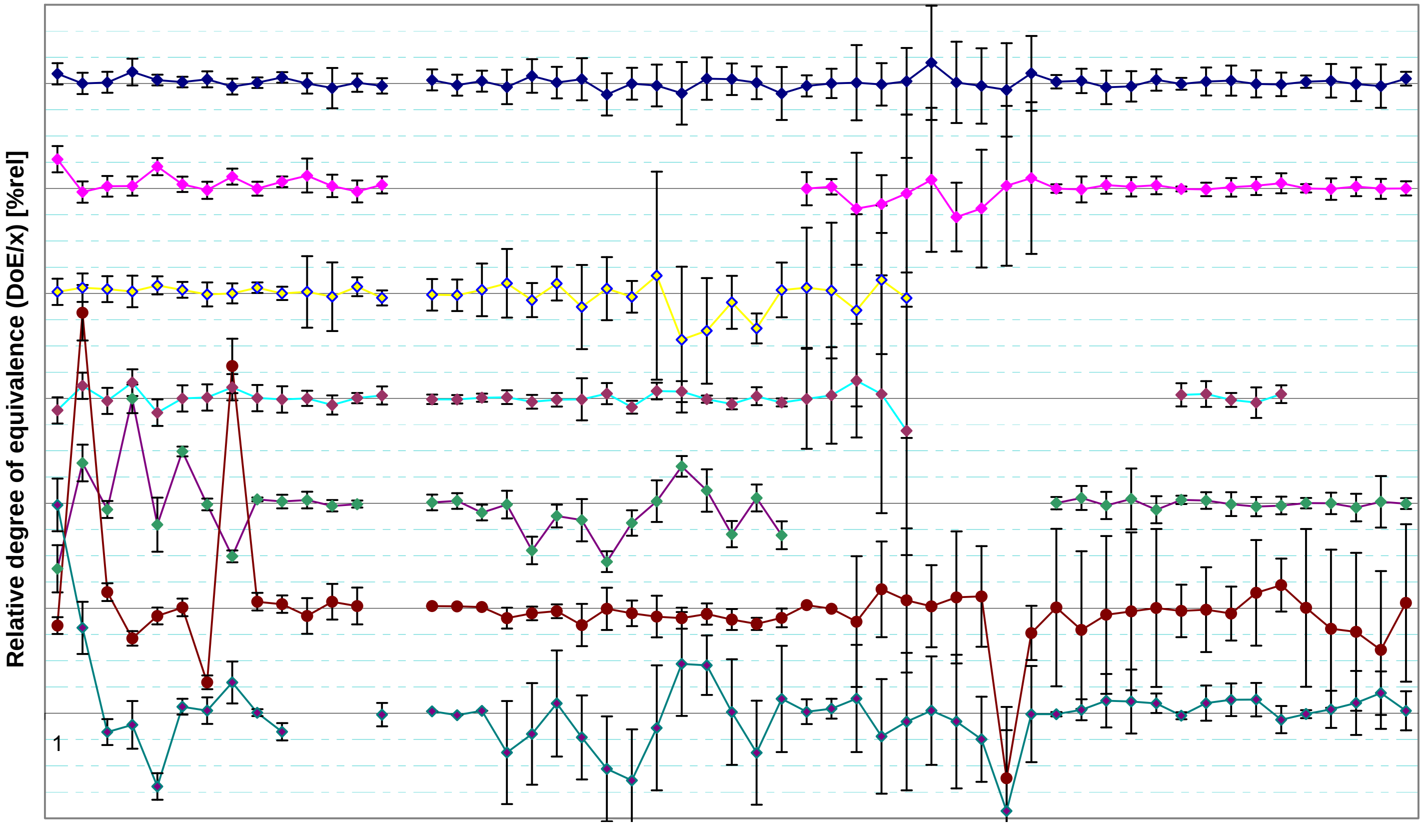
<u>So</u>

How far does the light shine?

