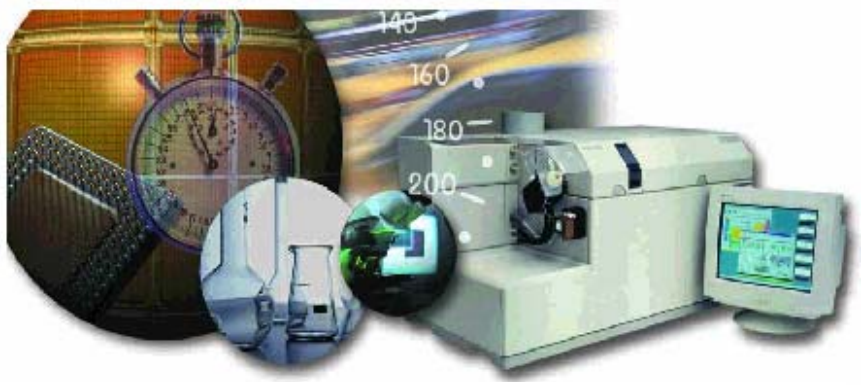


Academic exchange
University of Alicante,
March 2007

METROLOGY IN SPECTROCHEMICAL ANALYSIS

V. Kmetov
University of Plovdiv, BULGARIA

Why this topic?

* *AcadeMiC is an open European platform of analytical chemistry university lecturers, created to share ideas and best practice in teaching generic measurement science in analytical chemistry*

The Declaration underlines that there is a general lack of knowledge of metrology among researchers, laboratory practitioners, laboratory managers and legal experts.

To train good professionals, students in chemistry have to be educated in METROLOGY!

Rogaška Declaration

The signatories of this declaration who

- *lecture courses at European universities, dealing with analytical chemistry and its metrological basis*
- *or are representatives of organisations that have an interest in having such education provided to European students*
- *or who participated to the AcadeMiC* Summer School held in Rogaška Slatina (4-6th July 2005)*

and who are convinced of

- *the importance of chemical measurements to society (in sciences, trade, economy, quality of life)*
- *the need for reliable measurements to allow correct decisions and to avoid unnecessary spending of resources*
- *the necessity that those studying science should be taught the basics of measurement science, helping them to handle measurement technical issues related to ISO/IEC-17025 in the correct way*

hereby commit themselves

- *to promote such courses*
- *and to cooperate in making these available across Europe*

and recommend that Analytical Chemistry courses both at bachelor and master level should incorporate Metrology in Chemistry and Quality Assurance topics.

[list of signatories in annex]

signed by 59 university lecturers and a group of EU stakeholders from 21 countries and 5 international organisations.

http://www.acade-mic.eu/

TrainMiC

Training in Metrology in Chemistry

TrainMiC is organised and conducted by JRC-IRMM as a common international information platform to be used as a tool for the education in metrology in chemistry.

HEADLINES
four lectures + one practice

1. The new trends and the politics of the European Union concerning the
   Metrology in Chemistry
   Basic statistic needed for spectrochemical measurements

2. Uncertainty budget calculation in spectrochemical measurements
   Kragten approach

3. Validation of the measurement procedure
   Inter Laboratory comparison

4. Traceability of the measurement results,

5. Determination of gold in jewellery gold alloys by Flame Atomic Absorption Spectrometry - evaluation of the measurement procedure,
   TranMiC – example

REFERENCES

TrainMiC – Training Metrology in Chemistry - EUR Report 20841 EN
Office for Official Publications of the European Communities ISBN 92-894-6238-8

GUM-Guide to Expression of Uncertainty in Measurement, ISO, 1995

Eurachem/CITAC Guide: Quantifying Uncertainty in Analytical Measurement, 2nd Edition, 2000 http://www.eurachem.ul.pt/guides/QUAM2000-1.pdf

ISO/IEC 17025: General Requirements for the Competence of Testing and Calibration Laboratories, 1999

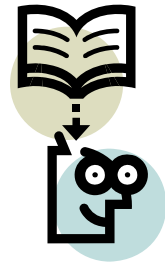
VIM - International Vocabulary of Basic and General Terms in Metrology, ISO, 1993 (2004)

J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, Ellis & Horwood, Chichester (ISBN 0-13-845421-3)

Kragten, J., The Analyst 119 (1994), 2161-2165

The topics today are :

1. Metrology - way to study it ?
2. How to present the measurement result?
3. Just to refresh the Basic statistics knowledge we need.
3. Confidence interval is this enough?
4. Single laboratory validation – some examples



The main goal of metrology is to prevent the problems between people in the process of quantity characterization !!

Metrology is the science of measurement

is about understanding the measurement procedure

(not about measuring with the smallest achievable uncertainty)

VIM (2004) (2.2) **metrology - field** of knowledge concerned with **measurement** includes all theoretical and practical aspects of measurement, whichever the **measurement uncertainty** and field of application.

Metrological aspects are part of any measurement even the simplest one

Metrology in chemistry (MiC)

is metrology applied to chemical analysis

The modern societies use measurements acount for 4 to 6 % of GDP

The importance of chemical measurements is enormous:

- in science
- in technology
- in trade
- when making regulation

(*about 40% EU directives involve measurements*)

Metrology is important and the EC supports it!

Between 5 and 30 % of chemical measurements are unsatisfactory!

Taylor, Leito, Majcen, Galdikas, Vassileva, Duta, Bulska “A strategy for a national metrology institute ...” *Accred. Qual. Assur.*, 2004

Directives requiring of measurements implementation

Design — **EC**

Adopt — **EU & National**

Implement equivalently across EU — **National**

measurements — **National**

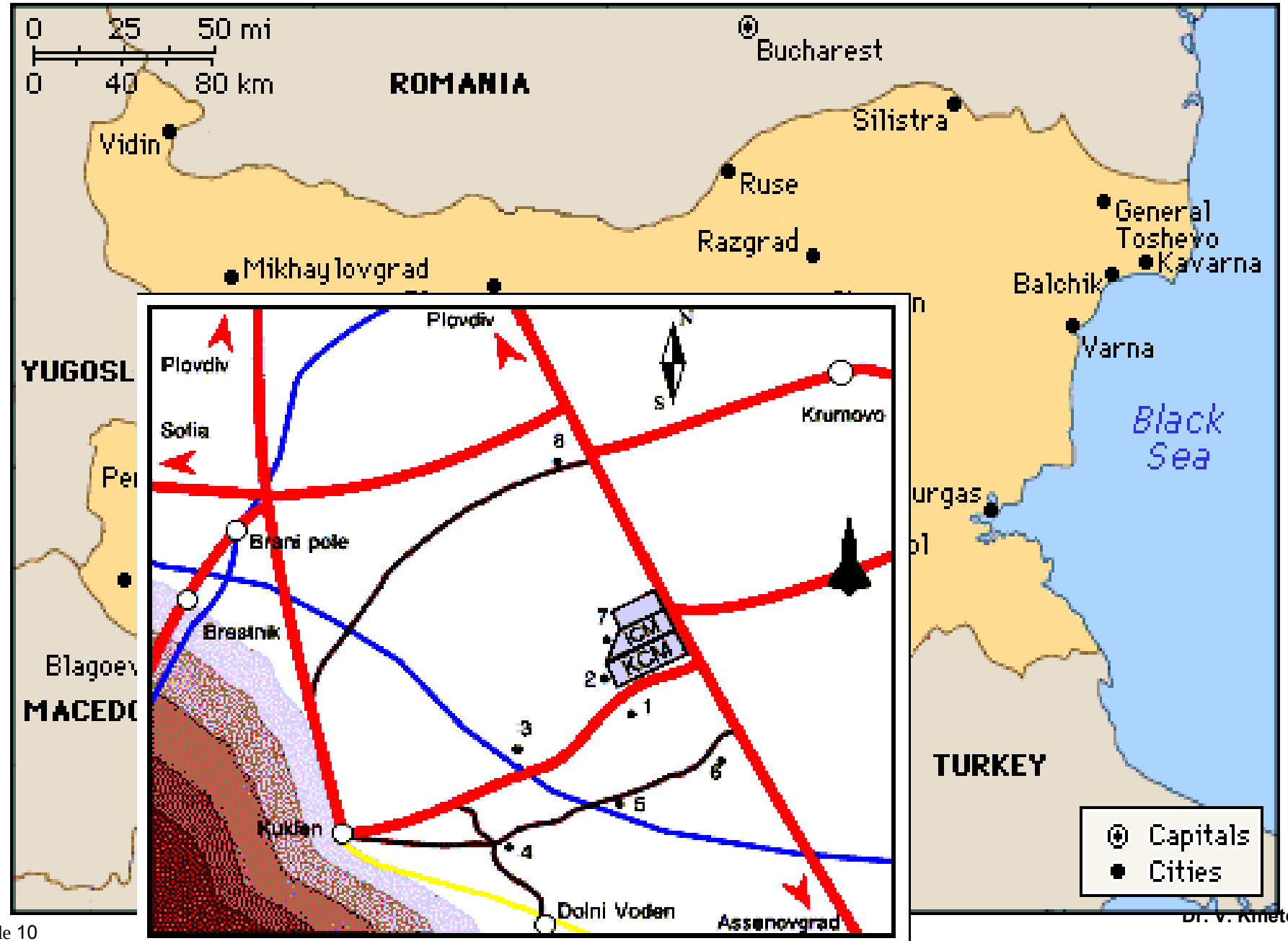
Miglena Kuneva
European Commissioner
for Consumers

Crucial link between directive and implementation uniform across EU

competent people

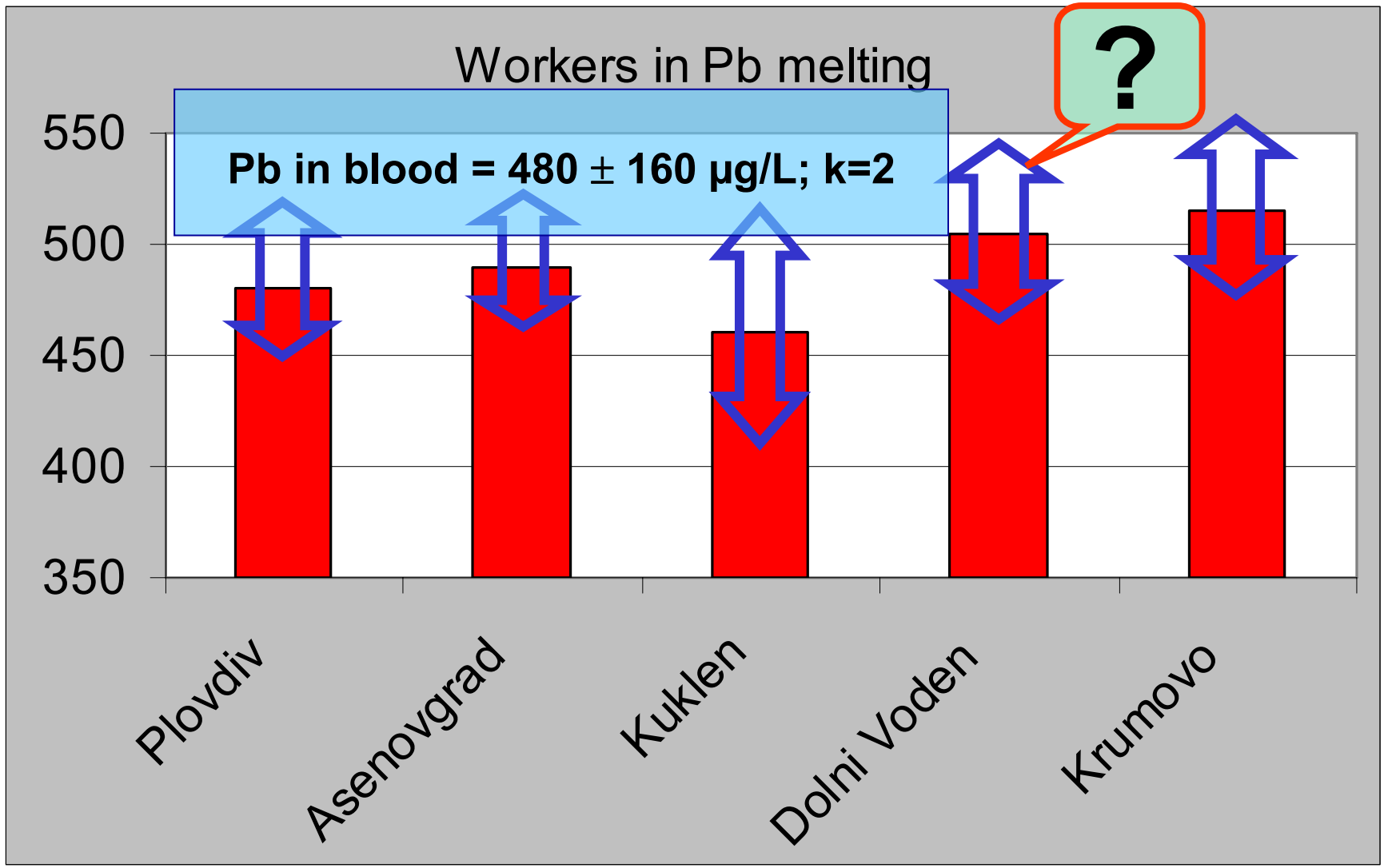
- Competent labs in the member states
- Competent national accreditation body
- Competent national reference labs

Decision makers affects - what is needed?



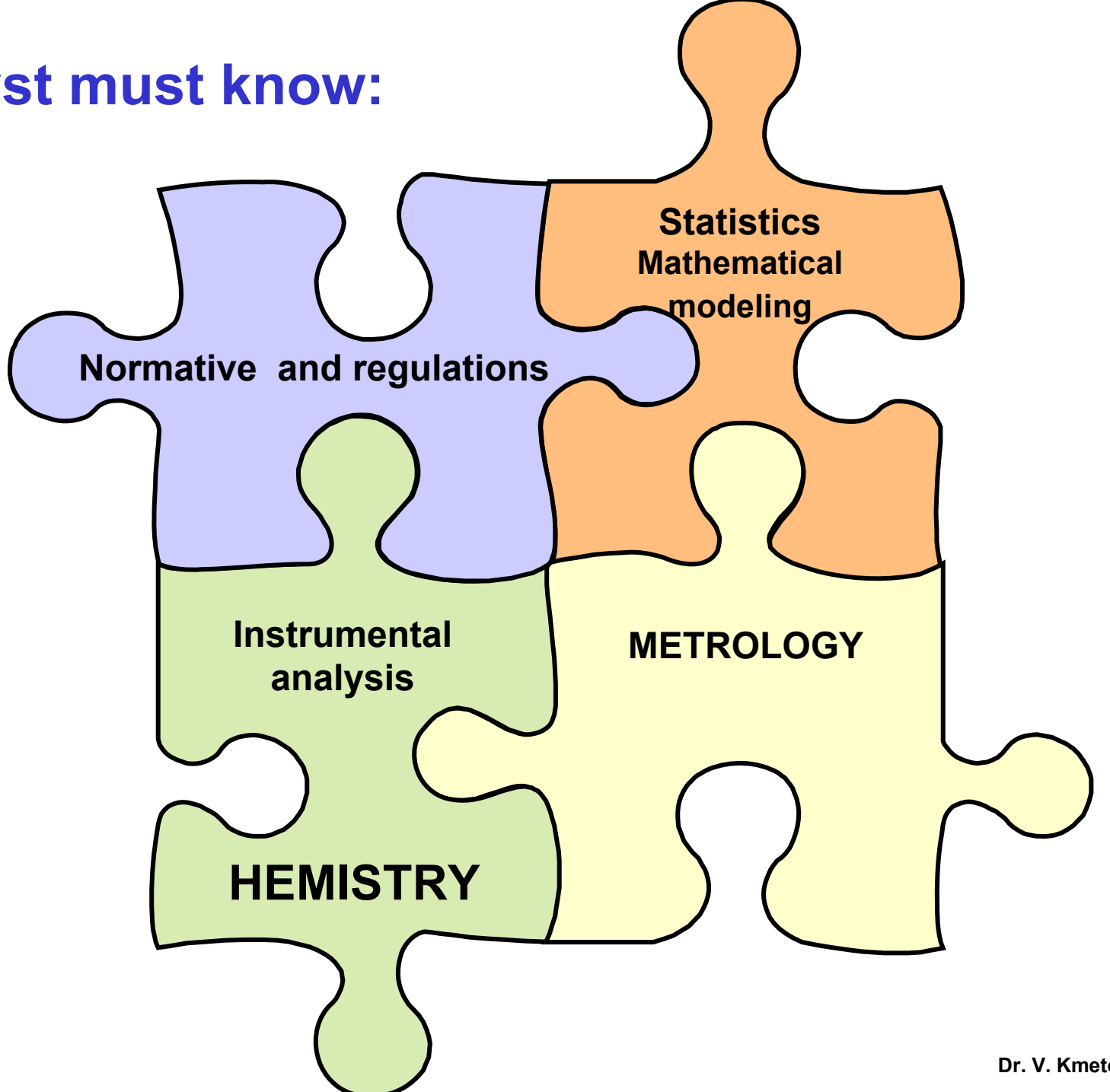
Decision makers affects - what is needed?

Dependence of leaving place on the Pb content in blood of workers (melting sector) from Non-Ferrous Factory KCM-Plovdiv



Dr. V. Kmetov Alicante 2007

The analyst must know:



"LEGISLATORS *VERSUS* ANALYSTS – THE DEMANDS OF CURRENT AND EMERGING FOOD LEGISLATION ON THE ANALYTICAL COMMUNITY"

Roger Wood, Food Standards Agency,
c/o Institute of Food Research, Norwich Research Park, Colney. Norwich NR4 7UA

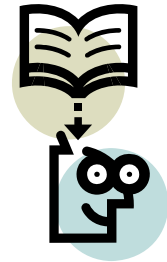
CONCLUSIONS

The analyst is increasingly being given more "freedom",

Is coming at an increasing cost to him?

It will be essential for him to develop and appreciate **statistical skills** in order to be able to use this new-found freedom effectively.

Definitions:



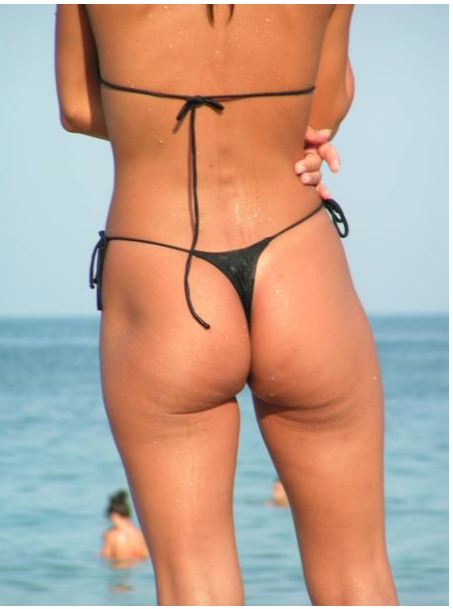
From Wikipedia, the free encyclopedia

Statistics is a mathematical science pertaining to the collection, analysis, interpretation, and presentation of data.

Statistic may also refer to:

Statistic, the result of applying a statistical algorithm to a set of data

Statistics (role-playng games), a piece of data which represents a particular aspect of a fictional character

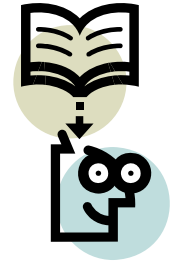


Statistics is like bikini – shows very much and interesting things, but the most important remains covered

Probability is the extent to which something is likely to happen or be the case

May we predict the result of analytical measurement ?

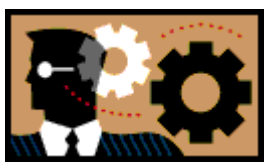
How to express quantitatively the probability ?



In mathematics, probabilities always lie between ZERO and ONE.

An impossible event has a probability of 0
transferred in percent **(0%),**
and

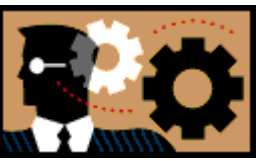
a certain event has a probability of 1
transferred in percent **(100%)..**



Give me example for 200 % probability.

Does a negative probability exists ?

