Metrology traceability of measurement in (bio)chemistry

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What is a reality ?

„We (not divided deity that operate in us) dreamed up this world. We have dreamed it as resistant, mysterious, visible, omnipresent in space and invariable in time; however we have admitted eternal gaps of madness into its architecture, so as to know it is not true“

(Jorge Luis Borges : La Perpetua Carrera de Aquiles y la Tortuga)

? Road map

Universe

materia (energy)	time	space	(attributes)
force, heat	repetition, descendance	distance, direction	(manifestations)
mass, temperature	*frequency, time*	*length, angle*	(quantities)

What are principal facts?
How to confirm them?

- Natural laws

(logical structures)

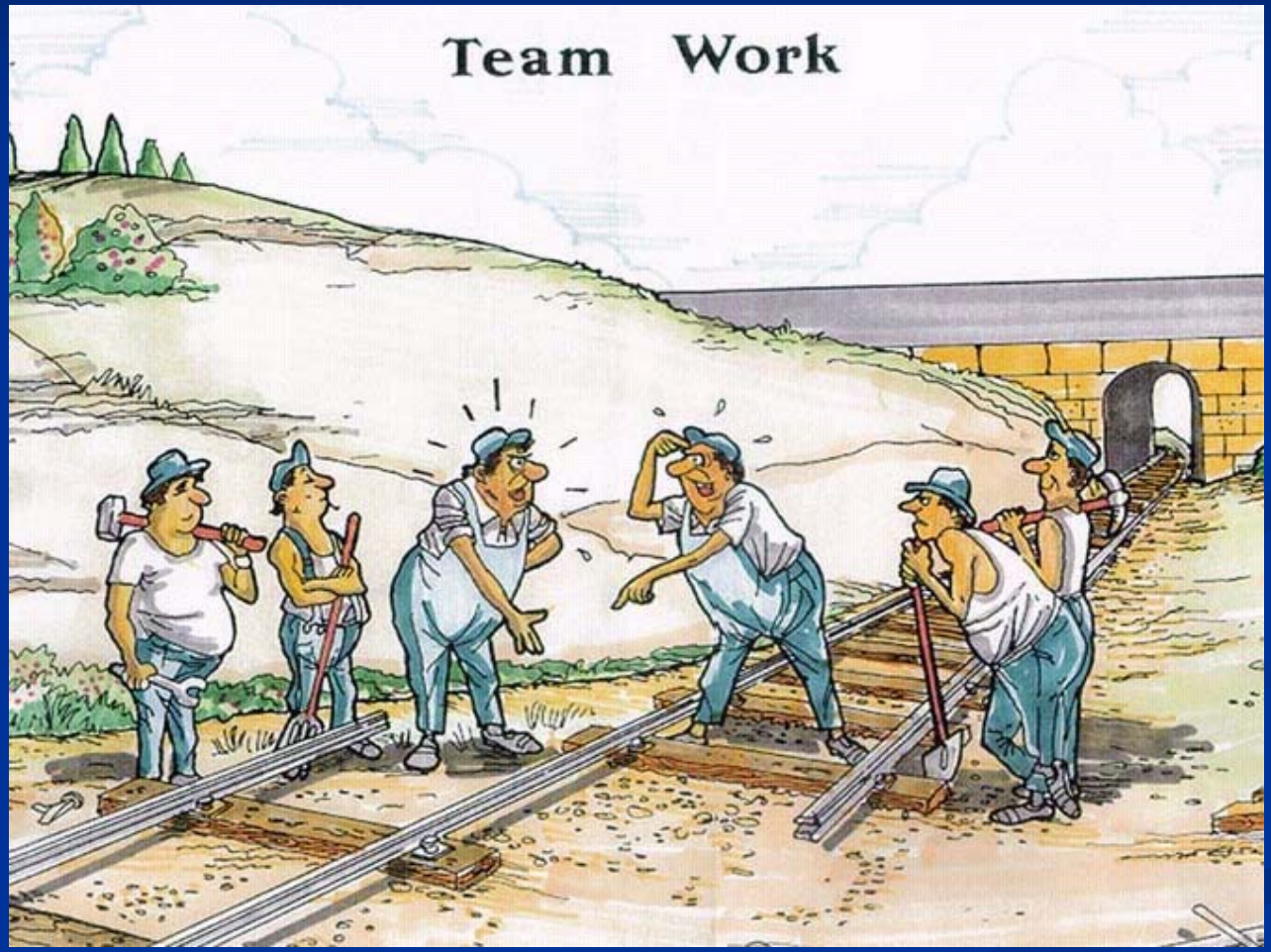
- experiments

(ways enabling selection of sensing entrance data that satisfy the laws requirements)

measurement

(process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity - VIM III)

Traceability 50%?