How far the light shines

Performance of 7 NMIs in the CCQM Gas Analysis Comparisons

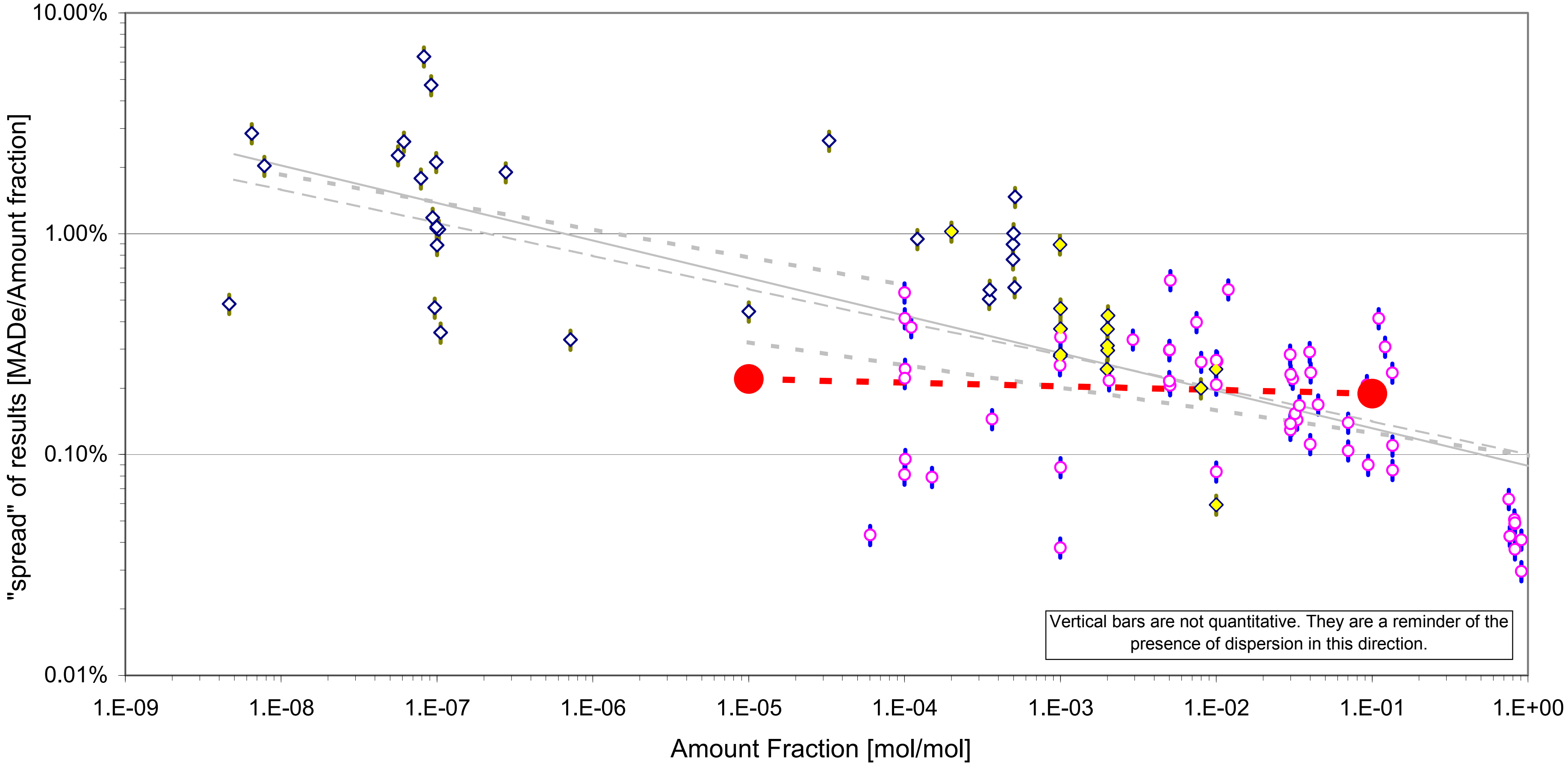
Gas analysis is an essential part of many different fields such as monitoring air quality, atmospheric composition and the contents of energy gases.



0.5% between gridlines - offset applied to each set

How far the light shines

How does performance vary with concentration?



There is negligible variation of performance with concentration. Therefore comparisons do not need to be repeated at different concentrations.

How will the worldwide measurement system evolve?