Predict the outcome sum of points from two dice



Stake on your favored result

Calculate all outcomes

Probability is related to the relative frequencies of one event when these frequencies become stable with infinite number of trails

$$\frac{M_A}{N} \cong p(A) = \frac{k_A}{k_\Omega}$$

Relative frequencies p

Number of outcomes kAi

k_A

Number of appearance

6 (7)

5 (6;8)

4 (5;9)

3 (4;10)

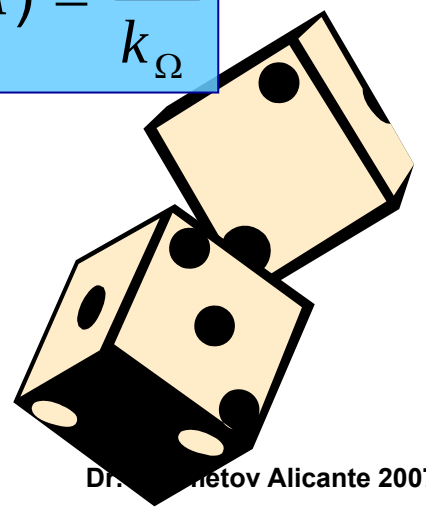
2 (3;11)

1 (1;12)

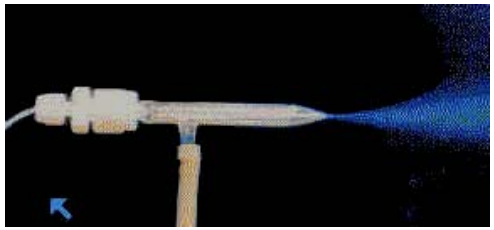
2	3	4	5	6	7	8	9	10	11	12
1+1	1+2; 2+1	1+3; 3+1; 2+2	1+4; 4+1; 2+3; 3+2	1+5; 5+1; 2+4; 4+2; 3+3	1+6; 6+1; 2+5; 5+2; 3+4; 4+3	2+6; 6+2; 3+5; 5+3; 4+4	3+6; 6+3; 4+5; 5+4	4+6; 6+4; 5+5	5+6; 6+5	6+6
P(2)=1/36	P(3)=2/36	P(4)=3/36	P(5)=4/36	P(6)=5/36	P(7)=6/36	P(8)=5/36	P(9)=4/36	P(10)=3/36	P(11)=2/36	P(12)=1/36

Relative frequencies p

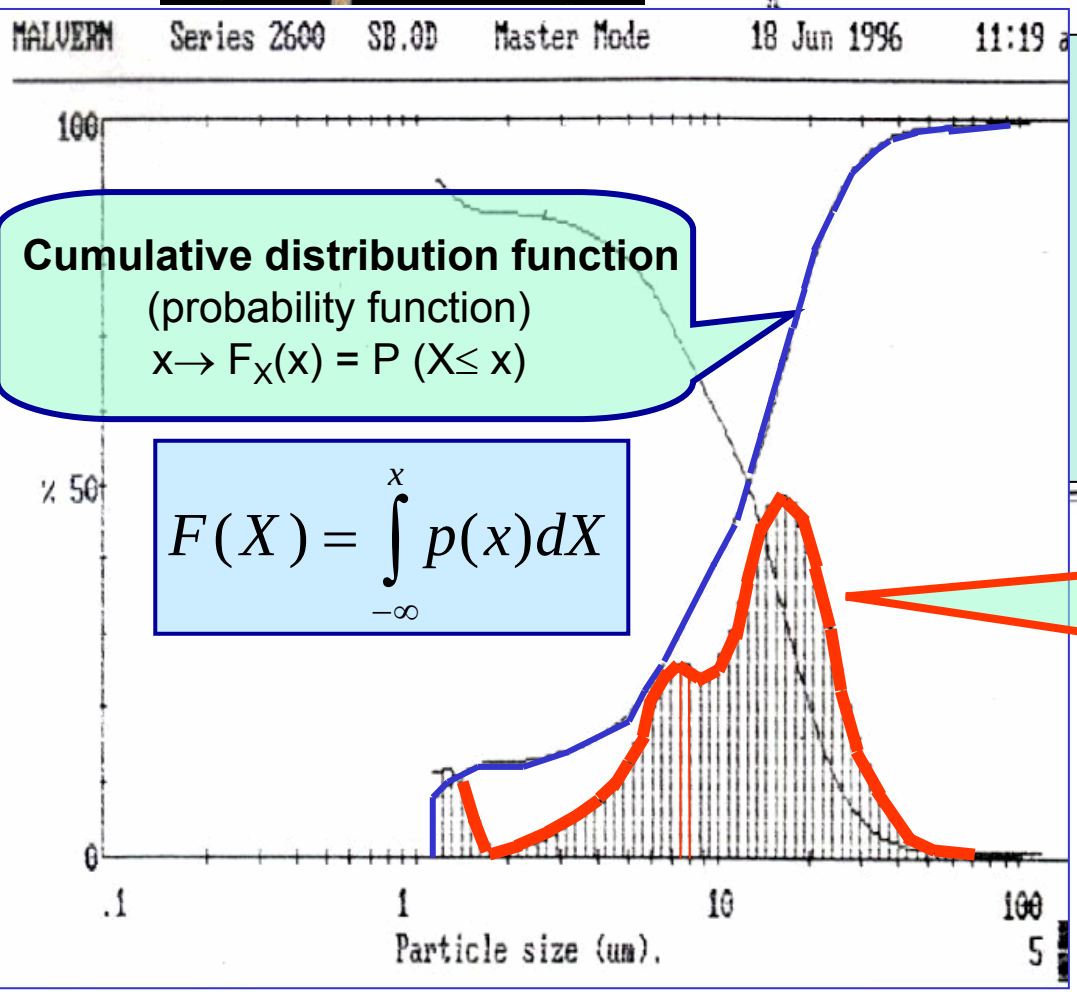
Total number of possible outcomes kΩ=36



Aerosol dimensions



Upper	p(X) in %	Lower	Under	Upper	p(X) in %	Lower	Under	Upper	p(X) in %	Lower	Under
				36.3	2.4	31.4	92.7	6.18	3.4	5.33	20.1
				31.4	5.3	27.1	87.3	5.33	2.3	4.60	17.8
				27.1	7.0	23.3	80.4	4.60	2.0	3.97	15.8
				23.3	6.9	20.2	73.5	3.97	1.8	3.42	14.0
100				20.2	7.5	17.3	66.0	3.42	1.4	2.95	12.6
100				17.3	7.4	15.0	58.6	2.95	0.8	2.55	11.8
100				15.0	7.1	12.9	51.5	2.55	0.5	2.19	11.3
100				12.9	7	11.1	45.8	2.19	0.2	1.90	11.0
99.7				11.1	2	9.63	40.6	1.90	0.3	1.64	10.8
98.5				9.63	5.6	8.31	35.1	1.64	1.3	1.41	9.5
96.9				8.31	6.2	7.16	28.8	1.41	2.0	1.22	7.5
95.1				7.16	5.3	6.18	23.5	1.22	7.5	0.32	0.0



Cumulative distribution function
(probability function)
$x \rightarrow F_X(x) = P(X \leq x)$

$$F(X) = \int_{-\infty}^{x} p(x)dX$$

Probability density function p(X)
The curve of the relative frequency
number of counts normalized by
the total number of events

$$P(-\infty;+\infty) = \int_{-\infty}^{+\infty} C.p(x)dX = 1$$

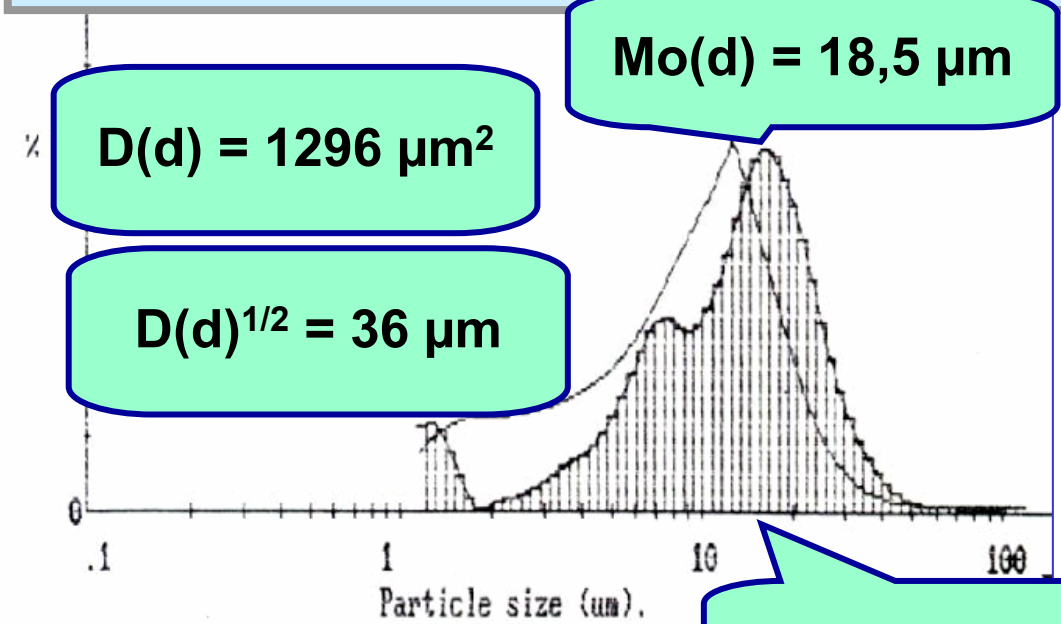
Definitions for mathematical expectation and the dispersion:

Variable	Mathematical expectation	Dispersion
Discrete	$M(X) = \sum_{k=1}^{\infty} p(X_k).X_k$	$D(X) = M\left[X - M(X)\right]^2$
Non discrete	$M(X) = \int_{-\infty}^{+\infty} X.p(X)dX$	$D(X) = \int_{-\infty}^{+\infty} p(X).\left[X - M(X)\right]^2 dX$

Mo(d) = 18,5 µm

D(d) = 1296 µm²

D(d)$^{1/2}$ = 36 µm



M(d) = 12,5 µm

The dispersion (variance) of a variable is defined as the mathematical expectation of the squared difference of the variable's value and its mathematical expectation

$$d_{3,2} = \frac{\int_0^{\infty} d_i^{\,3}\, p(d_i)\,dd}{\int_0^{\infty} d_i^{\,2}\, p(d_i)\,dd}$$

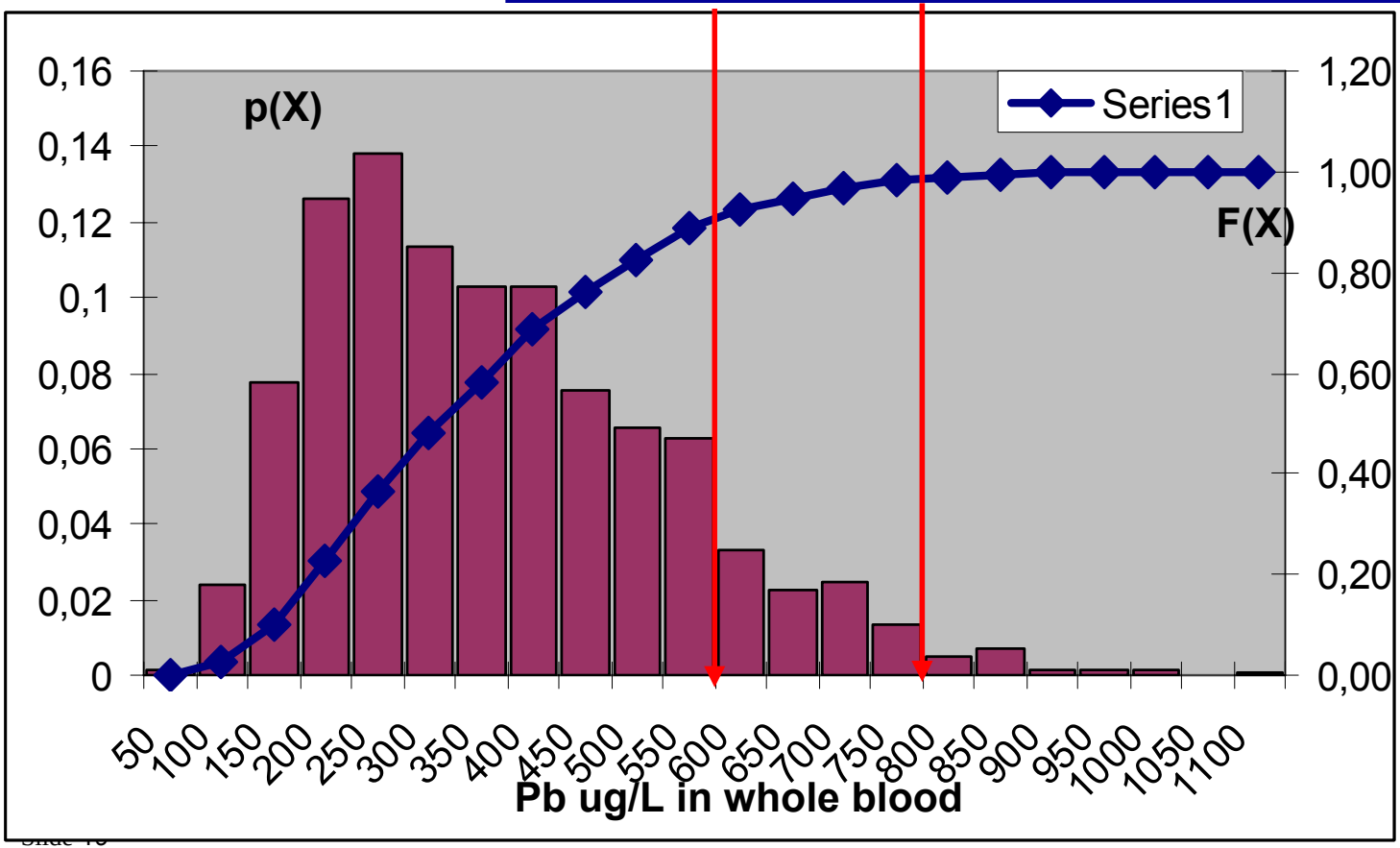
Variable characteristics

EDTA treatment should be implemented to workers with **Pb** content in blood between 600 - 800 µg/L.

You have tested the lead content in blood in 1763 workers

Define the probability one worker in the factory like this, to need a treatment with EDTA

$$P(600;800) = \int_{600}^{800} p(X)dX = F(800) - F(600) = 0{,}099$$



M(X) =

D(X) =

Mo(X) =

Md(X) =

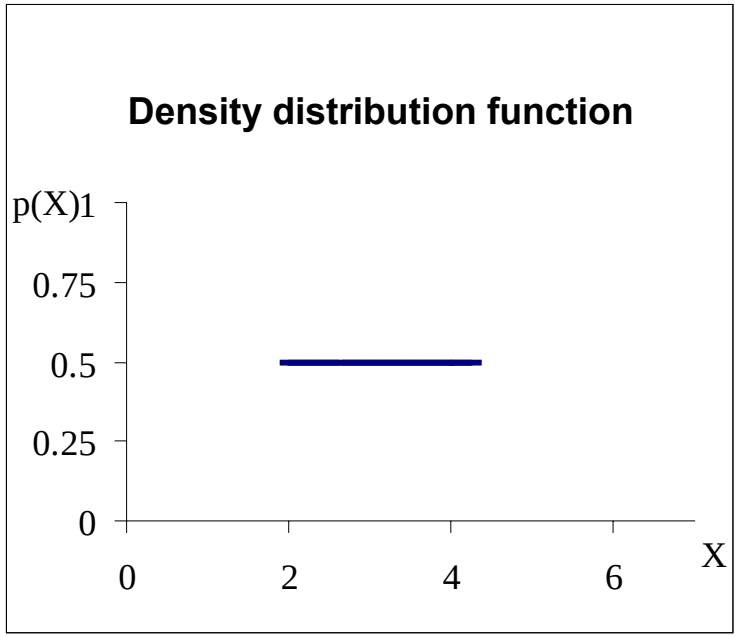
Rectangular distribution

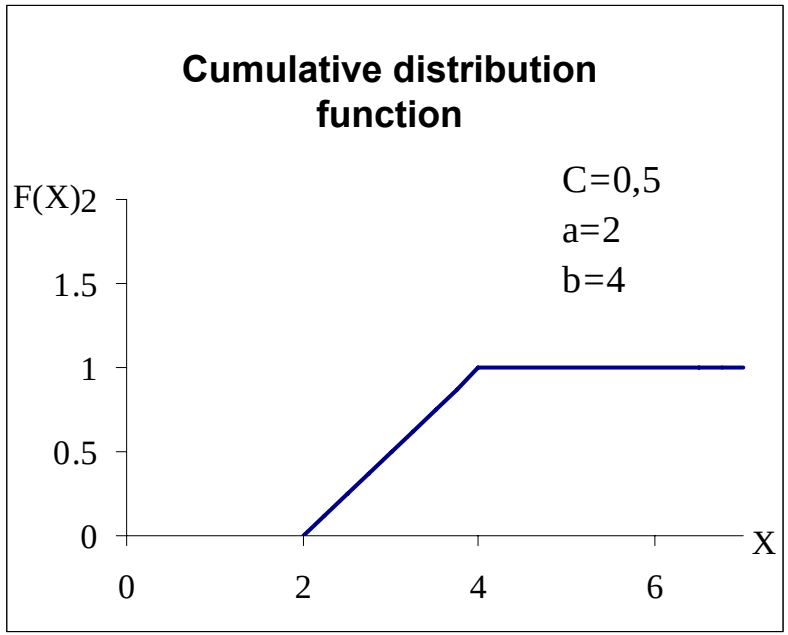
$$p(X) = \frac{1}{b-a}$$

$$M(X) = \frac{b-a}{2}$$

$$D(X) = \frac{(b-a)^2}{12}$$

$$u_s = S = \frac{b-a}{2\sqrt{3}}$$





Particulars:

- All possible outcomes are with the same relative frequency, and density $k\varpi_i$=const, $p(\varpi_i)$=const
- The population Ω is defined between *a* and *b*

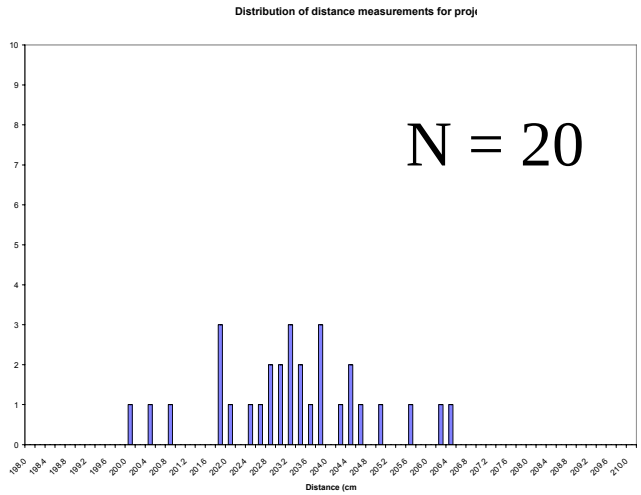
Density

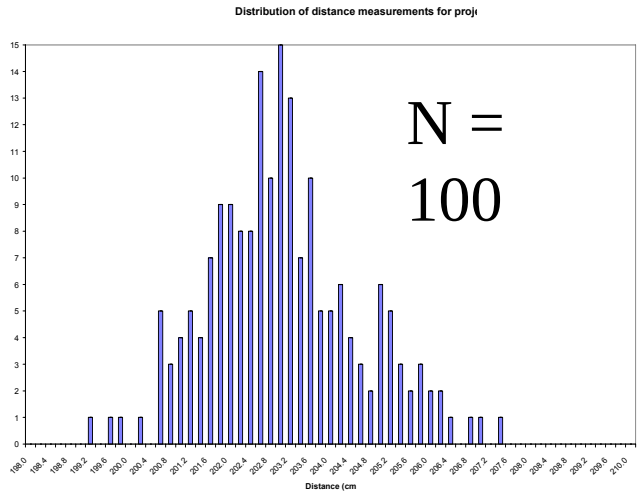
$$p(X) = \begin{cases} C : a \le X \le b \\ \mathbf{0} : X < a, X > b \end{cases}$$

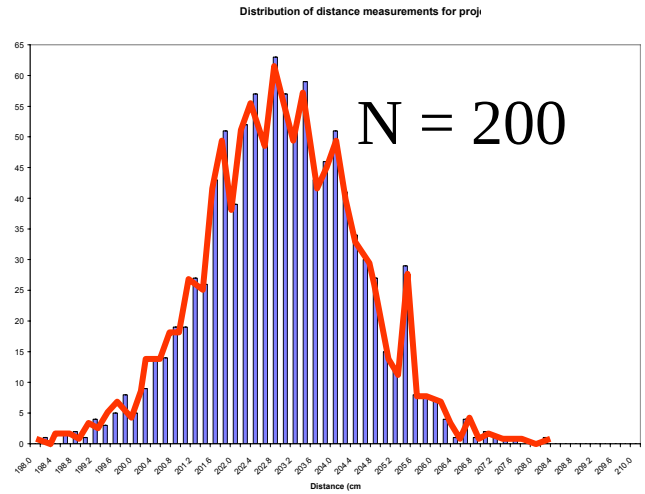
Distribution function

$$\int_{-\infty}^{+\infty} p(X)dx = \int_{-\infty}^{a} \mathbf{0}dx + \int_{a}^{b} Cdx + \int_{b}^{+\infty} \mathbf{0}dx = \int_{a}^{b} Cdx$$

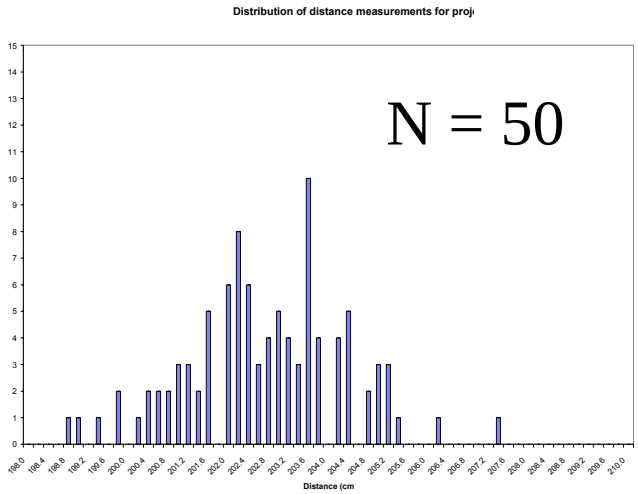
RESULTS DESTRIBUTION

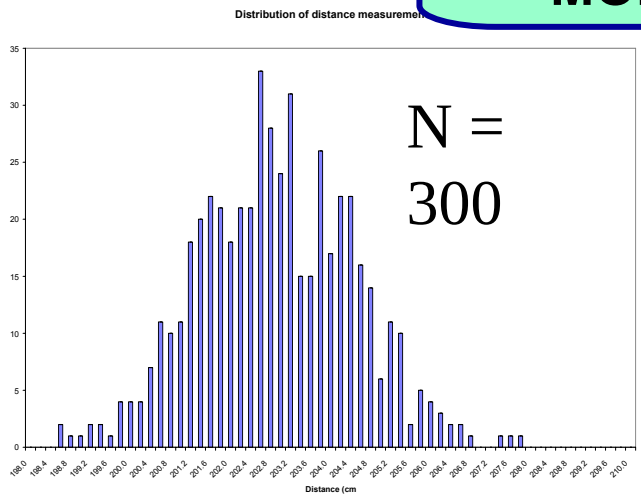


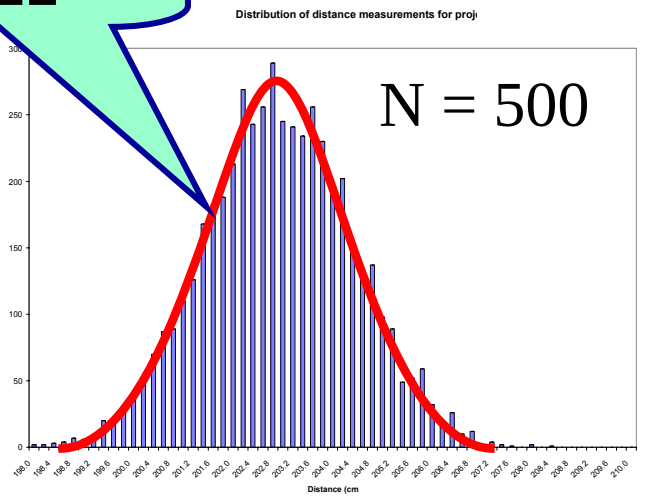




UNIVERSAL MODEL



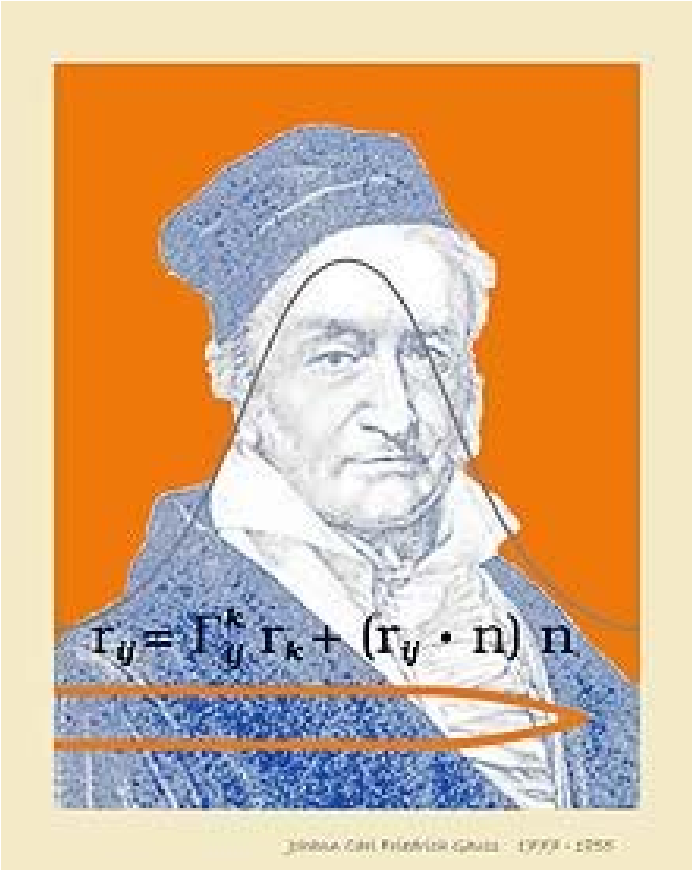




The Central Limit Theorem

The Central Limit Theorem states that if the sum of the variables has a finite variance, then it will be approximately normally distributed