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

VIM - III

Three assumptions :

- most accurate phys. unit realisation
- its international comparability
- its most accurate transfer into practical measurements

- Important – Attention - qualitative aspect of chemical measurements !!

Measurement versus testing

Measurement – process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity (VIM III)

Quantity – property of a phenomenon, body or substance, where the property has a magnitude that can be expressed as a number and a reference (VIM III)

Testing – Technical operation consisting of establishment one or more characteristics of given product, process or service, according to stated procedure **(ISO/IEC Guide 2)**

Characteristic – property which help to resolve a component of given population (ISO 3534)

Detection of characteristics – can be formulated a „measurement“ model ?

Calibration

Operation that, under specified conditions, in a first step establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication.
(VIM III)

- comparative method
- substitution method

! Traceability assurance is necessary but not sufficient condition of accurate results.

Indirect measurements

Measurement – comparing unknown value of phys. quantity to its known value - reference

$$X_x = F(c_x, c_i, \alpha_i, \beta_i, \gamma_i, \delta_i)$$

In calibration process besides individual quantity value of calibration function as well as validity of this function is confirmed.

Analytical function and its approximation

$$c_x = F^{-1} \quad (X_x\,,\, c_i\,, \alpha_i, \beta_i, \gamma_i\,,\, \delta_i) \rightarrow c_x = G\,(X_x\,,\, c_i\,, \alpha_i, \beta_i, \gamma_i\,,\, \delta_i)$$

General validity of such a dependence between relevant quantities is not a common matter.

The dependence shape is unknown in many cases, eventually depence can be staochastic.

Let the dependence is of functional character.

Weierstrass theorem : every continuous function can be uniformly approximated as closely as desired by a polynomial function

Selection of approximation

Let us take the dependence for continuous function.
G is substituted by other function φ (approximation for function).

$$\varphi(c) = a_0\, \varphi_0(c) + a_1\, \varphi_1(c) + \ldots + a_n\, \varphi_n(c)$$

By solving of such a problem one inhibits to dependence of only two variables , and it is focussed to relatively narrow range of the quantity values.

Question of approximation selection is connected with approximation error $E(c)$

$$E(c) = G(c) - \varphi(c)$$

How to evaluate it?

Two basic approaches

- continual
- discrete

Selection of approximation

Let us suppose, that for estimation of fitting error we have chosen a general criterion $|\rho(G, \varphi)| \geq 0$ such that

$$0 \leq \rho(G, \varphi^*) \leq \rho(G, \varphi)$$

In a continual case an approximation error is evaluated e.g. via quantity

$$\rho_p(G, \varphi) = \left(\int | G(c) - \varphi(c) |^p \, dc \right)^{1/p}, \; p \geq 1,$$

Selection of approximation

- The most used criterion in practice is

$$\rho_2(G, \varphi) = \left(\int \left| G(c) - \varphi(c) \right|^2 dc \right)^{1/2}$$

- According to this criterion the best approximation is called the best rational L_2 approximation.

$$\rho_{2,\omega}(G, \varphi) = \left(\int \left| G(c) - \varphi(c) \right|^2 \omega(c)\, dc \right)^{1/2}$$

Selection of approximation

In a discrete case function *F*, can be defined by different ways, e.g. measured values are given in form a table

$$\{(c_i, G(c_i)), i = 0, 1, \ldots, m\}$$

$$\rho_{m2}(G, \varphi) = (\Sigma | G(c) - \varphi(c) |^2)^{1/2}$$

$$\rho_{m2,\omega}(G, \varphi) = (\Sigma | G(c) - \varphi(c) |^2 \omega i)^{1/2}$$

The best approximations of this type are called the best discreet approximations .