In many respects, metrology in chemistry is different to metrology in physics:

- Traceability chains are very short,
- Dissemination is mainly through the distribution of reference materials
  - can be produced in large numbers ,
  - but a very wide possible range is needed.
- Strongly driven by regulation,
  - very few “metrology law” / legal metrology requirements in place
  - Many requirements for quality of food, air, water etc.
- Limited accreditation infrastructure,
- Expertise often spread widely (eg expert laboratories)

How will the worldwide measurement system evolve?

and, **what will be the role of the NMIs?**

What we learn from the *Comité consultatif pour la quantité de matière – métrologie en chimie*

- **Only a half of all Member States and Associates take part**
  - 24 out of 55 Member States have >10 CMCs in chemistry
  - 51 out of 55 Member States have CMCs in physics
  - 17 out of 37 Associates have CMCs in chemistry

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  - 24% of chemistry CMCs are from DIs
  - 14% of all CMCs are from DIs (but 32% in Ionizing Radiation)

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- **Key comparisons are mainly organized at the CCQM level**
  - 15% of Chemistry comparison are organized by the RMOs
  - 41% of physics comparisons are organized by the RMOs

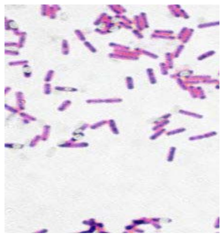
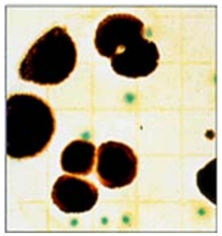
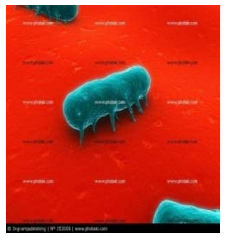
- **Key Comparisons are completed quickly**
  - 74% of CCQM comparisons are listed as "complete"
  - 61% of all comparisons are listed as "complete"

Do we expect a world-wide measurement system for biology to be like the system for chemistry?

- We already recognize the importance of human biology in the SI.
  - the candela, the grey

We learn from chemistry that

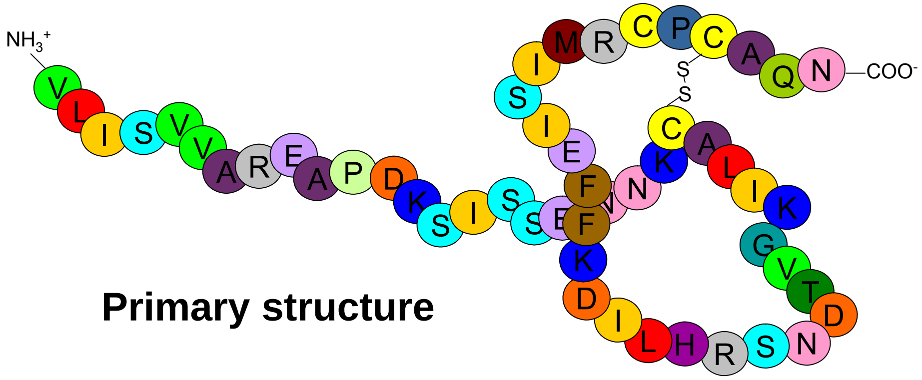
- The field will be very large (proteins, DNA, cells, microbiology …).
- Identification of the measurand (“the quantity intended to be measured”) is often very challenging.
- It will be essential to find some selected measurands/measurement methods for which “the light shines far”.
- There will be a continuum of capability – the different areas will “merge into” each other.