(i.e. following a normal or Gaussian distribution)



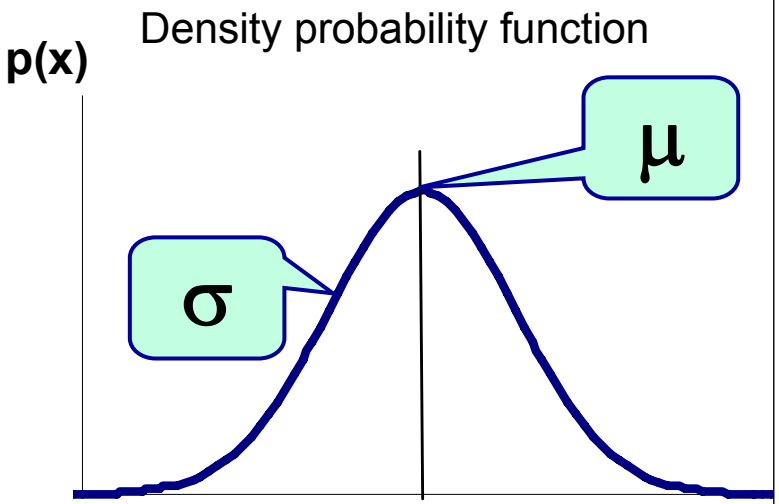
This is a theoretical model to which we refer the most of the measurement results in spectrochemical analysis

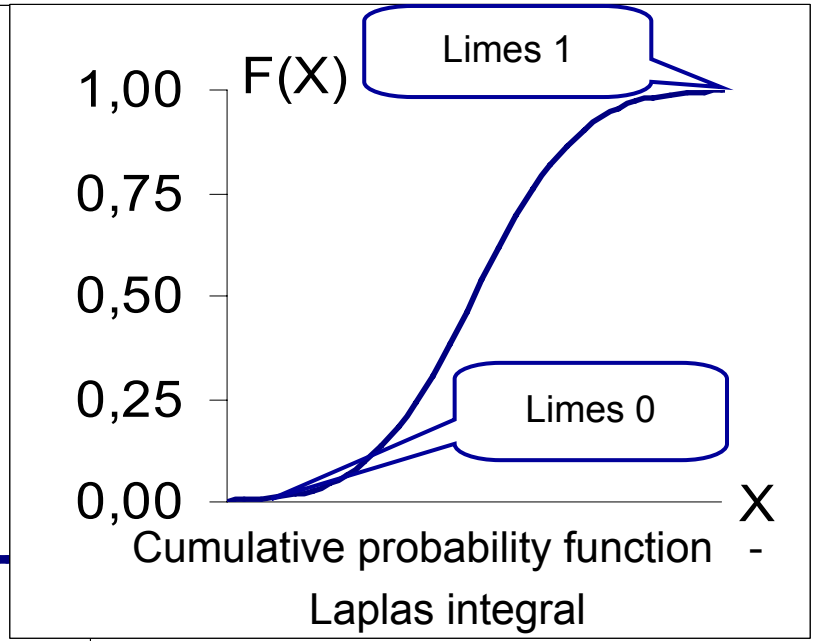
$$F(X) = \int_{-\infty}^{x} \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(X-\mu)^2}{2\sigma^2}} dx$$

Normal (Gauss) distribution N ($\mu;\sigma^2$)

$$M(X) = \mu$$

$$D(X) = \sigma^2$$





$$p(X) = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(X-\mu)^2}{2\sigma^2}}$$

$$F(X) = \int_{-\infty}^{x} \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(X-\mu)^2}{2\sigma^2}} dx$$

μ and σ are parameters and are enough to define the bell of any normal distributed population

The interval is open form - ∝ to + ∝

Important relations !

P – statistical probability **confidence level**

α – level of Significance

$$P = 1 - \alpha$$

α – level of Significance

a – quantil

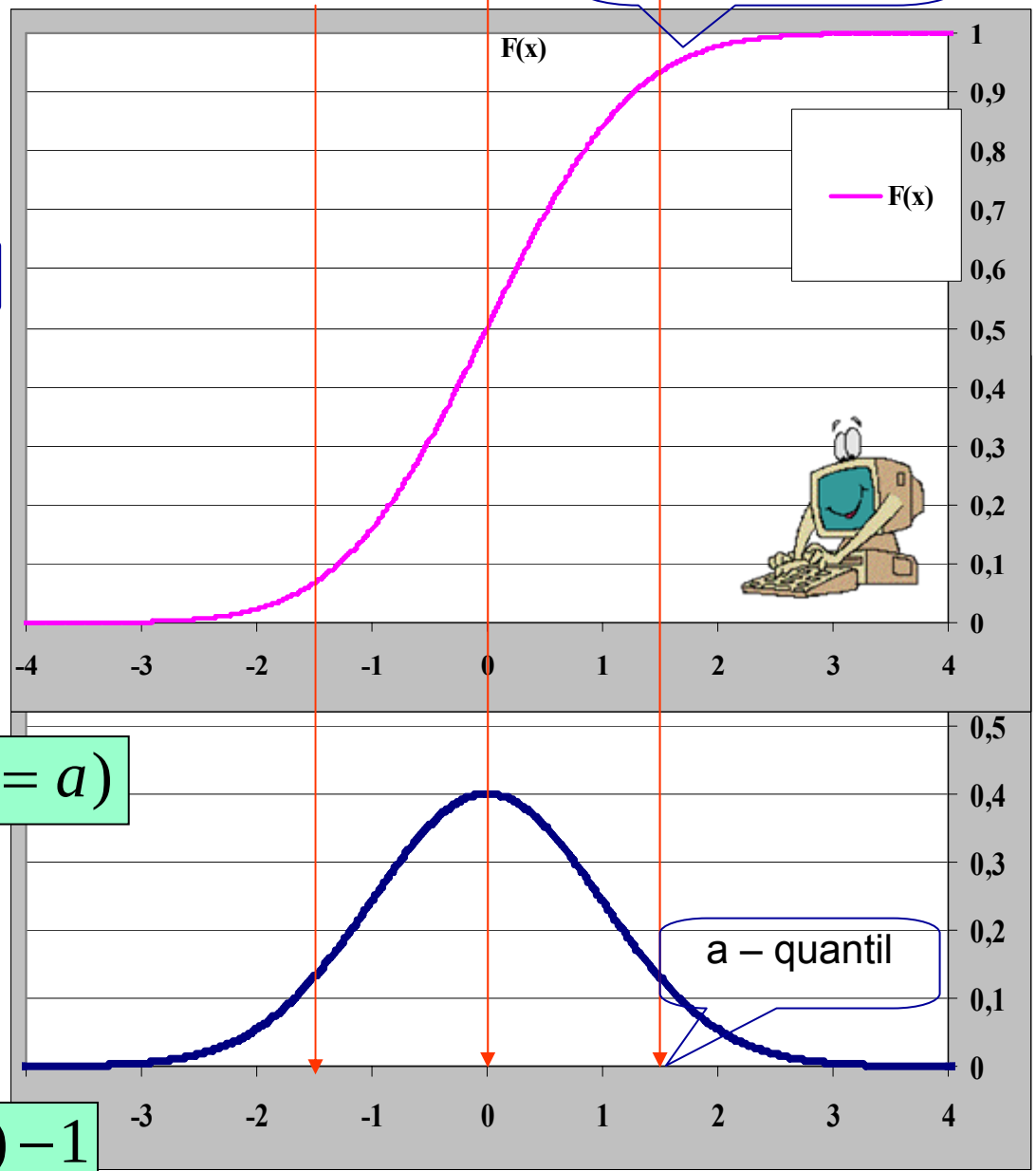
$$P(X \le a) = F(X = a)$$

$$P(X > a) = 1 - F(X = a)$$

$$P(a < X < b) = F(X = b) - F(X = a)$$

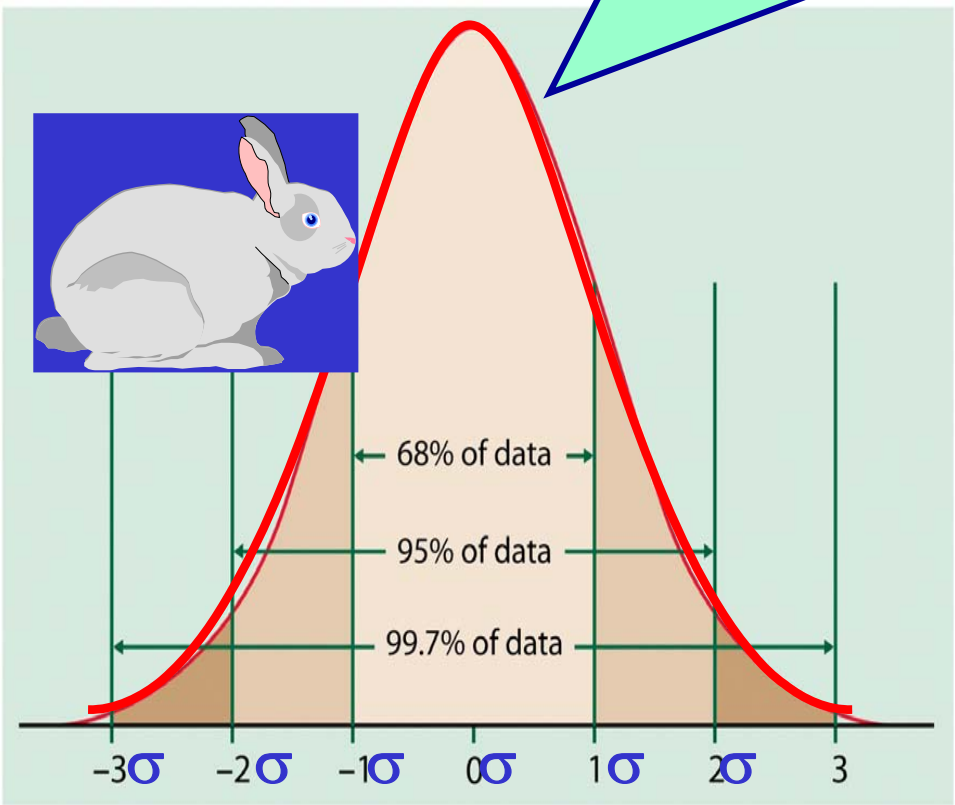
$$P(-\infty; -a) = 1 - F(X = a)$$

$$P(\mu - a < X < \mu + a) = 2F(X = a) - 1$$

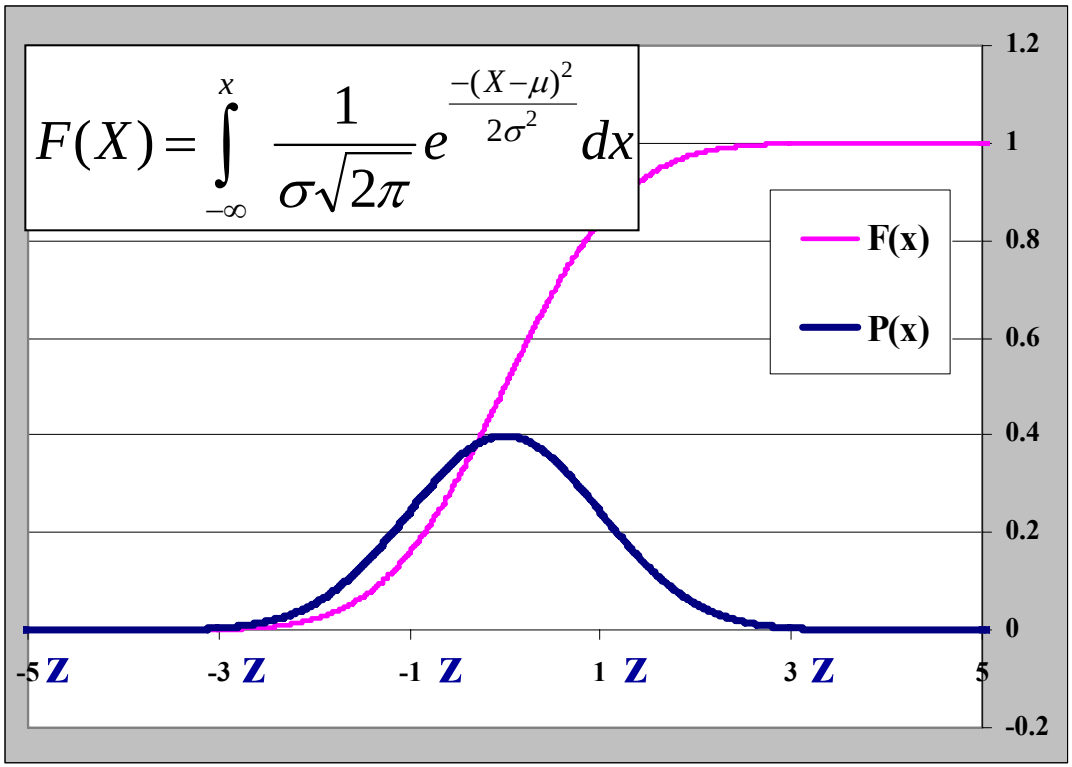


Z – transformation to standard distribution N(μ=0; σ_2= 1)

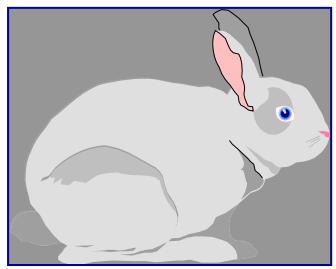
Every Normal distributed variable appears as a bell (surface=1) with equivalent profile in respect to μ and σ

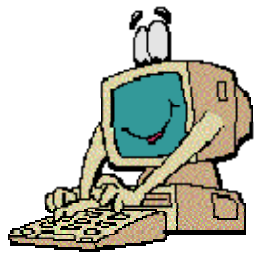


$$F(X) = \int_{-\infty}^{x} \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(X-\mu)^2}{2\sigma^2}} dx$$



$$Z = \frac{X - \mu}{\sigma}$$





STATISTICS ESTIMATIONS (unbiesed and consistent)

Parameters of the general population	Parameters of the normal distribution	Statistical estimations of a sample
M(X) Mathematic estimation	μ	$\overline{X}$
D(X) Dispersion	σ^2	S^2

Arithmetic mean

$$\overline{X} = \frac{1}{n}\sum_{i=1}^{n}(x_i)$$

$$S = \sqrt{\frac{\sum_i \left(X - \overline{X}\right)^2}{(N-1)}}$$

Sample Standard Deviation

With P = (1-α) the true value lies in the interval :

$$\overline{X} - \frac{t(f,\alpha).S}{\sqrt{N}} < \mu < \overline{X} + \frac{t(f,\alpha).S}{\sqrt{N}}$$

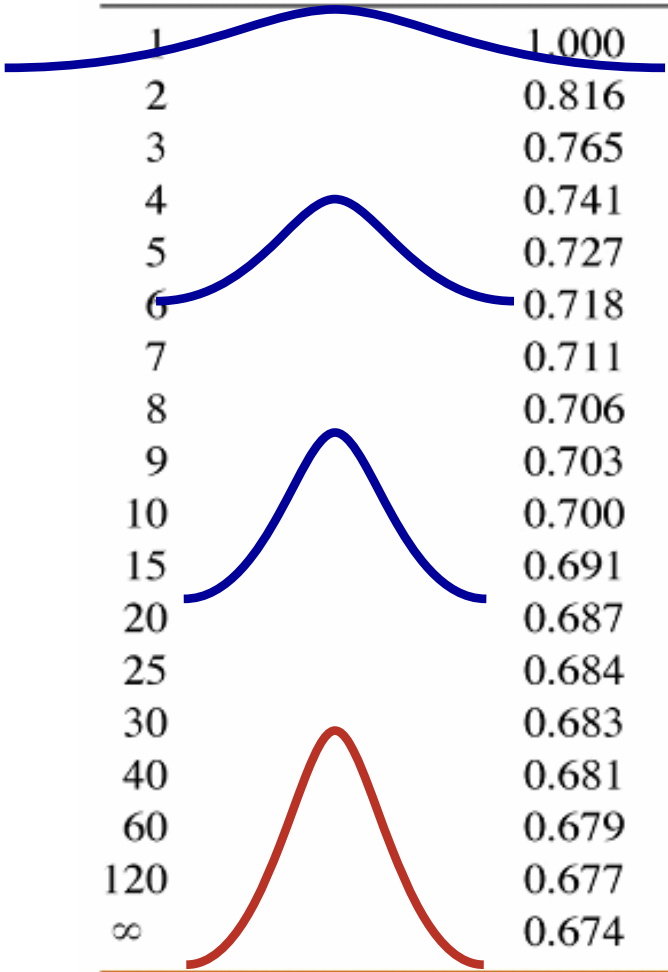
t-distribution

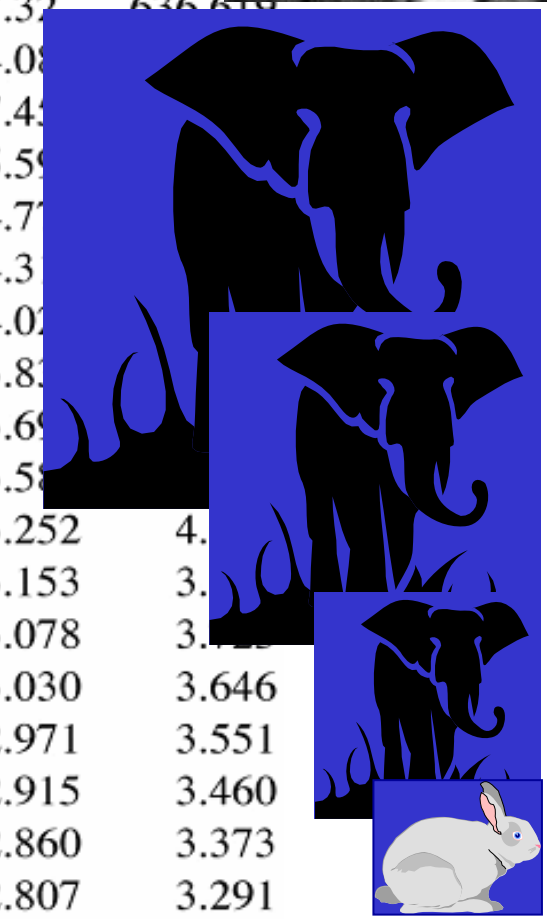
William Sealey Gosset

1908

Table 4-2 Values of Student's t

Degrees of freedom	Confidence level (%) 50	90	95	98	99	99.5	99.9
1	1.000	6.314	12.706	31.821	63.657	127.32	636.6
2	0.816	2.920	4.303	6.965	9.925	14.0	
3	0.765	2.353	3.182	4.541	5.841	7.4	
4	0.741	2.132	2.776	3.747	4.604	5.5	
5	0.727	2.015	2.571	3.365	4.032	4.7	
6	0.718	1.943	2.447	3.143	3.707	4.3	
7	0.711	1.895	2.365	2.998	3.500	4.0	
8	0.706	1.860	2.306	2.896	3.355	3.8	
9	0.703	1.833	2.262	2.821	3.250	3.6	
10	0.700	1.812	2.228	2.764	3.169	3.5	
15	0.691	1.753	2.131	2.602	2.947	3.252	4.
20	0.687	1.725	2.086	2.528	2.845	3.153	3.
25	0.684	1.708	2.060	2.485	2.787	3.078	3.
30	0.683	1.697	2.042	2.457	2.750	3.030	3.646
40	0.681	1.684	2.021	2.423	2.704	2.971	3.551
60	0.679	1.671	2.000	2.390	2.660	2.915	3.460
120	0.677	1.658	1.980	2.358	2.617	2.860	3.373
∞	0.674	1.645	1.960	2.326	2.576	2.807	3.291





Standard deviation of n independent measurements

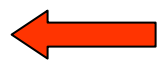
Several (n) independent measurements
with several instrumental replicates

$$R_i = \bar{x}_i \pm s_i$$

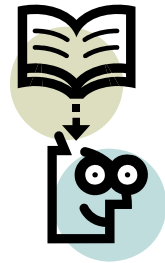
assuming that ALL s_i are similar *(= s)*

$$R_i = \bar{x}_i \pm s$$

$$R = (\bar{R}_i) \pm s_{mean} = (\bar{R}_i) \pm \frac{s}{\sqrt{n}}$$



Confidential interval (CI)



Low and high limits between which it is accepted one can find the true value of the measurand with appointed confidence level P=1-α, taking into account the degree of freedom and both statistics $\mathbf{X_{aver}}$ and **S** obtained from a sample with volume N = f+1

Low_limit < true value < High_limit

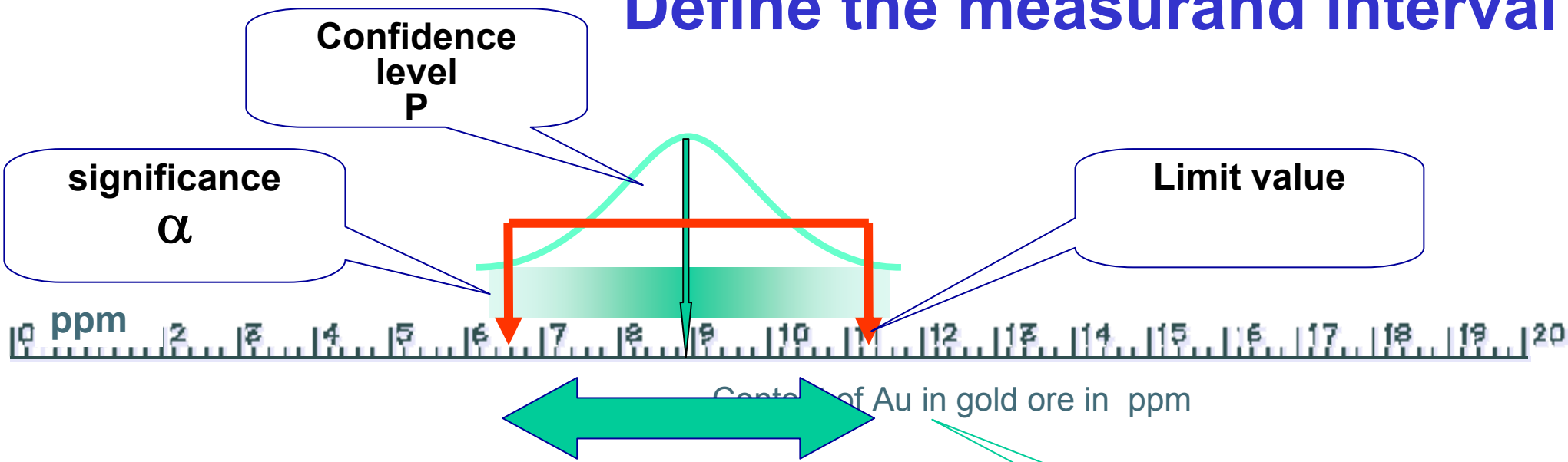
6,5 ppm < the best estimate < 11,5 ppm
for S = 2 ppm and convenience level P = 0,95 ; N = 5

For symmetrical interval around the average value, the **CI** could be given as $\pm$ of the half of the total

the true value is $\in$ 9 $\pm$ 2,5 ppm
for S = 2 ppm; N=5 and significance level α=0,05 (P=0,95)

$$\mu \in \overline{X} \pm \frac{t(f,\alpha).S}{\sqrt{N}}$$

Define the measurand interval



$(\mu - 3\sigma < x < \mu + 3\sigma)$

?