The best discrete L2 approximation is called method of least squares

Traceability ensured via original function

Amount of substance

Coulometry –Faraday's law (modified)

$$\varphi = [U_1.(t_1\text{-}cor_1) + U_2 .t_2 + U_3.(t_3 - cor_2)]/z.F.m'.cor.R$$

φ is amount of contents
U_1 is voltage during initial titration
t_1 is total time of initial titration
cor_1 is correction for difference between titration end point and time of solution introducing into separating part
cor_2 is correction for change of indication signal after displacing of solution from separating part
U_2 is voltage during main electrolyse
t_2 is time of main electrolyse
U_3 is voltage during final titration
t_3 is total time of final titration from end point of initial titration
m' is mass of weights balancing the sample in air
cor is air buoyancy correction
R is standard resistor resistance

Application

- Specification of analytical range
- Specification of measurement conditions
- Specification of mathematic function type

C=G(X)

linear function

$C = b_0 + b_1 X$

Polynomial of 2nd stage

$C = b_0 + b_1 X + b_2 X^2$

Polynomial of 3rd stage

$C = b_0 + b_1 X + b_2 X^2 + b_3 X^3$

Power function

$C = b_0 + b_1 X^{b2}$

exponencial function

$C = b_0 + b_1 exp(b_2 X)$

Application

- Specification of calibration points number *n*
- Selection (preparation) of calibration standards
- Estimate of their standard uncertainties *
- Determination of signals values of individual calibration standards x_i and estimation of their $u(x_i)$.
- Calculation of b_i parameters of mathematical function approximating analytical function.
- Parameters are calculated by general method of least squares

Application

- Determination of measured quantity signal (response) value
- Calculation of analyte contents $c_x = G(xx)$
- Calculation of $u(c_x)$ of measured quantity value

$$u(c_x)^2 = (\partial G/\partial x_x)^2\, u(x_x)^2 + \sum_{k=0}^{M} (\partial G/\partial b_k)^2 \; var(b_k) + 2 \sum_{k}^{M-1} \sum_{i}^{M} (\partial G/\partial b_k)\,(\partial G/\partial b_i) cov(b_k\, b_i)$$

$$var(b_k) = \sum_{i} (\partial b_k/\partial c_i)^2 var(c_i) + \sum_{j} (\partial b_k/\partial x_i)^2 var(x_i) + 2 \sum_{i} \sum_{j} (\partial b_k/\partial c_i)\,(\partial b_k/\partial c_j)\, cov(c_i\, c_j)$$

$$cov(b_k\, b_l) = \Sigma\, (\partial b_k/\partial c_i)\,(\partial b_l/\partial c_i)\, var(c_i) + \Sigma\, (\partial b_k/\partial x_i)\,(\partial b_l/\partial x_i)\, var(c_i) + \Sigma\, \Sigma\, [\, (\partial b_k/\partial c_i)\,(\partial b_l/\partial c_j)$$
$$+ \; (\partial b_l/\partial c_i)\,(\partial b_k/\partial c_j)]\; cov\,(\, c_i\, c_j\,)$$

Biochemical and clinical analysis

Research of analysed subject via measurement (quantity) depends from the level of knowledge

Requirement of accuracy is substituted by requirement of uniformity

⇒ Establishment of so called reference system (reference procedure, RM, reference laboratory)

⇒ Establishment of JCTLM (by CIPM, IFCC and EA) in 2002

- WG 1
- WG2

⇒ See www.bipm.org

Metrological assurance of IVMD

- very little quantitative evidence in the medical literature that the use of uncalibrated or poorly calibrated measurement systems leads to adverse outcomes

- ⇒ attention of healthcare administrators and practitioners would be drawn to traceable calibration

- besides it also correct measurement procedure, measurement conditions, optimal (validated) measurement method and a correct method of results evaluation are required

Metrological assurance of IVMD

- ISO 17 511:2003 IVMD Measurement of quantities in biological samples – Metrological traceability of values assigned to calibrators and control materials

- ISO 18153:2003 IVMD – Measurement of quantities in biological samples - Metrological traceability of values for catalytic concentration of enzymes assigned to calibrators and control materials

- ISO FDIS 15194 (TC 212) IVMD – Measurement of quantities in samples of biological origin – Requirements for CRM and the content of supporting documentation

- ISO 15193 IVMD – Presentation of reference measurement procedures

- ISO 15195 IVMD – Requirements for reference measurement laboratories

Metrological assurance of IVMD

a) ISO F DIS 15194

metrological traceability → calibration → CRM

Specification of requirements for CRM, their production and characterisation; the certificate contents and how to collect the data for quantity value determination

⇓

ISO Guide 35:2006

ISO Guide 34: 2000 (under revision)

ISO Guide 31:2000 (under revision)