The complexity of proteins

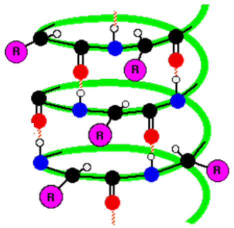
Protein measurements are needed for:

- Biopharmaceutical production & quality control
- Food safety, quality and authenticity
- Clinical pathology
- Medical diagnostics

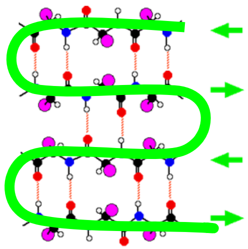


Primary structure

CCQM-P59

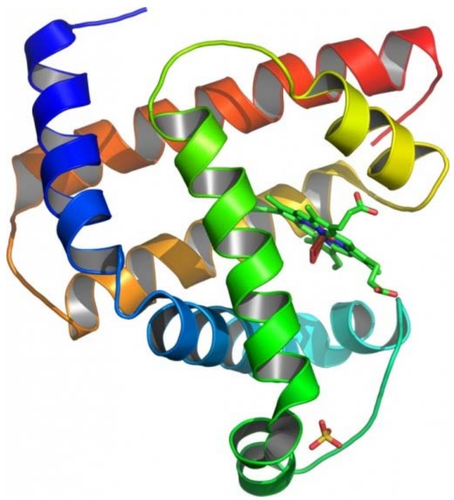


α-helix

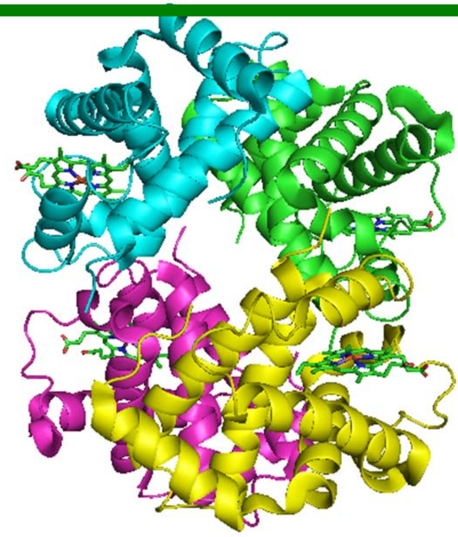


β- pleated sheet

Secondary structure



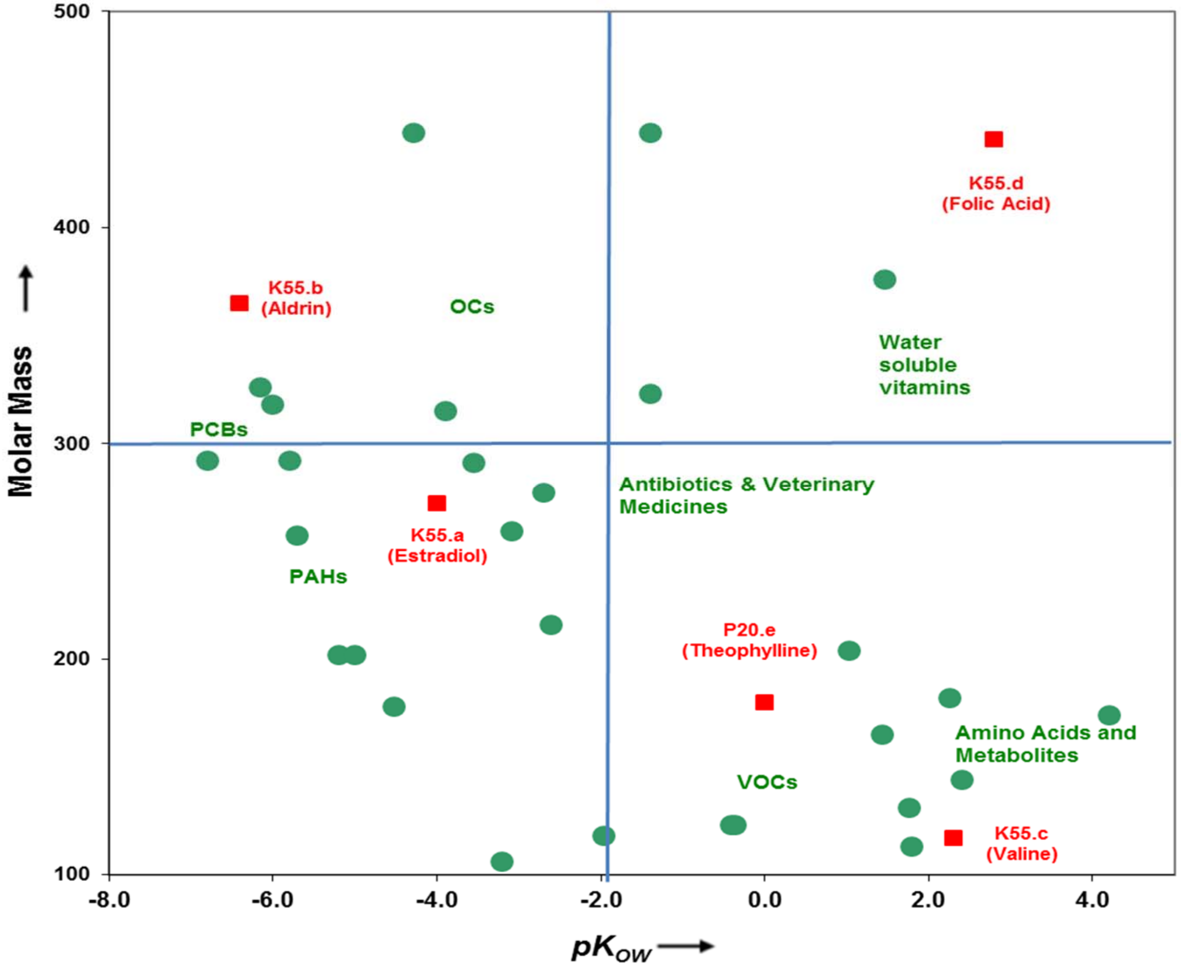
Tertiary structure



Quaternary structure

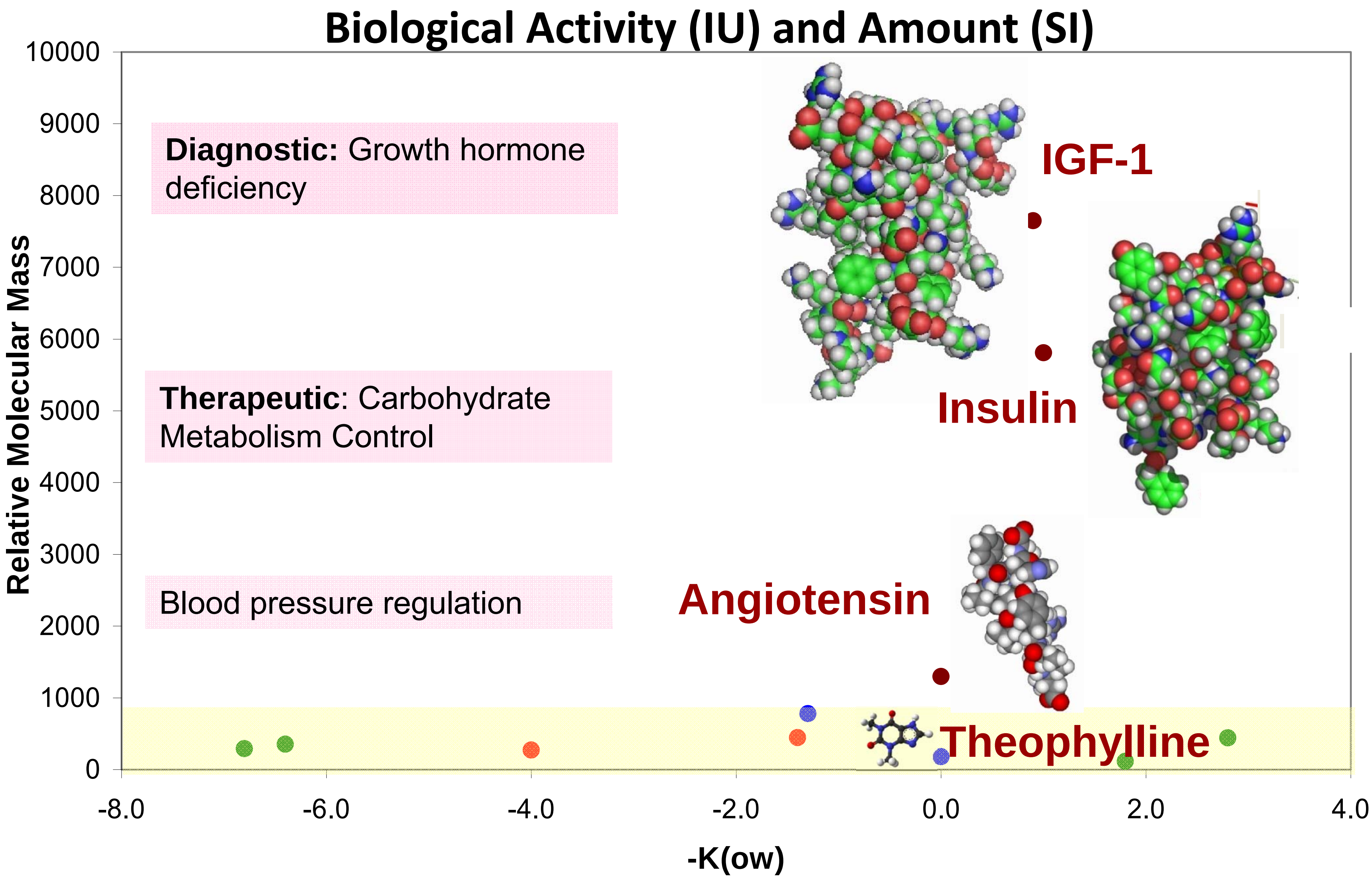
How far the light shines

Organizing the "universe" of possible organic compounds



Reference Measurement Systems for Peptides and Proteins

(joint activities by BIPM, NIST, other NMIs, WHO/NIBSC, IFCC, pharmacopoeia, industry)



Large Molecule Standards - Insulin

- 220 million people worldwide have diabetes
- 26% of adults diagnosed with diabetes take insulin