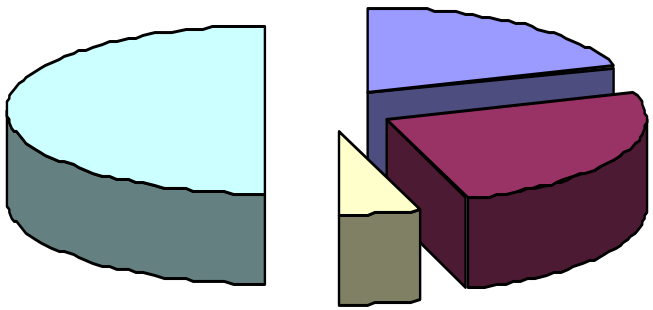
$$\overline{X} - \frac{t(f,\alpha).S}{\sqrt{N}} < \mu < \overline{X} + \frac{t(f,\alpha).S}{\sqrt{N}}$$

$(\ddot{X} - U < x < \ddot{X} + U)$; k=2

THE NEW METROLOGY CONCEPT

uncertainty: estimates all influencing parameters including the internal and external once

provable uncertainty statement



Identify and quantify

claim

Pb in blood concentration
490 µg/L ± 24 µg/L

With **P=(1-α) = 0.95** the true value is somewhere in the interval:

$$\mu \in \bar{X} \pm \frac{t(f,\alpha).S}{\sqrt{N}}$$

demonstrate

Uncertainty of Measurement Results

Validation *(method fit-for-purpose)*

Traceability

(my result is comparable - common reference)

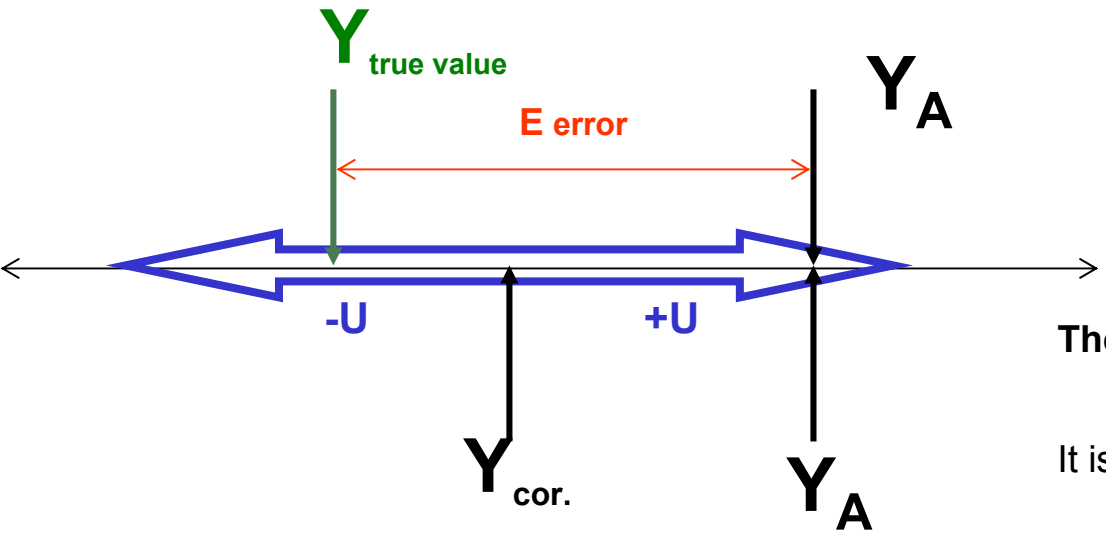
Uncertainty Budget

(How well I know the result)

Overview

- Definitions
- Uncertainty - what for?
- GUM procedure for uncertainty evaluation
- 10 Steps of uncertainty budgeting
- Kragten spread-sheet approach
- Examples

The GUM approach - difference between error and uncertainty



Before the GUM reliability of the measurements - by inconsistent or not generally accepted methods most based on an error-centered view.

The error - the difference between the unknown true value and the the actual measurement result.

It is a single value, so the known error can be applied as a correction to the result

- GUM is guide for a transparent, simple and standardised documentation of the measurement procedure
- Use uncertainty evaluations, such as *type A (measured in the lab)* and *type B (other)*

Do NOT use random & systematic errors !

In the GUM, a value of the measurand only exists as a result of an estimation process, usually called experiment, measurement or chemical analysis

☒The uncertainty cannot be used to correct a measurement result !

TOTAL UNCERTAINTY

Every PROCESS predetermines a PRODUCT

Every PRODUCT possess characteristics – qualities

Every quantitatively defined characteristics can be measured

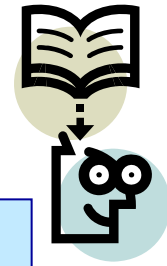
the total uncertainty:

Dispersion of the product characteristics
+

Dispersion of the measurement procedure

= total dispersion -> UNCERTAINTY

ISO Definition of Uncertainty



‘a parameter associated with the result of a measurement, that characterises **the dispersion** of the values that could reasonably be attributed to the *measurand* ’

Result = **Value ± Uncertainty Units**

(22.7 ± 4.8) mg/kg

The value is between 17.9 and 27.5 mg/kg
(cf. *range, interval*)

UNCERTAINTY OF MEASUREMENT COMPRISES MANY COMPONENTS

GUM - structured uniform methodology using transparency and reasonable estimations by including the knowledge from the test and all available pre-knowledge

The Statistical approaches are useful but are NOT ENOUGH !!

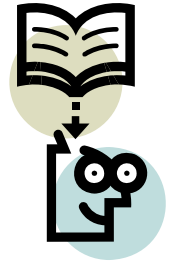
GUM (1995) amended (1995): ISO Guide on the expression of Uncertainty in Measurements

EURACHEM/CITAC

http://www.eurachem.ul.pt/guides/QUAM2000-1.pdf

Guide Quantifying Uncertainty in Analytical Measurement, 2000

TYPES of UNCERTAINTY



STANDARD uncertainty

u_s (A or B)

Uncertainty of the results of the measurement expressed as a **standard deviation**. Could be type **A** or type **B**

COMBINED standard uncertainty

u_c

Standard uncertainty of a result obtained from values of known number of other quantities.

Using the low of uncertainty propagation!

EXPANDED uncertainty

U (K=2)

Defining an interval that may be expected to encompass a large fraction of the distribution of values that could be reasonably be attributed to the measurand.

It could be calculated by multiplying the Combined uncertainty with a coverage factor K (most often K=2)

Why do we need uncertainty?

- It is required by ISO 17025 - Accreditation
- The U demonstrates the metrological QUALITY of the measurements
  (not measuring with the smallest achievable uncertainty)
- It improves the knowledge about the measurement procedure
- In laboratory → document in transparent way the measurement procedure
- For end-user → give the result with proper confidence
- It allows comparison of results
- A well documented U statement underpins your results and provides transparency!
- Identify major uncertainty contributors - find out ways to improve the procedure
- Demonstrate compliance with limits (legal or contractual) and the establishment of acceptance criteria

⇨ Your best defence in discussions!

When should you evaluate uncertainties of measurement results ?

- When a procedure is introduced inside your laboratory
- When a critical factor changes in the procedure (instrument, operator, …)
- During / together with procedure validation

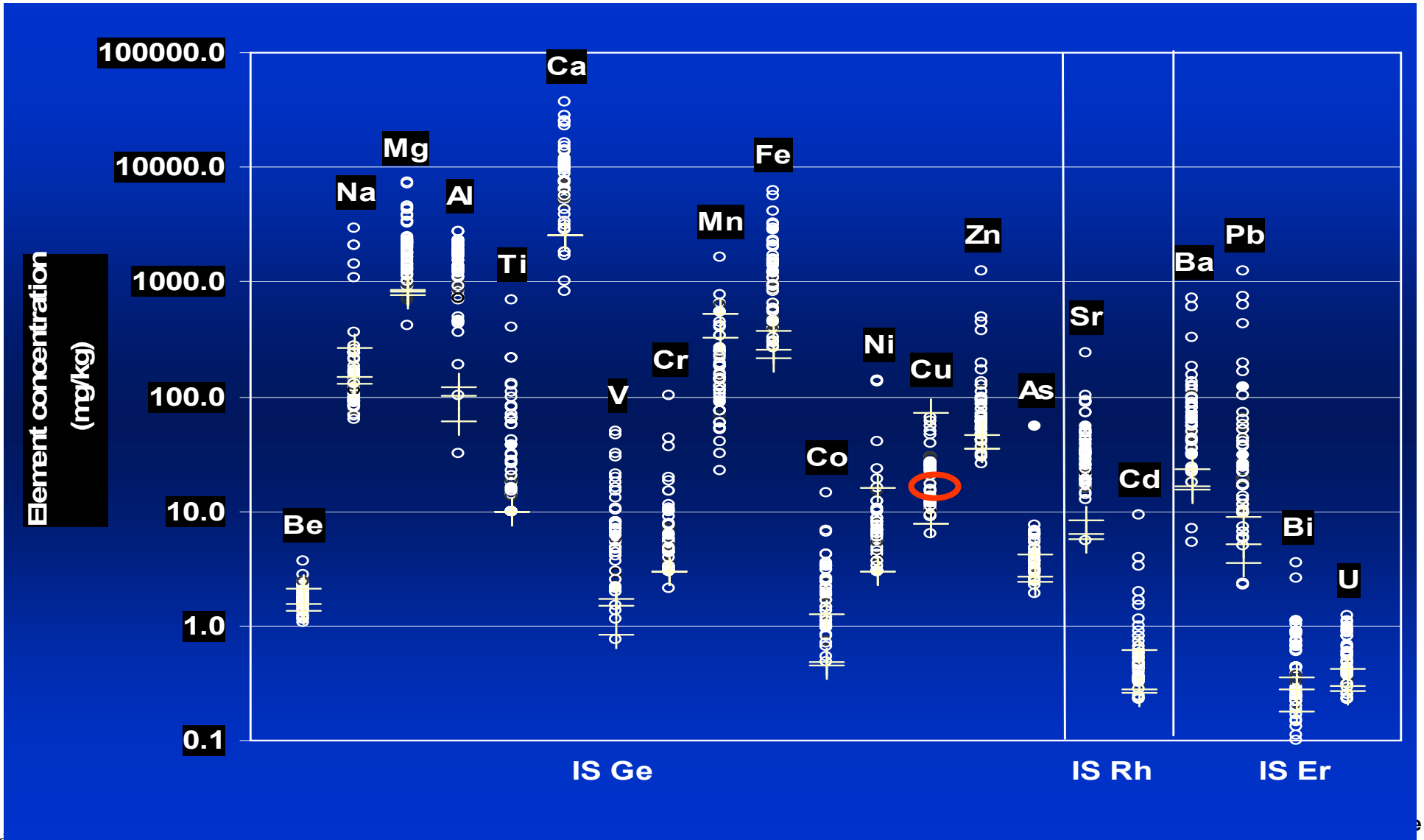
➔ An individual evaluation process is NOT needed for every individual result produced !

☒ Repeating the measurement 2, 10 or 100 times does not give you all information to have reliable results!

V. Kmetov, V. Stefanova, D. Hristozov, D. Georgieva, A. Canals+

Talanta Volume 59, 1 (2003) 123-136

Determination of calcium, iron and manganese in moss by automated discrete sampling FAAS as an alternative to the ICP-MS analysis 62 moss samples 21 elements



Are results different?

No results without uncertainty !

R1 = 20.6 mg/kg
R2 = 21.6 mg/kg

- Traditional approach: precision

R1 = (20.6 ± 0.6) mg/kg
R2 = (21.6 ± 0.7) mg/kg

- GUM approach: uncertainty propagation (combined unc.)

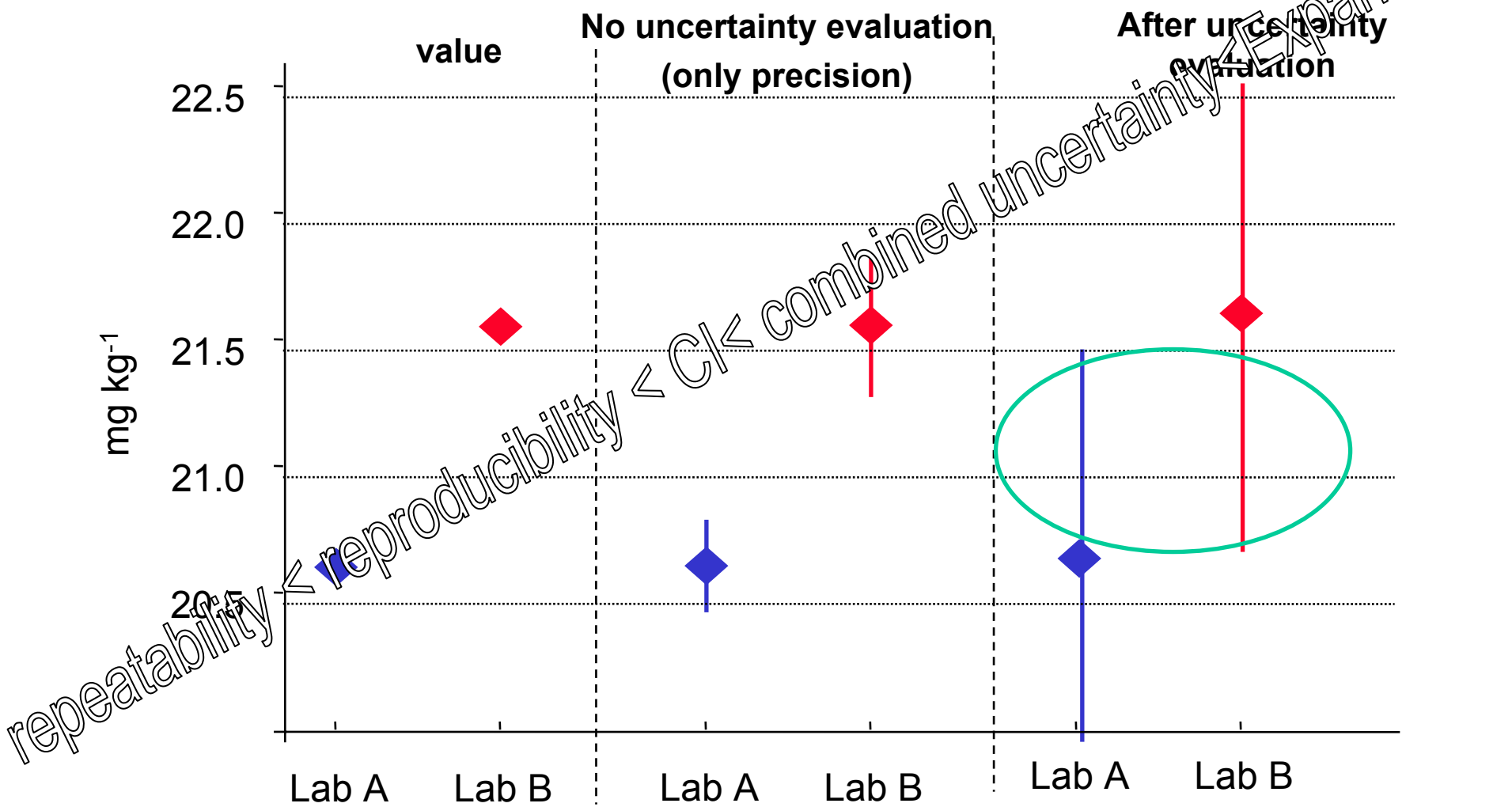
to take into account the contribution of all components

R1 = (20.6 **± 2.1**) mg/kg
R2 = (21.6 **± 2.3**) mg/kg

No statistical tests required by GUM *(almost)* …/…

cf. Visual comparison ➔ overlapping ranges Y/N ?

Are these results different?



Understanding the measurement !

"…The evaluation of uncertainty is neither a routine task nor a purely mathematical one; it depends on detailed knowledge of the nature of the measurand and of measurement…"

[GUM § 3.4.8]

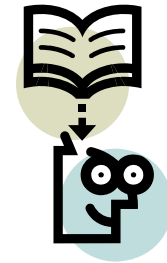
GUM does not require
statistical tests unless you need it …

What do you need to know ?

some basic statistics

- average of the set of data;
- standard deviation;
- law of propagation;
- distribution (normal, rectangular, triangular…)

Uncertainty "Type"



Type A standard uncertainty is measured from repeatability experiments and is quantified in terms of *the standard deviation* of the measured values

Type B evaluation of uncertainty:

by *other means* than statistical analysis

"…The pool of information may include:

previous measurement data; *[GUM, 1993]*

validation data

experience with or general knowledge of the behaviour and properties of relevant materials and instruments;

manufacturer's specifications

data provided in calibration and other certificates;

uncertainty assigned to reference data taken from handbooks"

[GUM § 4.3.1]

The 10-steps GUM Sequence

1 - Define the Measurand

2 - Describe the Model Equation *(for the measurement procedure)*

3 - Identify (all possible) sources of uncertainty

4 - Evaluate all input quantities

5 - Evaluate the standard uncertainty (1s) of each input quantities

6 - Calculate the value of the measurand (using the equation model)

7 - Calculate the combined standard uncertainty of the result

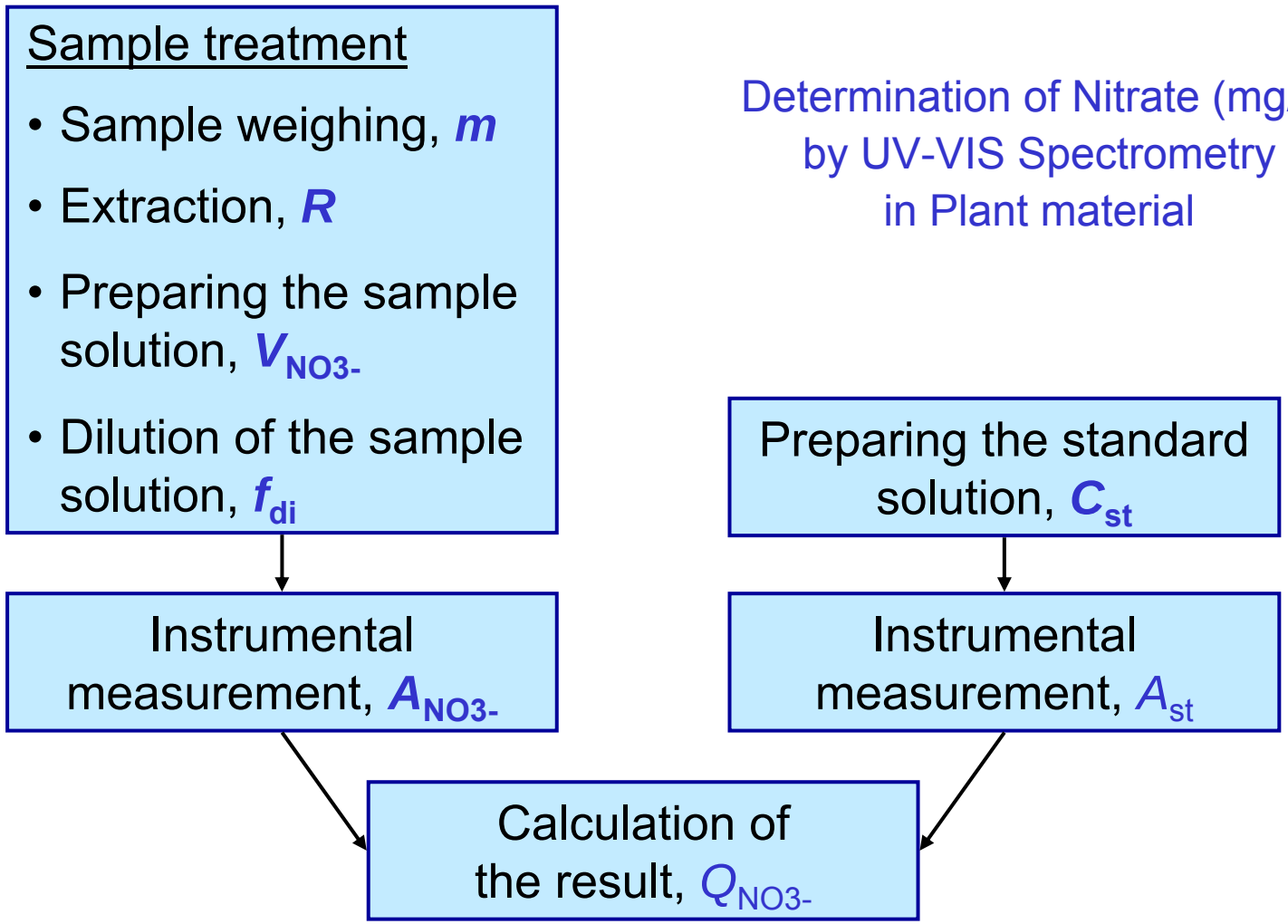
8 - Calculate the expanded uncertainty (with a selected k)

9 - Analyse the uncertainty contribution index *(THINK !!)*

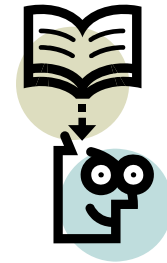
10- Document all steps in a Report.