Metrological assurance of IVMD

a) ISO F DIS 15194

ISO Guide 31:2000 – Certificate contents:

- Name and address of the certifying body
- Title of the document
- Name of material
- Reference material code and batch number
- Certified values and their uncertainties
- Traceability
- Description of the CRM
- Intended use
- Instruction for correct use of the CRM
- Hazardous situations
- Values obtained by individual laboratories or methods
- Uncertified values

Metrological assurance of IVMD

- ISO F DIS 15194
- ISO Guide 31:2000 – Certificate contents:
  - Hazardous situations
  - Level of homogeneity
  - Date of certification
  - Period of validity
  - Further information
  - Names and signatures of certifying officers

Metrological assurance of IVMD

b) ISO 17511:2003

- How to assure the metrological traceability of values assigned to calibrators and control materials

- Calibrator – primary, secondary unit of measurement with smallest u_C and its value is assigned by a primary reference procedure (CRM)
  secondary →...
  - it shall be characterised by following information: recognition (international, national...), issuing authority, certificate, origin, molecular form, matrix, phase...

- Methods of validation (wording of this standard is very close to language of biochemical and biological community)

Metrological assurance of IVMD

c) ISO 18153:2003

- Catalytic activity – property of component corresponding to the catalysed substance rate of conversion of a specified chemical reaction, in a specified measured system.

- Metrological traceability and calibration hierarchy
  - primary ref. measurement procedure
  - primary calibrator
  - secondary ref. measurement procedure
  - secondary calibrator
  - end users routine measurement procedure

- Validation of metrology traceable calibration
  - its analytical specificity (it must be demonstrated that all meas. procedures in the calibration hierarchy have essentially the same analytical specificity)

Analytes - measurands

CATEGORY I Traceable to SI	CATEGORY II Non-traceable to SI
creatinine	glycoprotein
cholesterol	coagulation factors
electrolytes	proteins
glucose	peptide-bond
steroids	epitopes

Estimation of measurement results u_C

- measurement – application of GUM
- determination of characteristics – individual approach

Main contributing factors :

- definition of measurand (property)
- sampling
- transport , storage and manipulation with sample
- sample treatment
- measurement (testing) conditions
- experience of operator
- deviations from declared procedure
- quality of used measuring instrument
- quality of applied reference standard
- Software (procedure) limitations
- Corrections for systematic effects

Estimation of measurement results u_C

a) Indirect measurements with application of approximate function – u_C cannot be completely estimated from measurement model

- $\Rightarrow$ need of validation

b) Determination of characteristics – can „ measurement model " be formulated ?

- yes – similar approach as in measurement case
- non – defining main sources and estimation of their uncertainties (strict keeping of procedure including conditions), eventual use of test results indicators (suitable physical quantity- e.g. temperature, resp. flash time – indicator of inflammability)

Reference standards and RM

- Reference standards
- Reference materials

a) for calibration purposes

b) for quality assurance (validation, PT, ... (matrix CRM)

c) evaluation of parameters (repeatability of method or measuring instrument, operators capability) → ISO Guide 80 (in house materials (Precision Control Materials)

Reference standards and RM

a) Requirement of covering of potential concentration range of analyt in samples

- CRM → requirement of certificate content (ISO Guide 31) (traceability, uncertainty of certified value, measurement conditions, minimum sub-sample weight, certificate validity...

b) CRM → requirement for certificate content (the same as in the case of CRM for calibration purposes except of traceability)

c) PCM → neither certificate nor traceability is required; required is evaluation of homogeneity and stability

Traceability of measurement results for non-SI traceable quantities

- Strategy for introducing traceability has to be different (for large number of analytes for which no defined molecular structure can be stated)

- The first step (the most important) – definition of quantity before establishing any reference system → whenever possible, should be achieved a global consensus

- The second step – establishment of reference system (reference material, reference method...)

- Establishment of calibration hierarchy

Standardisation needs for internat. acceptability

- Characterisation of analyte
- Clinical needs
- Reference procedure
- Reference material
- Reference laboratories

Conclusion

- Application of approximation function in calibration process is a limiting factor of uncertainty type B estimation
- Relevant calibration methods are not of the same qualitative level as methods working with original function
- Traceability declaration is not fully clear
- Systematic error of method can be reduced by better calibration to be a a part of internationally accepted reference systems
- If non SI quantities are determined → agreed reference procedure is acceptable