  - (4 million people in the US)

- Current World Health Organisation (WHO) standards for insulin are assigned in **International Units (IU)** and not based on biosynthetic human insulin used to treat patients.

Biological Standardisation

- The **International Unit (IU)** is "a unit of measurement for the amount of a substance, based on biological activity or effect".
  - Used for vitamins, hormones, vaccines and blood products,
  - The measurand is defined on the basis of what it does in the human body and not on the basis of what it is.
- The WHO provides a reference preparation of the agent and arbitrarily sets the number of IUs contained in that preparation.
- The number of IUs contained in a new substance is set arbitrarily
  - There is no equivalence between IU measurements of different biological agents.

Large Molecule Standards - Insulin

- Regional Pharmacopoeias have established their own reference standards
- Relative assigned property values for these compendial standards can differ.
- From the standpoint of the patient, these issues could result in differences in the dose of this lifesaving medication in different regions of the world
- Industry led call for the establishment of a new Insulin International Reference Standard value assigned in SI and not IU

- This is one example of a trend away from biological standardization, towards “chemical” standardization.

- Mass spectrometry; mass balance approaches; production of stable reference materials.

Progress in Biotechnology under the CCQM

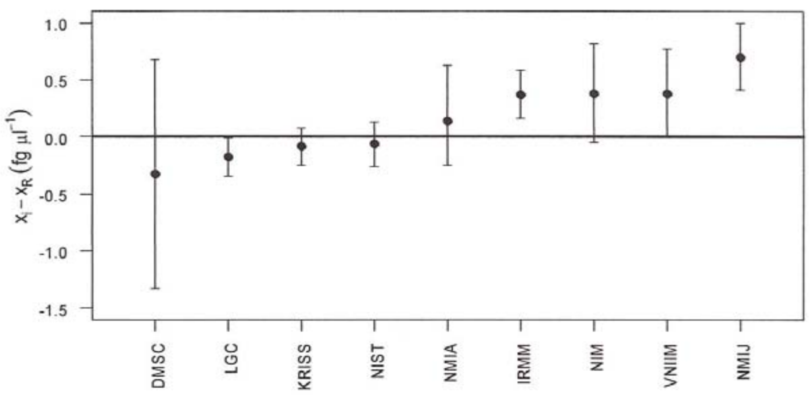
Key Comparisons

- **Quantitative PCR (*completed*)**
- **Relative quantification of genomic DNA (*completed*)**
- Relative quantification of Bt63 in GM rice matrix sample
- Peptide purity determination - synthetic human C peptide (HCP)
- ***+ 20 other studies completed***