Experimental Protocol

Determination of Nitrate (mg/g)
by UV-VIS Spectrometry
in Plant material



Step 1 - Definition of "Measurand"



Analyte: Article that is the subject of a measurement *(GLP)*

e.g. cholesterol;
Au ;
Pb

Measurand: Particular quantity subject to measurement (VIM,2.6)

e.g. concentration of cholesterol in serum;
Au in gold alloy; Pb in whole blood

In this example: **content of NO_3^- in (mg/g) in fresh plant material (lettuces)**

Step 2 - Model Equation

The model of the measurement procedure is a functional relation between input quantities and output quantity (result)

You have it already

$$Y = f(X_1, X_2, ..., X_n)$$

Measurement MODEL is the equation you use for the calculation of your result !

$$C_{work\_sol} = C_{stock} \cdot \frac{V_{i\_stock}}{V_{i\_1000}}$$

$$C_{NaOH} = \frac{1000 \cdot m_{KHP} \cdot P_{KHP}}{M_{KHP} \cdot V_T}$$

$$Q_{NO_3^-} = C_{st} \frac{A_{NO_3^-} \cdot V_{NO_3^-}}{A_{st} \cdot m} \times f_{di} \times \frac{1}{R}$$

What are input quantities?

The output quantity Y depends on input quantities $X_1, X_2, \ldots, X_n$:

$$Y = f(X_1, X_2, ..., X_n) \qquad [GUM\ 4.1.2]$$

Input quantities (X_i) may be quantities whose value and uncertainty are directly determined in the current measurement (Type A, *statistical analysis of series of observation*) or brought into the measurement from external sources (Type B, *previous experiments, literature data, information from manufacturer*)

Model Equation

$$Q_{NO_3^-} = C_{st} \frac{A_{NO_3^-} \cdot V_{NO_3^-}}{A_{st} \cdot m} \times f_{di} \times \frac{1}{R}$$

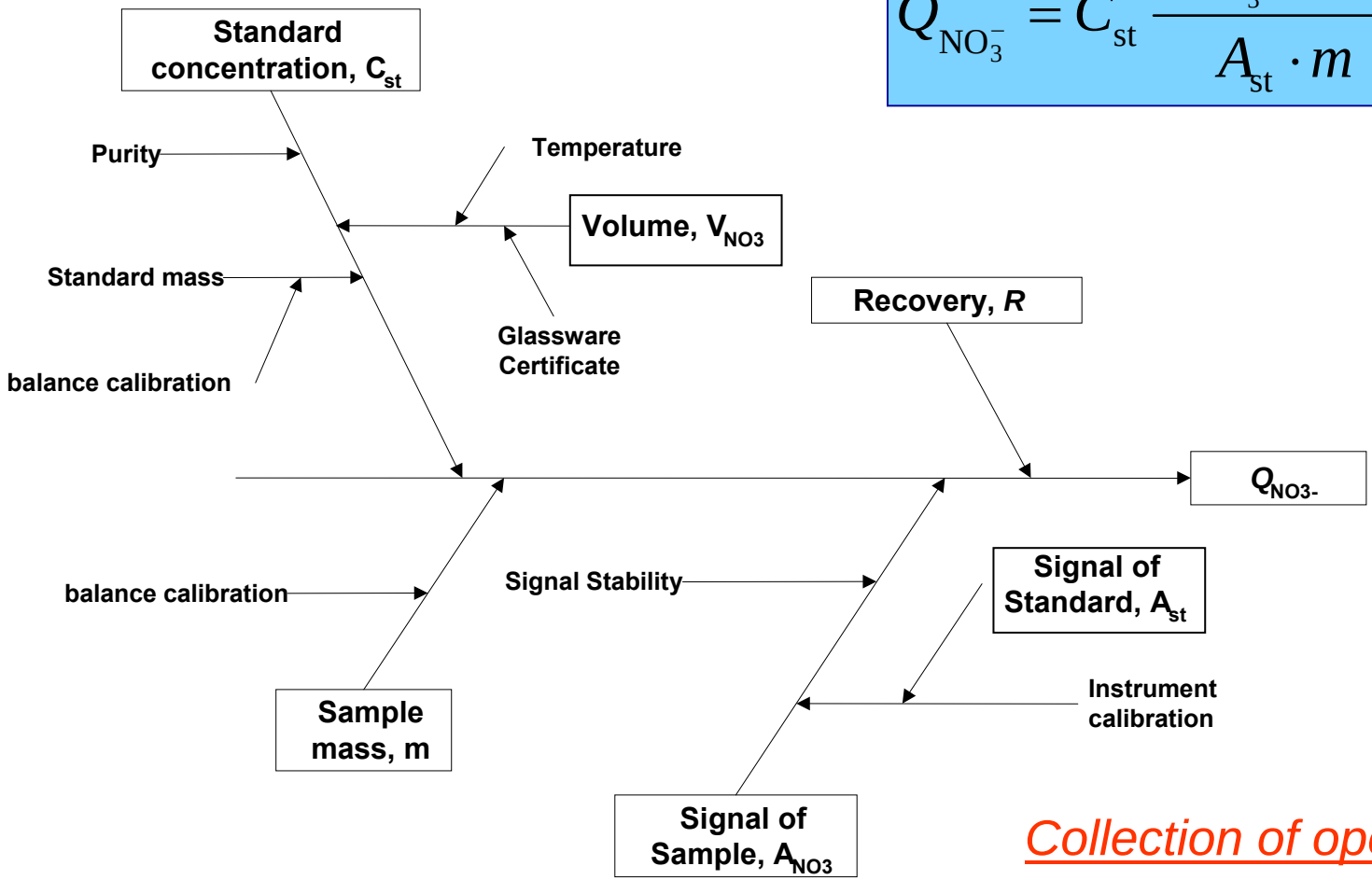
Q_{NO3-}	*nitrate content of the sample (mg/g)*
C_{st}	*nitrate concentration in standard solution (mg/l)*
A_{NO3-}	*intensity of the signal (AP) for sample solution*
A_{st}	*intensity of the signal (AP) for standard solution*
V_{NO3-}	*volume of sample solution (l)*
m	*mass of the sample (g)*
f_{di}	*dilution factor (no units);*
R	*recovery factor (cf. sample preparation)*

Step 3 - Possible Sources of Uncertainty

- ☑ recovery of analyte from a complex matrix
- ☑ storage conditions
- ☑ reagent purity
- ☑ assumed stoichiometry
- ☑ sampling
- ☑ measurement conditions
- ☑ instrument response
- ☑ bias of instrument
- ☑ instrument resolution
- ☑ uncertainty of standards and CRM's
- ☑ variations in repeated observations

Step 3 - Possible Sources of Uncertainty

$$Q_{NO_3^-} = C_{st} \frac{A_{NO_3^-} \cdot V_{NO_3^-}}{A_{st} \cdot m} \times f_{di} \times \frac{1}{R}$$



Collection of operations

Step 4 - Input Quantities Uncertainty (evaluation type A & B)

- repeated observation (A)
- validation experiments (A and/or B)
- manufacturers' specifications (B)
- calibration certificates (B)
- results of interlaboratory method validations (B)
- from experience and/or literature (B)

Step 5 - Convert to Standard Uncertainties

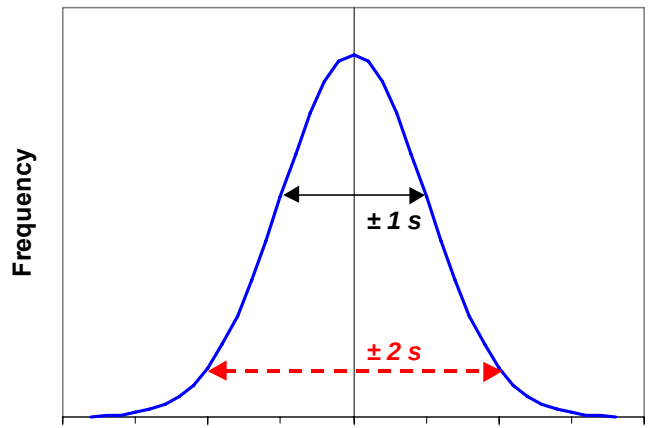
$$R = \overline{x} \pm \Delta x \Rightarrow \mathbf{s}$$

But what is Δx ?

Before combining, all uncertainty contributions must be expressed/converted as "estimated" standard uncertainty

when available as:

- standard deviation: use as is
- confidence intervals: convert
- stated range: convert
- expanded uncertainties: convert



Rectangular distribution

The Value is between the limits

$$a_{-} \ldots a_{+}$$

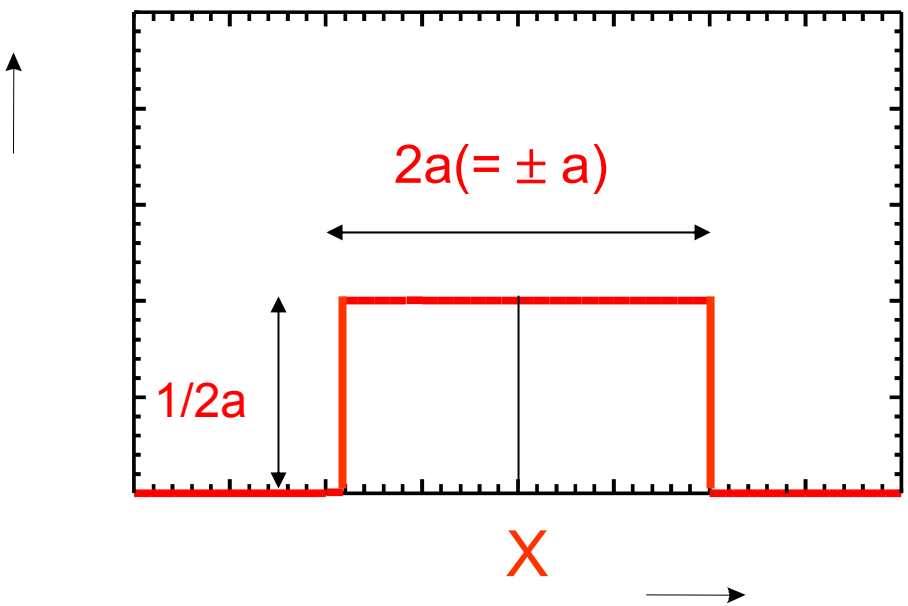
The expectation

$$y = x \pm a$$

Assumed standard deviation:

$$s = a / \sqrt{3}$$

$$u_s = S = \frac{b-a}{2\sqrt{3}}$$



One can only assume that it is equally probable for the value to lie anywhere within the interval

Example of Rectangular distribution

"*It is likely that the value is somewhere in that range*"

Rectangular distribution is usually described in terms of:
the average value and the range (±a)

Certificates or other specification give limits where the value could be, without specifying a level of confidence (or degree of freedom).

Examples:

Concentration of calibration standard is quoted as (1000 ± 2) mg/l
Assuming rectangular distribution the standard uncertainty is:

$$s = u(x) = a / \sqrt{3} = 2 / \sqrt{3} = 1.16 \ \ mg / l$$

The purity of the cadmium is given on the certificate as (99.99 ± 0.01) %
Assuming rectangular distribution the standard uncertainty is:

$$s = u(x) = a / \sqrt{3} = 0.01 / \sqrt{3} = 0.0058 \ \%$$

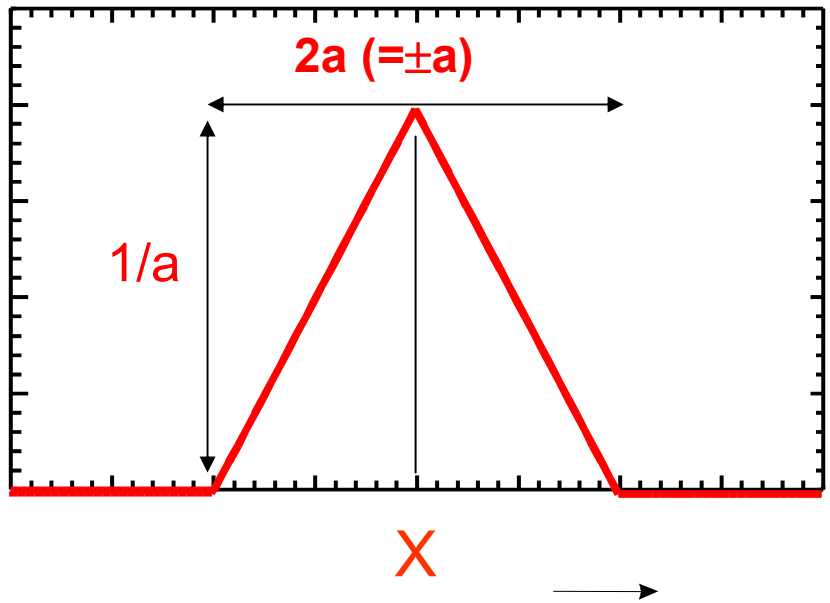
Triangular distribution

Distribution used when it is suggested that values near the centre of range are more likely than near to the extremes

$$y = x \pm a$$

Assumed standard deviation:

$$s = a / \sqrt{6}$$



Example of Triangular distribution

Values close to x are more likely than near the boundaries

The available information concerning the value is less limited than for rectangular distribution.

Example *(volumetric glassware)*

The manufacture quotes a volume for the flask of
(100 ± 0.1) ml at T = 20° C.
Nominal value most probable!
Assuming triangular distribution the standard uncertainty is:

$$s = u(x) = a \cdot 1/\sqrt{6} = 0.1/\sqrt{6} = 0.04\ ml$$

In case of doubt, use the rectangular distribution

Step 6 - Calculate Value of Measurand

Use model equation to calculate the value of output quantity Y (Q_{NO3-})

Example

$$Q_{NO_3^-} = C_{st} \frac{A_{NO_3^-} \cdot V_{NO_3^-}}{A_{st} \cdot m} \times f_{di} \times \frac{1}{R}$$

$$Q_{NO_3^-} = 0.801 \times \frac{0.0131 \times 0.1000}{0.0232 \times 1.142} \times 10 \times \frac{1}{0.78}$$

$$Q_{NO_3^-} = 0.508 \quad mg / g$$

Step 7 - Combined Standard Uncertainty

When there is no correlation between input quantities the combined standard uncertainty is evaluated as the square root of the combined variance according to:

$$u_c^2(Y) = \sum \left(\frac{\partial f}{\partial X_i} \right)^2 \cdot \left(u(X_i) \right)^2$$

Law of Uncertainty propagation

where

$u_c(Y)$ = combined standard uncertainty

$u(X_i)$ = standard uncertainty of each input quantity

Can be done by spreadsheet or by dedicated software!

Law of "Uncertainty Propagation"

without correlation

$$Y = f(X_1, X_2, ..., X_n)$$

$$u_c^{\,2}(Y) = \sum \left(\frac{\partial f}{\partial X_i} \right)^2 \cdot \left(u(X_i)\right)^2$$

$$C = (a + b)$$
$$C = (a - b)$$

$$\Rightarrow \quad u(C) = \sqrt{u(a)^2 + u(b)^2}$$

$$C = (a * b)$$
$$C = (a / b)$$

$$\Rightarrow \quad \frac{u(C)}{C} = \sqrt{\left(\frac{u(a)}{a}\right)^2 + \left(\frac{u(b)}{b}\right)^2}$$

Combined Standard Uncertainty

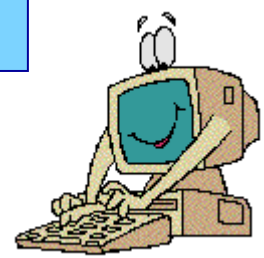
$$Q_{NO_3^-} = C_{st} \frac{A_{NO_3^-} \cdot V_{NO_3^-}}{A_{st} \cdot m} \times f_{di} \times \frac{1}{R}$$

$$u_{c,r}(Q_{NO_3^-}) = \sqrt{\begin{array}{l} RSu(C_{st})^2 + RSu(A_{NO_3^-})^2 + RSu(A_{st})^2 + \\ + RSu(V_{NO_3^-})^2 + RSu(m)^2 + RSu(f_{di})^2 + RSu(R)^2 \end{array}}$$

where $RSu(X_i) = u(X_i)/X_i$ ***(relative standard uncertainty)***

$$u_{c,r}(Q_{NO_3^-}) = \sqrt{\begin{array}{l} \left(\frac{0.00058}{0.801}\right)^2 + \left(\frac{0.0003}{0.0131}\right)^2 + \left(\frac{0.0006}{0.0232}\right)^2 + \\ + \left(\frac{0.0003}{0.1000}\right)^2 + \left(\frac{0.00058}{1.1420}\right)^2 + \left(\frac{0.023}{10.000}\right)^2 + \left(\frac{0.04}{0.78}\right)^2 \end{array}}$$

$$u_c(Q_{NO3-}) = u_{c,r}(Q_{NO3-}) \times Q_{NO3-} = 0.031 \text{ mg/g}$$



Kragten approach

❶ Model: $Y = X_1 * X_2 / (X_3 * X_4)$ *part 1*

❷

RSD	stdev	value	description
??	0,02	2,46	**X1**
3,0%	??	4,32	**X2**
??	0,11	6,38	**X3**
2,3%	??	2,99	**X4**

❸

RSD	stdev	value	description
0,8%	0,02	2,46	**X1**
3,0%	0,13	4,32	**X2**
1,7%	0,11	6,38	**X3**
2,3%	0,07	2,99	**X4**

❹

RSD	stdev	value	description
0,8%	0,02	2,46	**X1**
3,0%	0,13	4,32	**X2**
1,7%	0,11	6,38	**X3**
2,3%	0,07	2,99	**X4**
??	**??**	**0,557**	Result

Kragten approach

❶ Model: $Y = X_1 * X_2 / (X_3 * X_4)$ *part 2*

❺

RSD	stdev	value	description	X1	X2	X3	X4
0,8%	0,02	2,46	**X1**		2,46	2,46	2,46
3,0%	0,13	4,32	**X2**	4,32		4,32	4,32
1,7%	0,11	6,38	**X3**	6,38	6,38		6,38
2,3%	0,07	2,99	**X4**	2,99	2,99	2,99	
??	??	**0,557**	Result				

❻

RSD	stdev	value	description	X1	X2	X3	X4
0,8%	0,02	2,46	**X1**	2,48	2,46	2,46	2,46
3,0%	0,13	4,32	**X2**	4,32	4,45	4,32	4,32
1,7%	0,11	6,38	**X3**	6,38	6,38	6,49	6,38
2,3%	0,07	2,99	**X4**	2,99	2,99	2,99	3,06
		0,557	Result	0,562	0,574	0,548	0,544
4,2%	**0,024**						

$x+\Delta x$

❼

diff	0,005	0,017	-0,009	-0,013	0,001

$$u_c = \sqrt{\sum_i (y_i - y)^2}$$

$\text{sumsq}(\text{diff}_i)$

Kragten approach

❶ Model: $Y = X_1 * X_2 / (X_3 * X_4)$ *part 3*

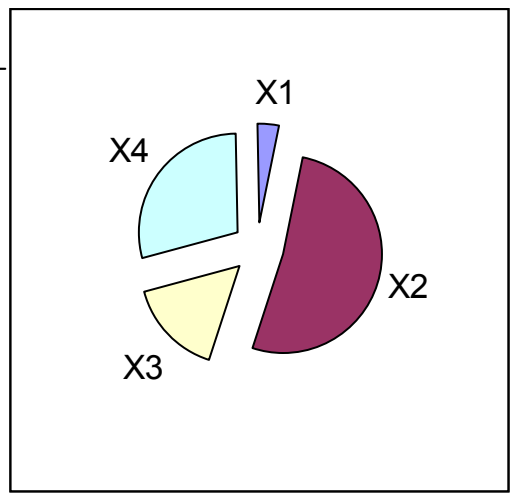
RSD	stdev	value	description	X1	X2	X3	X4	
0,8%	0,02	2,46	**X1**	2,48	2,46	2,46	2,46	
3,0%	0,13	4,32	**X2**	4,32	4,45	4,32	4,32	
1,7%	0,11	6,38	**X3**	6,38	6,38	6,49	6,38	
2,3%	0,07	2,99	**X4**	2,99	2,99	2,99	3,06	
4,2%	**0,024**	**0,557**	Result	0,562	0,574	0,548	0,544	
			diff	0,005	0,017	-0,009	-0,013	0,001

❽

index	3,7%	**50,8%**	16,1%	**29,4%**	100,0%
					sum

$$index = \frac{(y_i - y)^2}{\sum_i (y_i - y)^2}$$

Think!