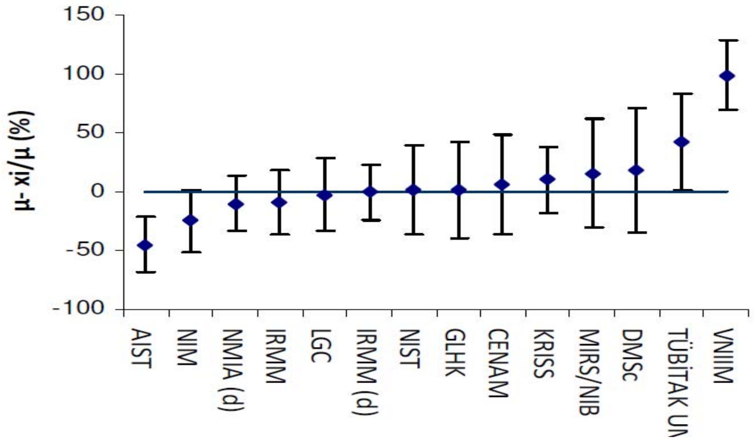
Studies being planned

- Fragments extracted from a biological tissue
- Number and geometrical property of cells adhered to a solid substrate
- Quantification of cells with specific phenotypic characteristics
- Cell viability measurement
- Absolute quantification of DNA
- Gene expression biomarker profiling
- Multiple cancer cell biomarker measurement

CCQM-K61- Quantitation of a linearised plasmid DNA in a matrix of non-target DNA.



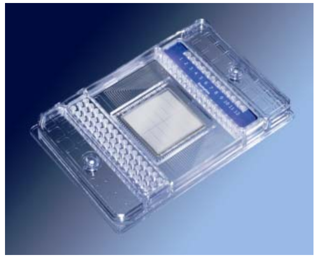
CCQM-K86 - Relative quantification of genomic DNA fragments extracted from a biological tissue

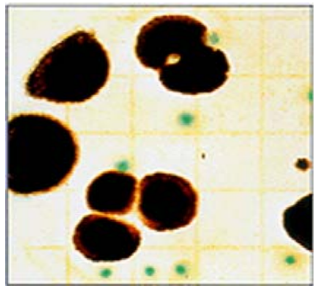


Progress in Biotechnology under the CCQM

Now focusing in four areas

- **Proteins** - (for medical diagnostics, disease monitoring, biopharmaceutical production & quality control)
- **DNA** - (for GM ingredients in food, gene expression biomarkers - clinical diagnostics)
- **Cells** - (for cancer diagnosis & therapy, infectious disease monitoring, regenerative medicine, toxicology)
- **Microbiology** – (for food safety, medical diagnosis, bio-manufacturing)
  - *Annually in the US there are 128 000 hospitalizations and 3000 deaths from food-borne pathogens.*
  - *In the EU in 2009 there were 198 252 cases of campylobacter.*





Conclusions

- Application of metrology to chemistry and biology has potentiel for great impact.
- The fields are enormous - we can only ever hope to provide traceability (or a measurement infrastructure) for measurands that are representative of whole areas of similar measurements.
- Our approach to chemical measurement is enabling us to approach biological measurements
  - as molecular weights get larger, the « IU » can be made traceable.
- Chemical and biological measurements are very method dependant
  - this threatens our concept of coherent measurements
  - but, many methods are being developed with properties that are well suited for use in metrology:
    - CRDS, IDMS, q-NMR, d-PCR …
- These methods only solve a very limited number of the challenges in chemical and biological measurement, but they indicate what can be done.

Thank you for your attention

Dr Martin Milton

- BA Hons (First Class), Physics, Oxford University
- PhD, Physics, Southampton University
- MBA (Distinction), London Business School
- Honorary Professor, Department of Chemistry, University of York.
- Joined the National Physical Laboratory in 1981,
- Appointed as an NPL Fellow in 1998.
- Led the "Gas Metrology and Trace Analysis" Group
- Appointed to the International Bureau of Weights and Measures (BIPM) as Director Designate on 1st October 2012,
- Director of the BIPM since 1st January 2013.