Major Contributor :

- Type B? ☹
- Type A? ☺
- Replicates?
- Much work?
- Control Charts?

Step 8 - Expanded Uncertainty

The expanded uncertainty, U, is obtained by multiplying the combined standard uncertainty $u_c(y)$ by a coverage factor k:

$$U = k * u_c$$

The result is then expressed as:

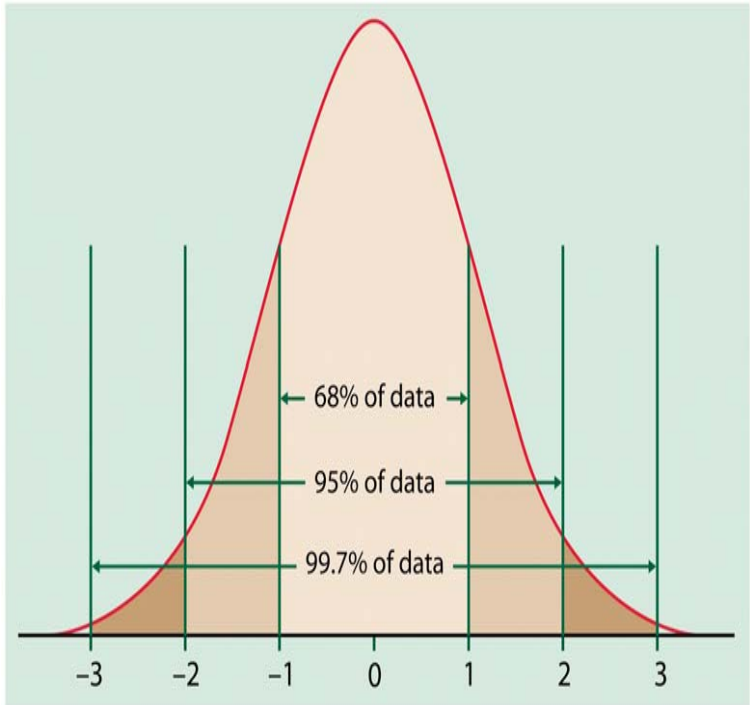
$$\text{Result} = y \pm U \ (k = ??)$$

For the example: $Q_{NO3-} = (0.51 \pm 0.06)$ mg/g , $k = 2$

- the best estimate of the value attributed to the measurand is "y",
- the interval $[y - U , y + U]$ is the range that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand.

Step 8 - Expanded Uncertainty (2)

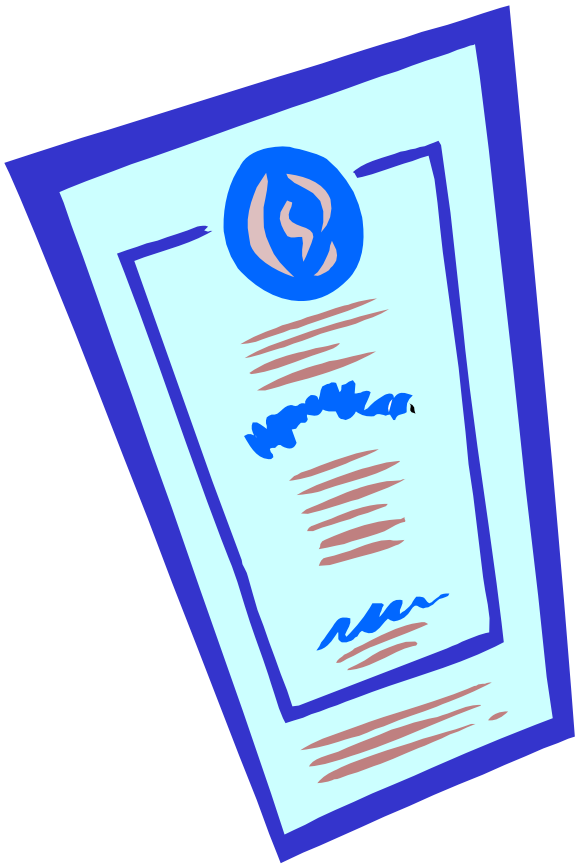
- Expanded uncertainty gives a more realistic range of possible values.
- The coverage factor usually used is $k = 2$, representing a coverage of about 95%, if the distribution is normal



Standard uncertainty should be used inside the laboratory
(to apply uncertainty propagation)

Expanded uncertainty is more realistic range
given for the end-users of the results

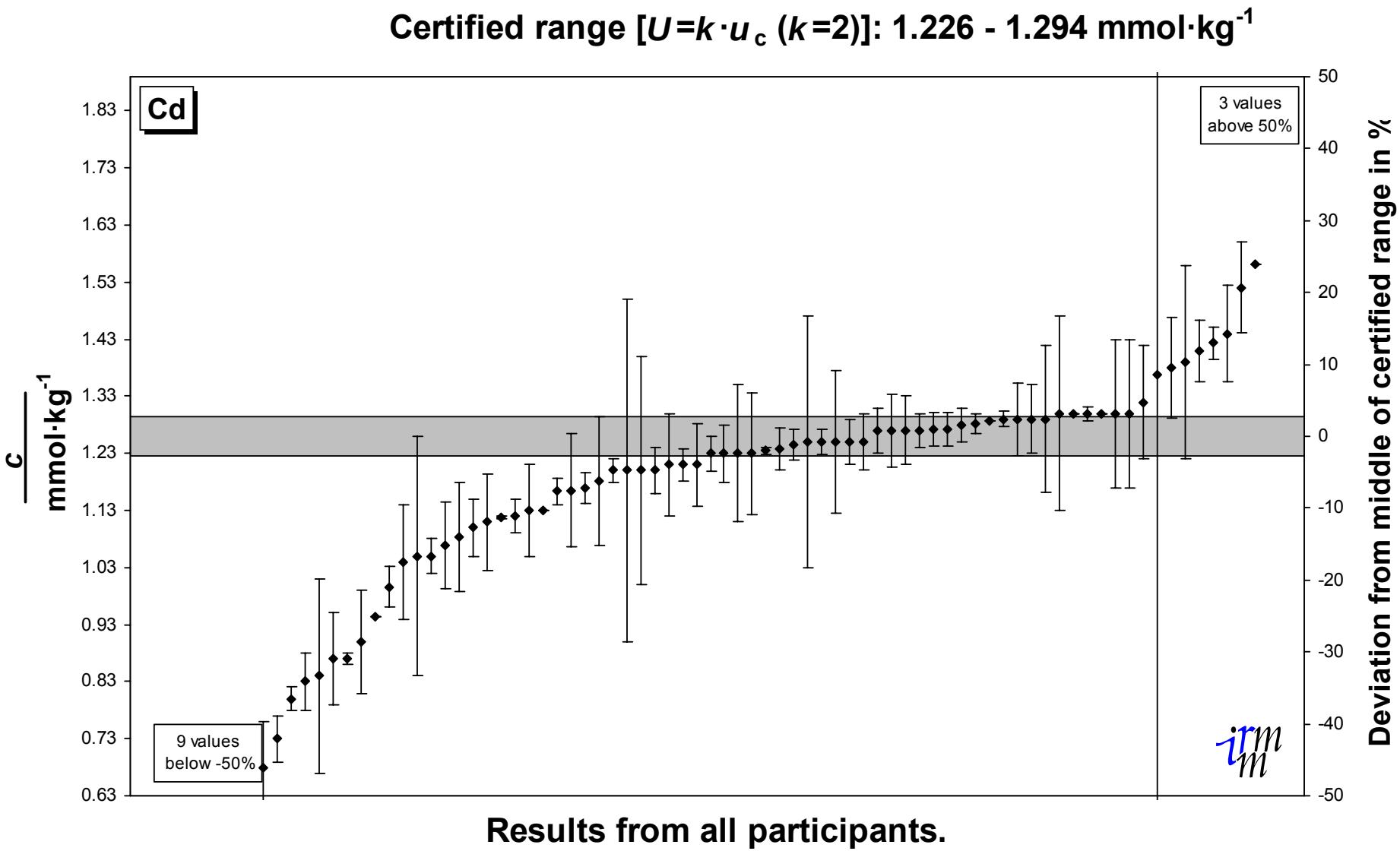
Step 10 - Reporting Results



$$Q_{NO3\text{-}} = (0.51 \pm 0.06)\ \text{mg/g}\ (*)$$

(*)

the reported uncertainty is an expanded uncertainty calculated using a coverage factor of $k = 2$, which gives a level of confidence of approximately 95%



Metrologists obsessed by small uncertainties ?

Learning how to apply GUM:

Better sell your results with reliable uncertainty statement !

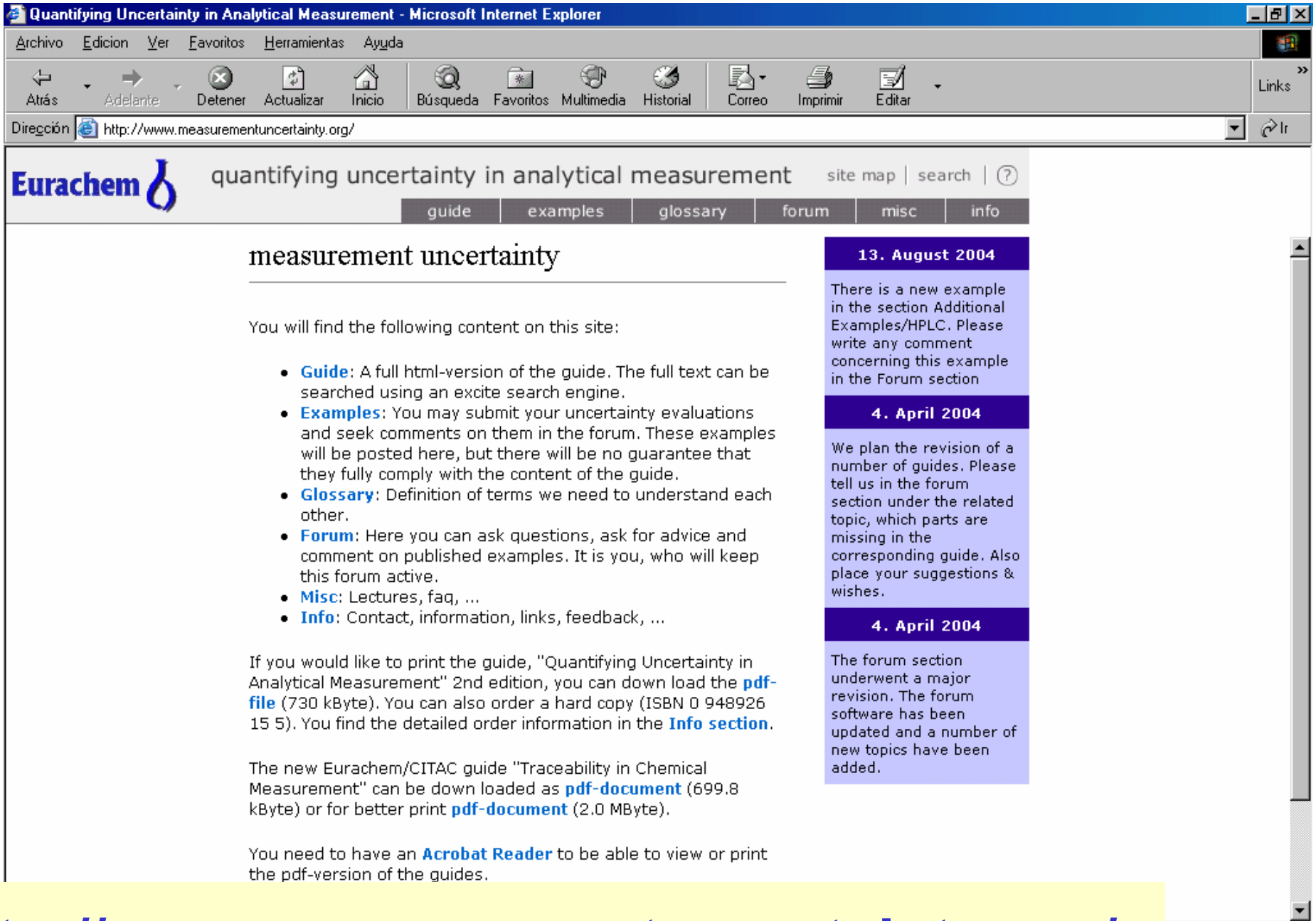
Conclusion: about uncertainty

- Uncertainty budgeting according to the GUM is a useful and accepted concept to evaluate results of a measurement;
- It allows others (e.g. assessors) to understand what & how things were done
- It allows the analyst to combine prior knowledge and observations in a consistent and well defined way;
- It doesn't requires to measure with smallest achievable uncertainty, but with **the most realistic one**

Uncertainty adopted and accepted by ...

- Many international institutions, such as NMIs and BIPM
- Is required under ISO 17025 for accreditation
- IUPAC, OIML and accreditation community such as EA and ILAC have accepted this concept
- CEN is incorporating these concepts

YOU have to know it and work with it!


Quantifying Uncertainty in Analytical Measurement - Microsoft Internet Explorer
Archivo Edicion Ver Favoritos Herramientas Ayuda
Atrás Adelante Detener Actualizar Inicio Búsqueda Favoritos Multimedia Historial Correo Imprimir Editar
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You will find the following content on this site:
Guide: A full html-version of the guide. The full text can be searched using an excite search engine.
Examples: You may submit your uncertainty evaluations and seek comments on them in the forum. These examples will be posted here, but there will be no guarantee that they fully comply with the content of the guide.
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Misc: Lectures, faq, ...
Info: Contact, information, links, feedback, ...
If you would like to print the guide, "Quantifying Uncertainty in Analytical Measurement" 2nd edition, you can down load the pdf-file (730 kByte). You can also order a hard copy (ISBN 0 948926 15 5). You find the detailed order information in the Info section.
The new Eurachem/CITAC guide "Traceability in Chemical Measurement" can be down loaded as pdf-document (699.8 kByte) or for better print pdf-document (2.0 MByte).
You need to have an Acrobat Reader to be able to view or print the pdf-version of the guides.
13. August 2004
There is a new example in the section Additional Examples/HPLC. Please write any comment concerning this example in the Forum section
4. April 2004
We plan the revision of a number of guides. Please tell us in the forum section under the related topic, which parts are missing in the corresponding guide. Also place your suggestions & wishes.
4. April 2004
The forum section underwent a major revision. The forum software has been updated and a number of new topics have been added.
http://www.measurementuncertainty.org/
US
14:45

Validation of measurement procedures

Validation *(method fit-for-purpose)*

Quality of measurement result

Traceability

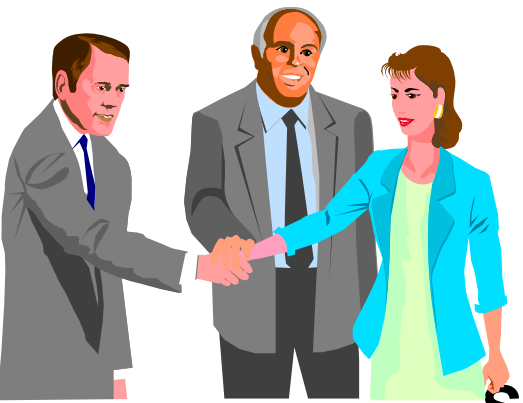
(my result is comparable - common reference)

Quality: The ability of the qualitative (analyte identification) and quantitative (accuracy, precision) data to satisfy the requirements of their purpose.

Uncertainty Budget

(How well I know the result)

How to GUARANTEE reliability of the measurement result

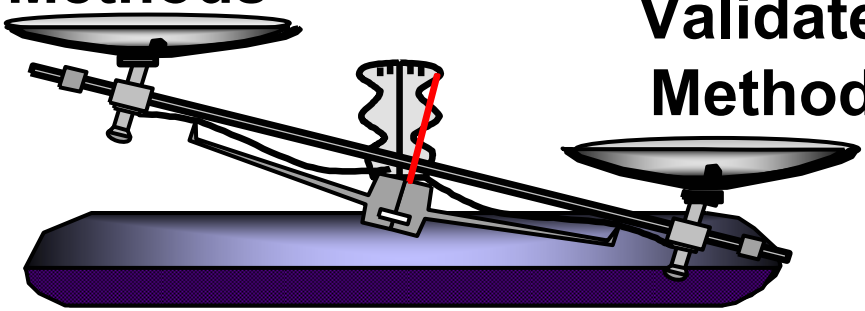


Accreditor (ISO17025) :
uncertainty
traceability
validation

Fully documented measurement procedure by a transparent, clear and standartirized approach

Standartirized Methods

Validated Methods



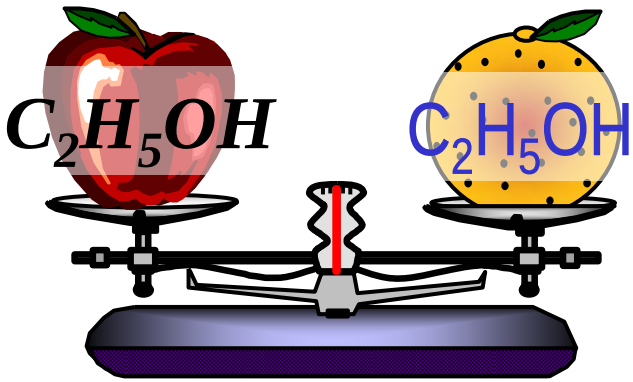
THE TRUST

Content

- What is validation of a measurement procedure ?
- Why procedure validation ?
- Approach to procedure validation ?
- How to perform validation ?
- Step by step examples?
- How the ILC and PT should be evaluate?

The goal of the measurement is to assign a value to an unknown quantity

THE ANALYSIS IS COMPARISON !



The result of the chemical measurement is little more than a DECLARATION of an isolated figure

until /or someone does not prove the contrary !

The analysis concept

What is that?

How much is it?

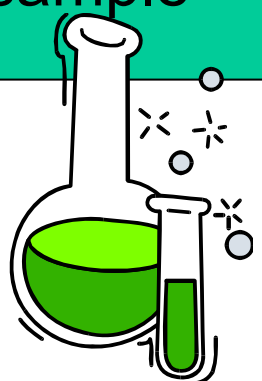
What its properties are?

TO WHICH EXTEND
the procedure is stable and relationships are constant ?

Provocation
Stimulus

Reagent, titrant, coagulant
electrons, photons, atoms,
ions, molecules , heat

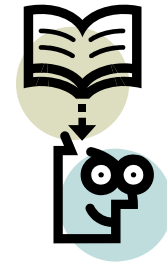
Analytical sample



Response

Phisico-Chemical quantity that could be registered and quantified as a magnitude

Beware



Difference in terminology between ISO/IEC 17025 and VIM
(Internat. Vocabulary of basic and general terms in Metrology)

- ISO/IEC 17025 uses “method” ➔ *method validation*
- VIM uses “(measurement) procedure” ➔ *procedure validation*
- GLP uses “standard operating procedure”, SOP ➔ *SOP validation*

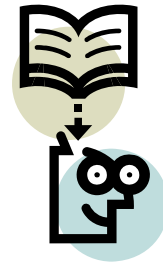
Measurement procedure - set of operation, described specifically, used in the performance of particular measurement according to a given method

[VIM 2.5]

Method of measurement - Logical sequence of operation, described generically, used in the performance of measurements

[VIM 2.4]

What is validation ?



Validation includes

- ☒ analytical requirements
- ☑ determination of procedure characteristics
- ☒ check that requirements can be fulfilled by the procedure
- ☒ statement on validity

Validation is the confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled

(ISO/IEC 17025)

Validation of measurement procedure

Process of establishing includes:

- performance characterisation
- scope & limitation of a measurement procedure
- identification of the influences which may change the characteristics and to what extent.

- Which analyte can it determine, in which matrices, in the presence of which interference?
- Within these conditions (to be defined) what uncertainty can be achieved?

The process of verifying that a procedure is

fit for purpose

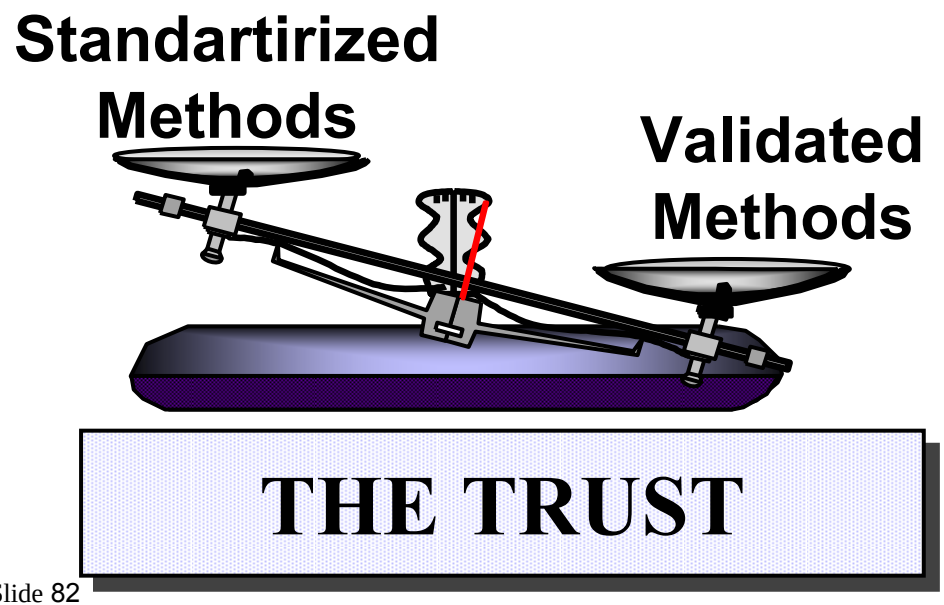
(i.e. for solving a particular analytical problem)

Intended use

- By providing information on procedure performance characteristic we Increase the confidence:
  - for users of the procedure (analyst)
  - for users of measurement results (customer)

better understanding

💣 validation is a study of the procedure,
NOT of the analyst or of the laboratory performance !



- compliance with regulations
- maintain quality and process control
- make regulatory decisions
- support national and international trade
- support research

Standard & non-standard methods (procedures)

Sources of standard method (procedures):

- procedures published in international, regional, national standards *(ISO, EN, DIN, BS, ASTM, …)*
- procedures published by reputable organizations in their publications *(AOAC for food and agriculture; ICH for clinical analysis,….)*
- not in scientific literature!

Why do we need it ?

Laboratories should demonstrate that they operate within quality system, are technically competent and are able to generate technically valid results

(ISO/IEC 17025)

Three milestones of ISO/IEC 17025:

- ✓ procedure validation
- ✓ traceability of results
- ✓ uncertainty of results

Validation is essential EVEN IF you are not going for accreditation

Often Encountered Terms

- Full Validation:
  where all relevant parameters of the procedure are investigated
- Degree of Validation:
  where only some of the performance parameters are investigated
- Confirmation:
  used in relation to (already validated) standardised procedures. No need for additional validation, just a “confirmation” in your lab.

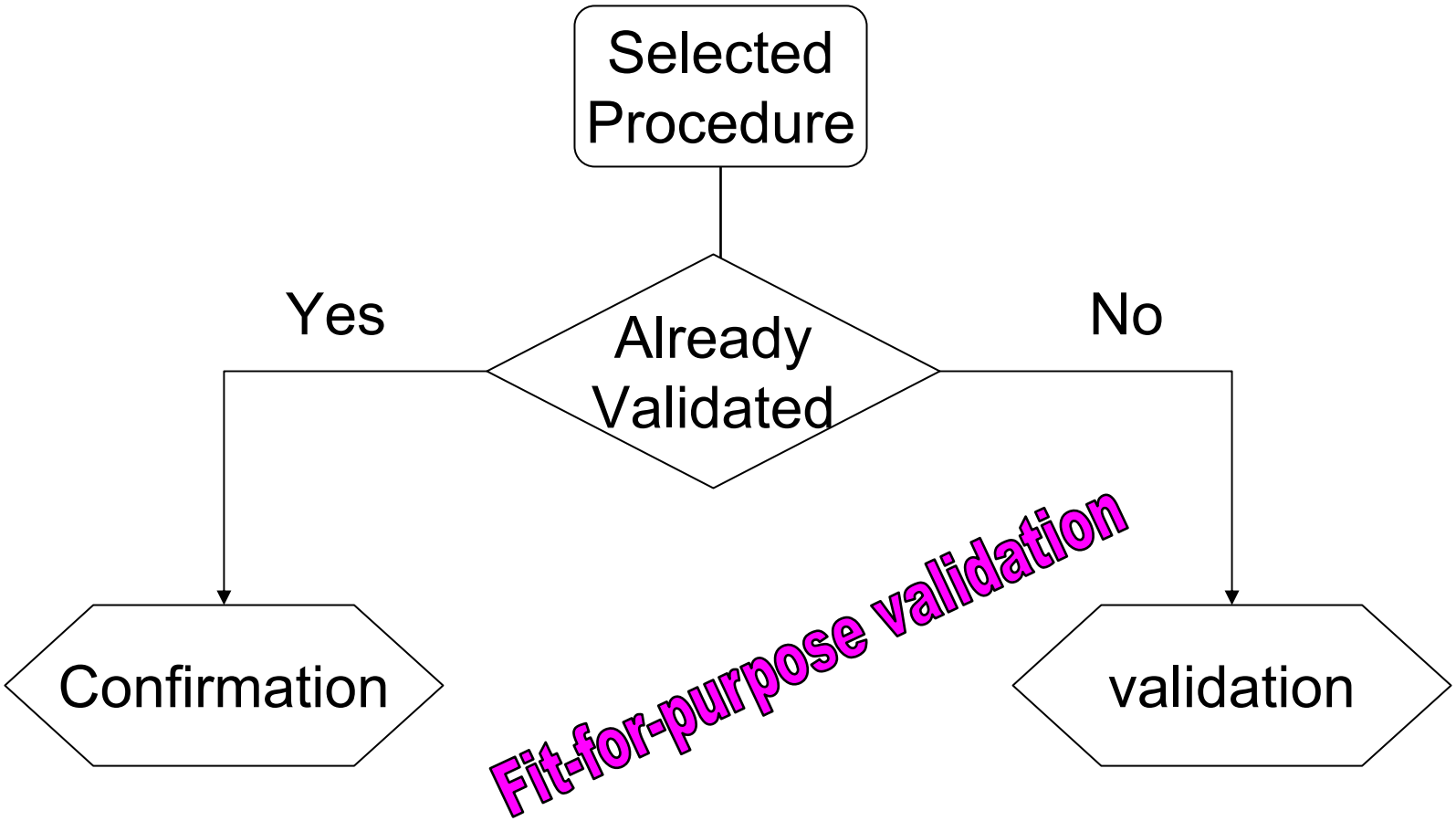
Which procedures should be validated?

- non-standard
- in-house developed
- standard ones used outside their intended scope
- modified standard

Will a validated procedure “automatically” work in my lab?

- (First) No, confirmation needed
- (Then) Yes, within the specified conditions

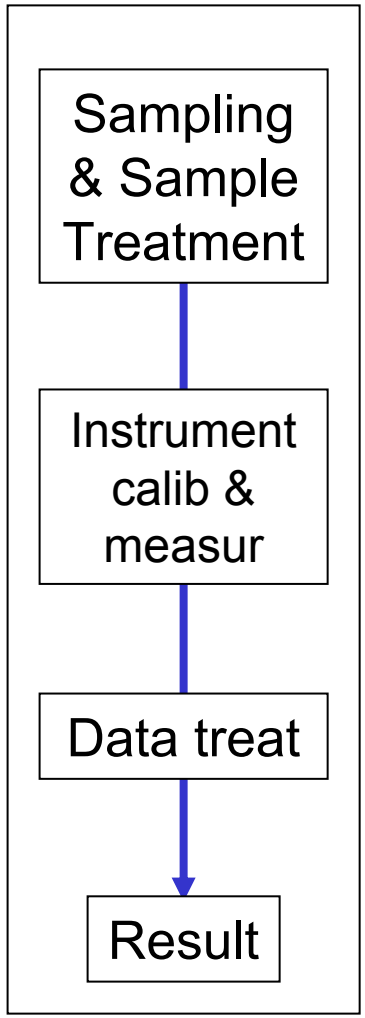
When is a procedure validation needed?



The scope of validation

- validate whole procedure
  (from sample preparation to measured signal)
- validate full concentration range
  (intended use!)
- validate all intended types of matrices

Put the effort where it is needed



Required degree of validation

Decide which characteristics are most relevant for your validation (spend effort accordingly!!)

- cholesterol in serum,
  LOD not important (NO),
  uncertainty is important (YES)
  (e.g. better uncertainty of the results ⇨ USA saves 100 M$/year)

- survey of environmental contamination [to find hot spots]:
  range and linearity YES,
  LOD and size of uncertainty NO

- doping control (against limit):
  LOD is critical,
  uncertainty is extremely important;
  range, linearity is not important

Validation technique...

… recommended by ISO/IEC 17025

- evaluation of uncertainty =
  systematic assessment of the quantities influencing the result
- measurement of CRM
- participation in inter-laboratory comparison
- comparison of results achieved with other procedures

Use...

- ✓ Standards and/or reference materials
- ✓ Investigate blanks
- ✓ Artificially prepared samples (e.g. spiked)
- ✓ Statistics
- ✓ Common sense

The Validation Menu

The Validation Checklist

- ☐ selectivity/interference
- ☐ sensitivity
- ☐ repeatability
- ☐ recovery
- ☐ linearity, working range
- ☐ LOD, LOQ
- ☐ within-lab reproducibility
- ☐ robustness
- ☐ quality control
- ☐ exp uncertainty
- ☐ traceability

Check list

Performance parameters of the procedure

(qualitative): ☐ selectivity, specificity

(*quantitative*):

- ☐ sensitivity
- ☐ detection & quantification/determination limits
- ☐ working (linear) range

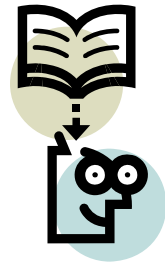
Property of the result obtained with this procedure

☐ traceability *(cf. other module)*

☐ uncertainty, considering e.g.

- recovery
- robustness
- repeatability
- reproducibility

Selectivity, Specificity



Selectivity refers to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behaviour

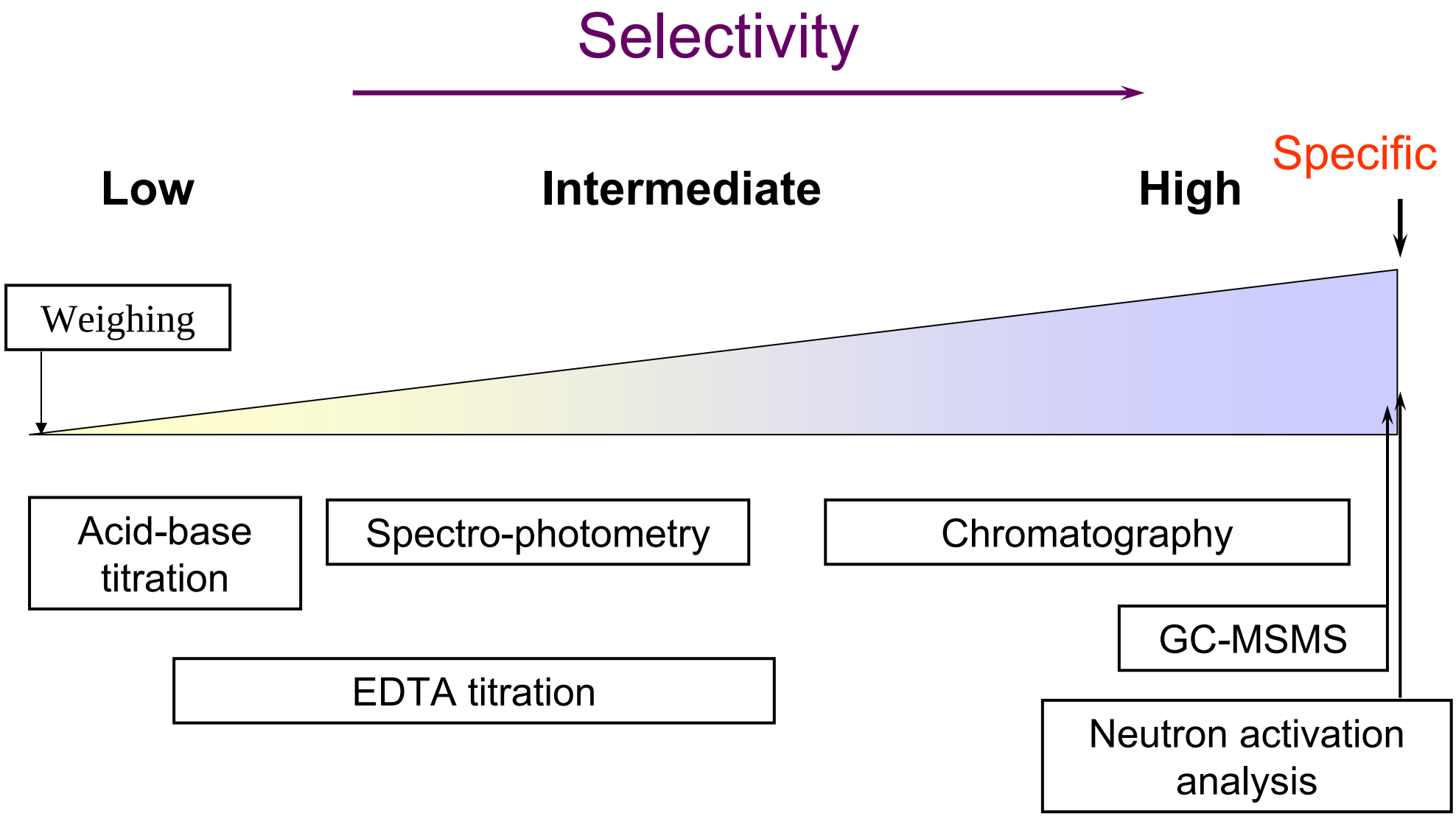
(IUPAC, 2001)

Potentially interfering substances must be chosen and relevant blank samples must be analyzed to estimate such effects.

- Specificity is 100% selectivity
- Few, if any methods are specific
- IUPAC recommends that the term specificity should be avoided

(IUPAC, 2001)

Selectivity, Specificity



Sensitivity

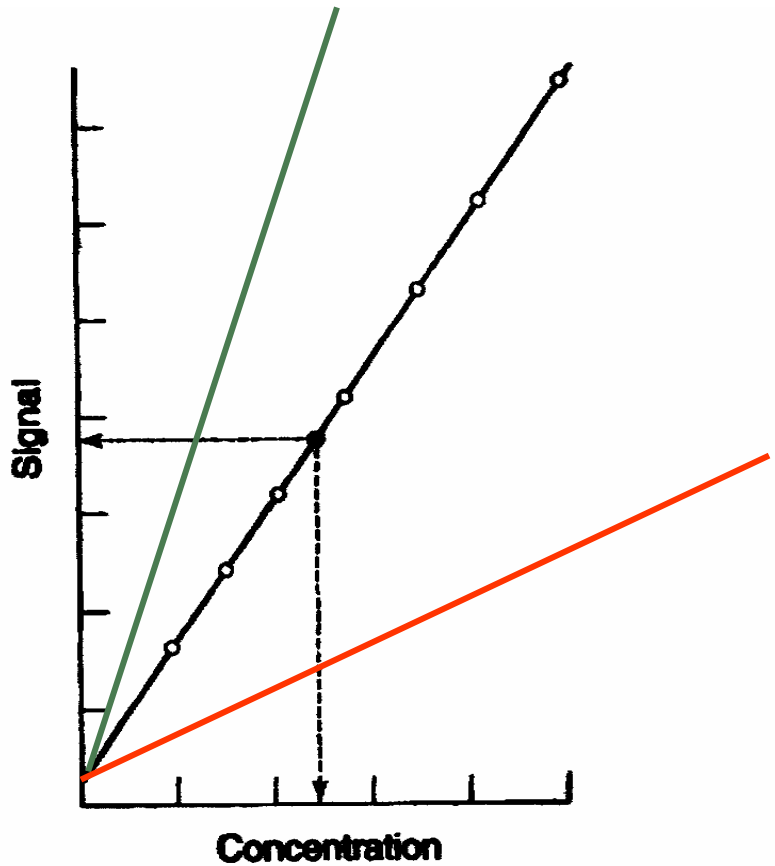
Definition:
The change in the response of a measuring instrument divided by the corresponding change in the stimulus

(VIM 1993)

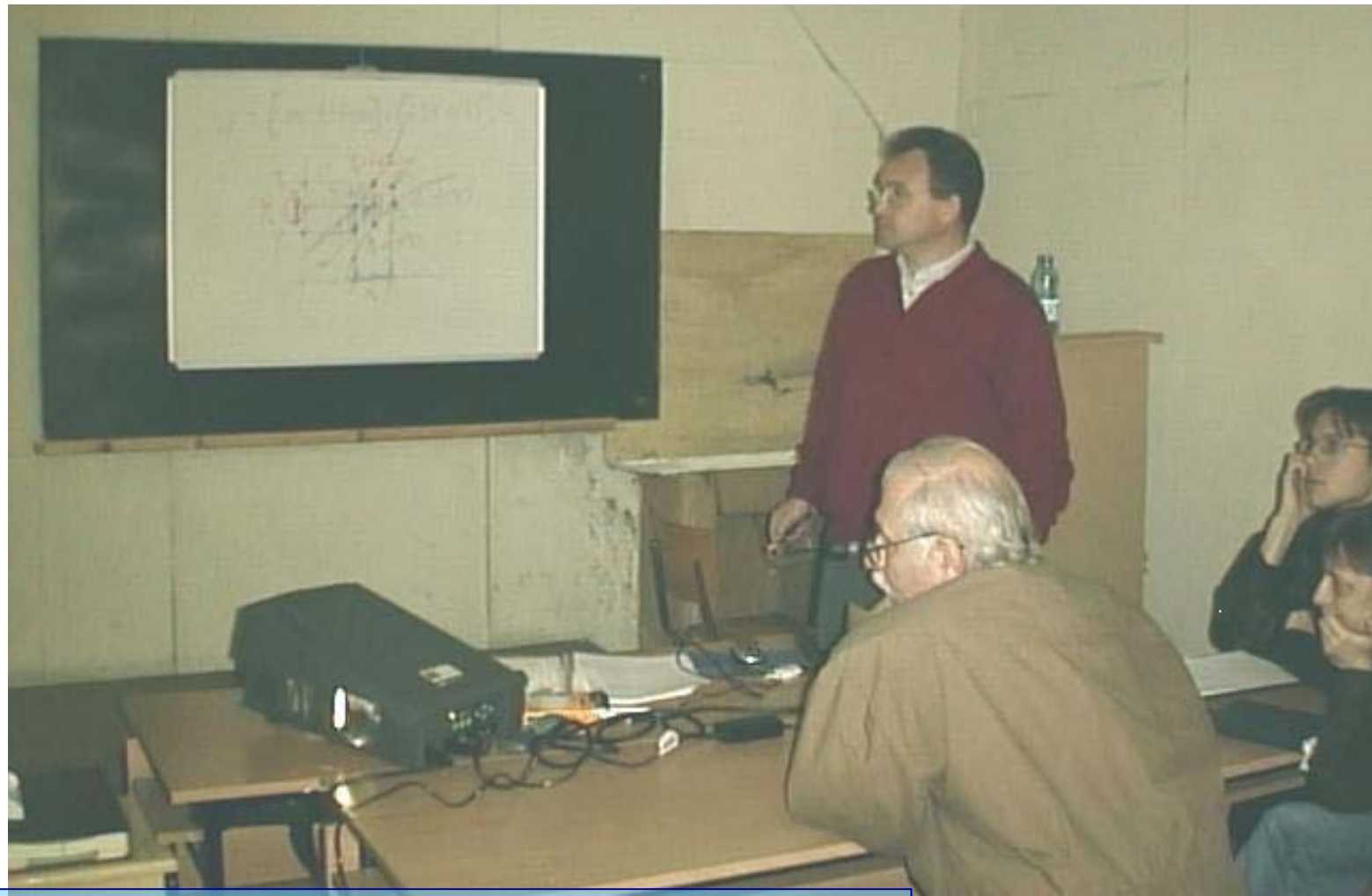
- *What it means*:
  It is a measure of the gradient (slope) of the calibration graph

$$Y = b_0 + b_1 X$$

$$b_1 = m = \frac{dSig}{dC} = tg\alpha$$

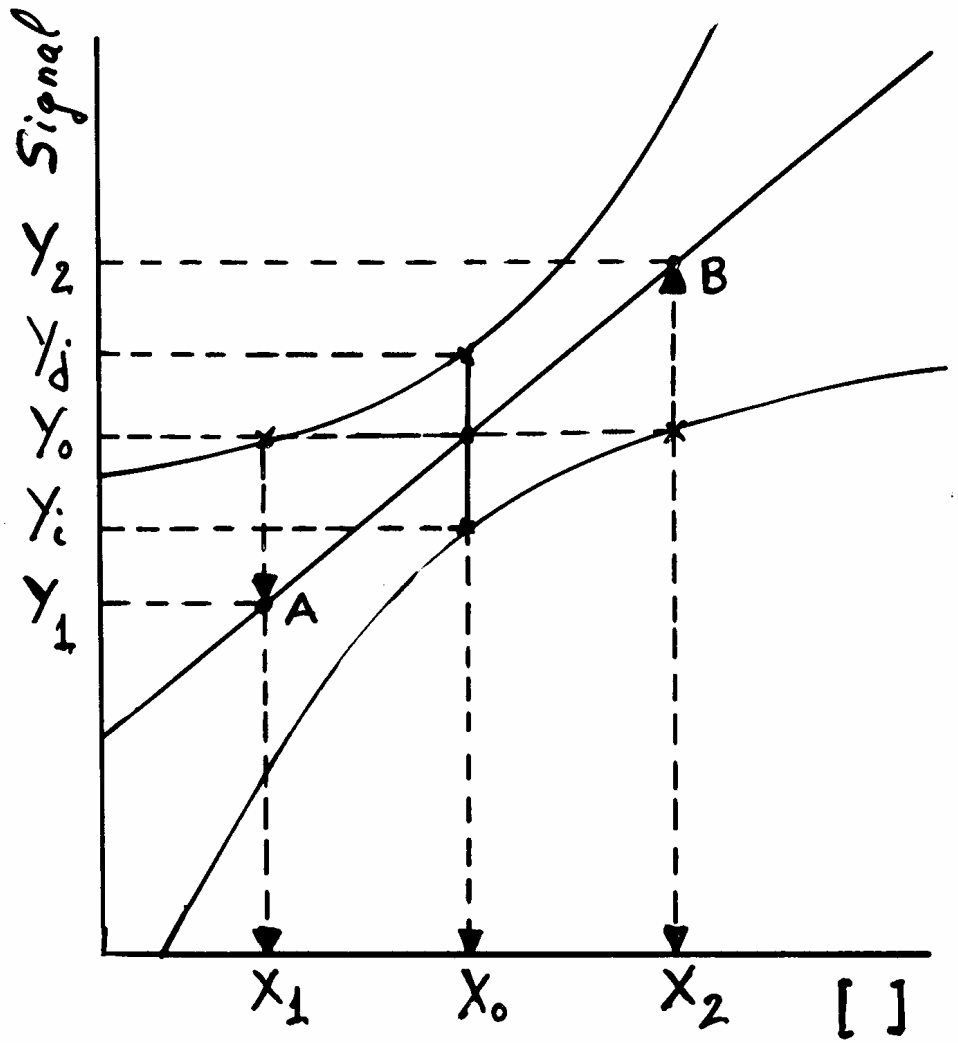


Prof. Dr. Antonio Canals Hernández



Univariate Methodological Calibration on Instrumental Analysis

Standard deviation of predicted values



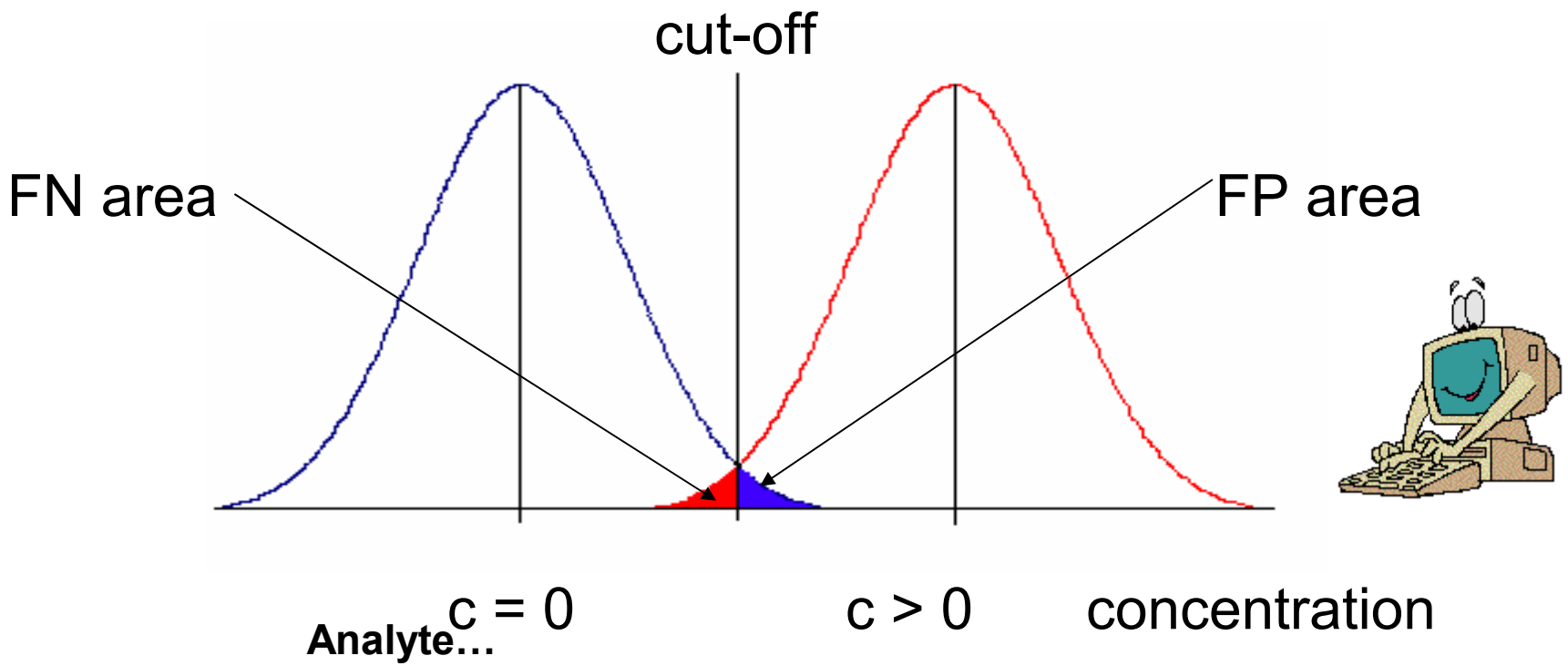
$$s_{x/y} = \sqrt{\frac{\sum (y_i - \hat{y}_i)}{n-2}}$$

$$s_{b_1} = \frac{s_{y/x}}{\sqrt{\sum (x_i - \bar{x}_i)^2}}$$

$$s_{b_o} = s_{y/x} \sqrt{\frac{\sum x_i^2}{n \sum (x_i - \bar{x}_i)^2}}$$

Reporting of LOD

Non-presence of analyte	Presence of analyte



Analyte…	Not present	Present
Not detected	True Negative	False Negative ❶
Detected	False Positive ❷	True Positive

❶ Insensitive method? Interference?
❷ Interference? Contamination

Dr. V. Kmetov Alicante 2007

Limits of...
Detection (LOD)
Quantification (LOQ)

'Blank'

- instrumental
- from sample treatment *(e.g. contamination in digestion, purification)*

Calibration equation:

$$Signal = b_0 + b_1 * c$$

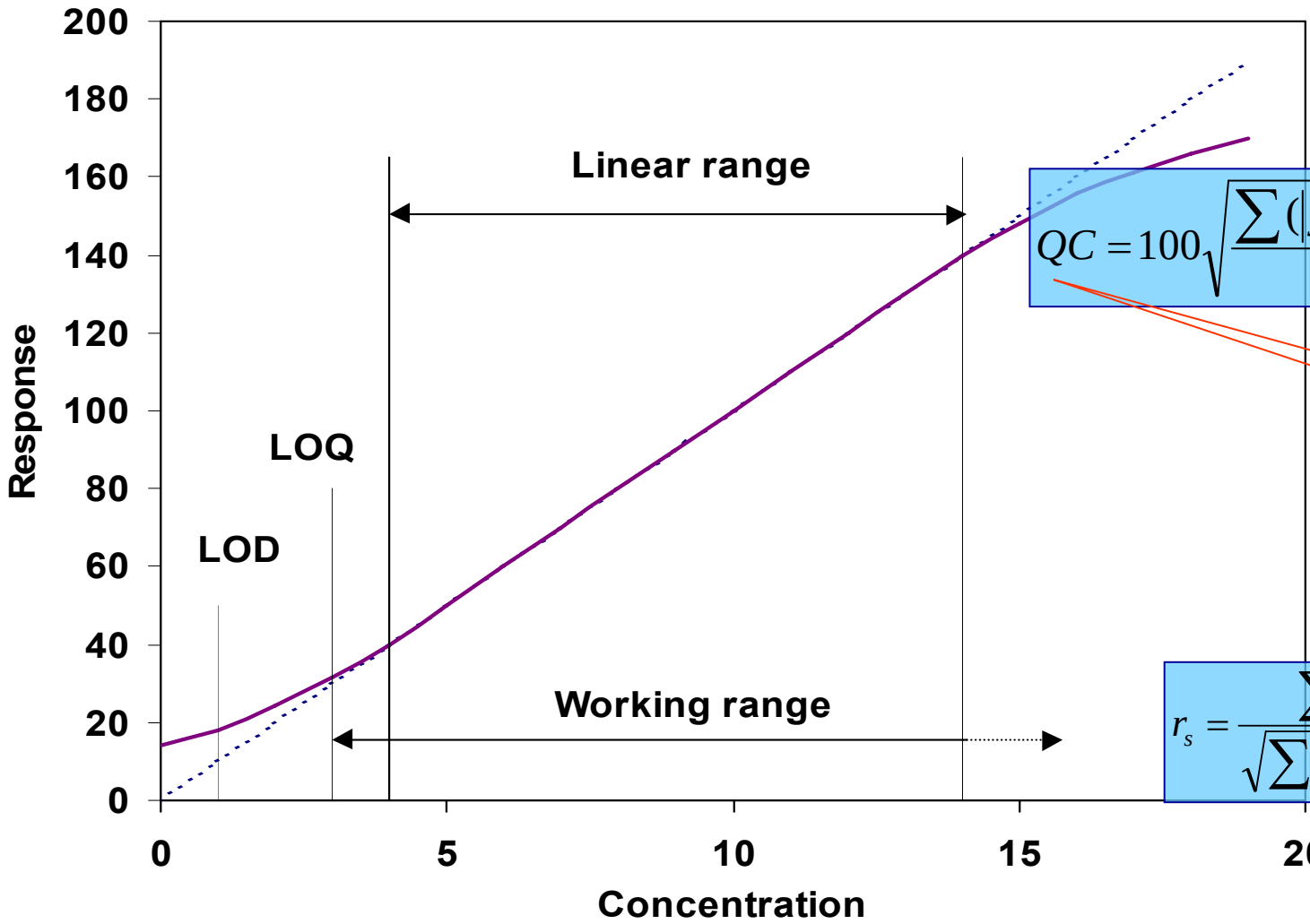
Y_{bl} = Signal of the 'blank' ;

s_{bl}= stdev of the 'blank' in signal domain

$Y_{LOD} = Y_{bl} + 3\ s_{bl}$ ➔ $LOD = (Y_{LOD} - b_0)/b_1$

$Y_{LOQ} = Y_{bl} + 10\ s_{bl}$ ➔ $LOQ = (Y_{LOQ} - b_0)/b_1$

Linearity Working Range



$$QC = 100\sqrt{\frac{\sum(|y_i - \hat{y}_i| / \hat{y}_i)^2}{n-2}}$$

Quality coefficient Massart

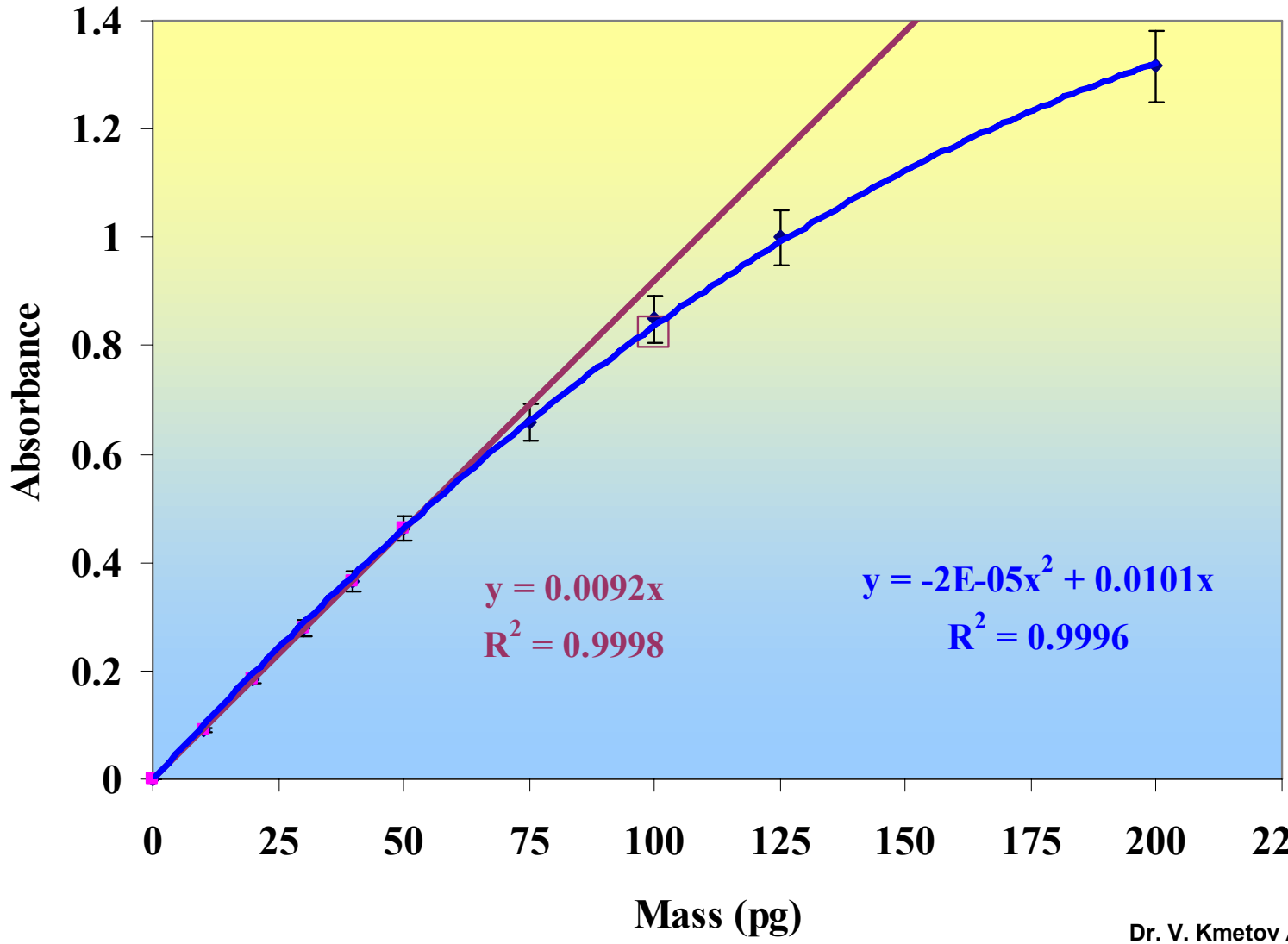
$$r_s = \frac{\sum(x_i - \bar{x}_i)(y_i - \bar{y}_i)}{\sqrt{\sum(x_i - \bar{x}_i)^2 \sum(y_i - \bar{y}_i)^2}}$$

- **Working Range** is the interval between the upper and lower levels of analyte (inclusive) that have been demonstrated to be determined with precision, accuracy and linearity. Within this interval, the method can be regarded as validated.

V. Kmetov, A. Sanchez, A. Canals, D. Georgieva, V. Stefanova

5th European Furnance Symposium and 10th International Solid Sampling Colloquium with Atomic Spectrometry, 1-4 September 2002, South-West University "Neofit Rilski",Blagoevgrad, P28, 90

Determination of Pb and Cd in wool and goat's hair from animal population in the vicinity of Plant for non-ferrous metals (Plovdiv) by ETAAS and ICP-MS methods.



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Recovery (1)

A measure of the trueness of a (measurement) procedure

$$R = \frac{observed\ \_value}{reference\ \_value}$$

(IUPAC 1999)

Reference value from:

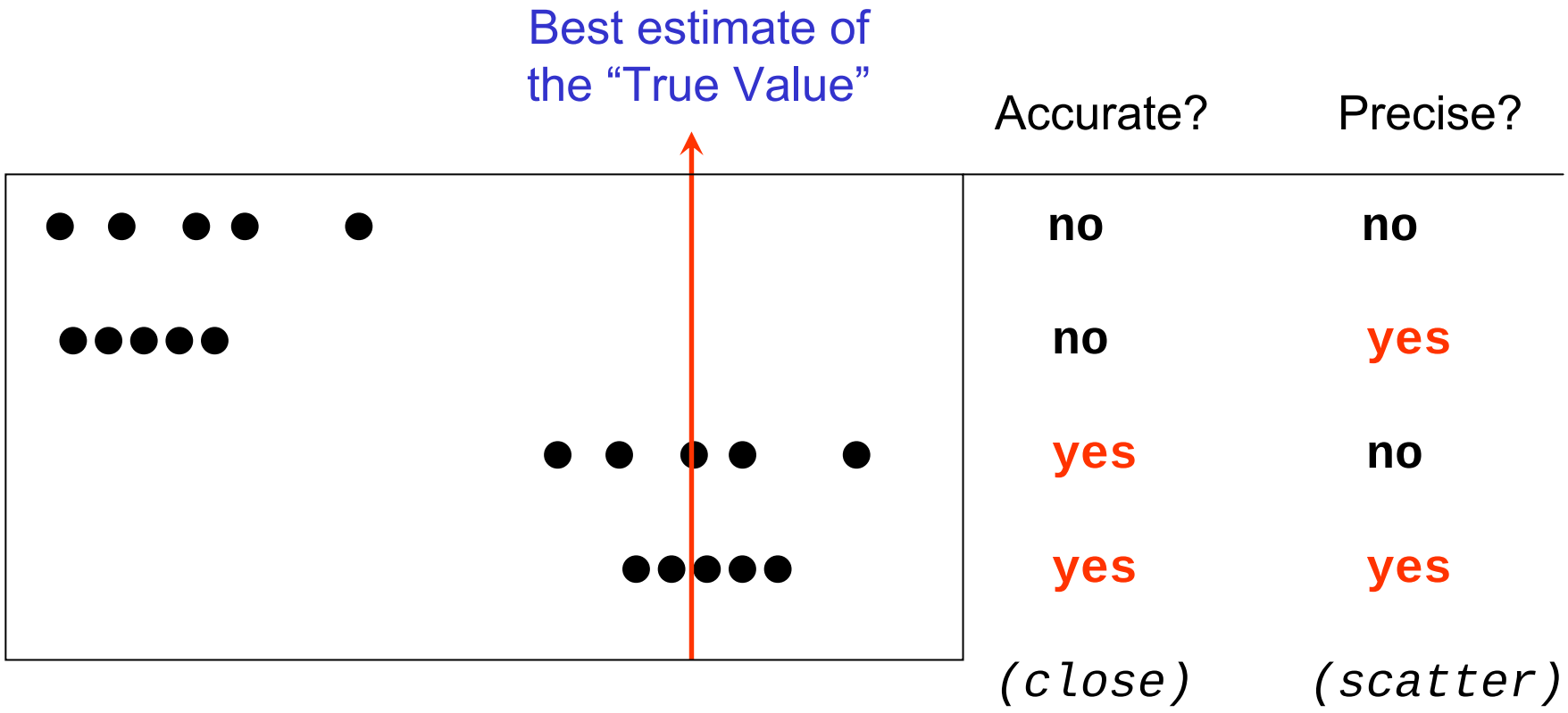
$$R = \frac{C_{observed}}{C_{CRM}}$$

- CRM
- spike of pure substance

$$R = \frac{C_{observed} - C_{matrix}}{C_{spike}}$$

The closer R is to 1, the smaller the bias in the procedure

Recovery is the estimate of trueness through the addition of a known mass of the analyte to the test portion.



Precision: The closeness of agreement between independent test results obtained under stipulated conditions [ISO 5725]

It is normally expressed as **the percent relative standard deviation** for a statistically significant number of samples.

Precision ↗ ⇒ Scatter ↘ ⇒ uncertainty ↘

Repeatability

Precision recorded under repeatability conditions:

– same laboratory, analyst, equipment, time (short interval)

Typically used for studying variation

within a batch or between replicated measurements.

Within-run precision = Repeatability

Reproducibility

Precision recorded under reproducibility conditions:

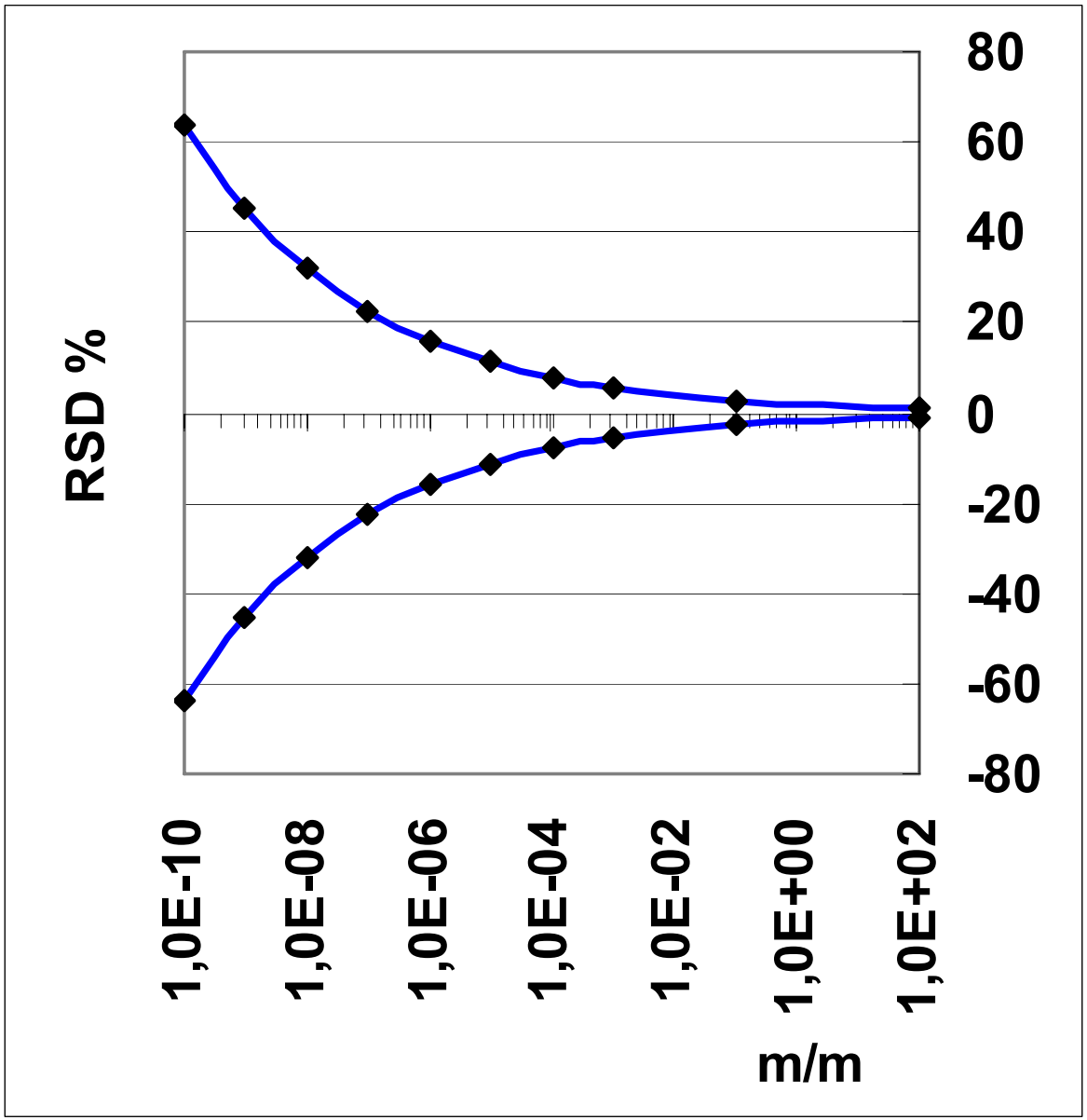
– different laboratory, analyst, equipment, time (short interval)

Typically used for studying variation

on measurements made between laboratories.

Between-run precision = Reproducibility

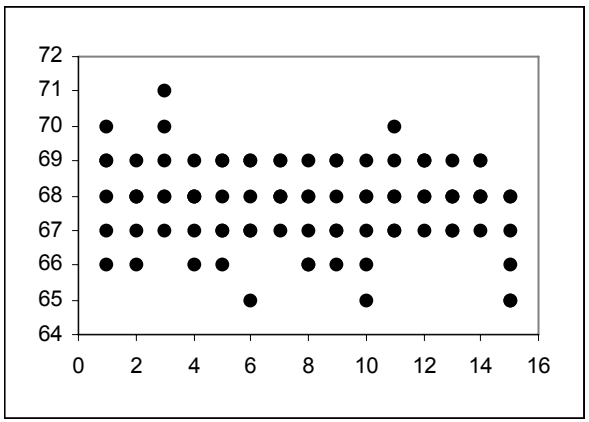
Trumpet of Horwitz



$$RSD = \pm 2^{(1-0,5\log C)}$$

Anova Single factor

Replicates		1	2	3	4	5	6
Vials	1	66	68	67	69	70	69
	2	66	67	68	68	68	69
	3	71	67	68	69	68	70
	4	66	68	67	68	68	69
	5	67	67	66	69	69	68
	6	65	67	67	69	68	69
	7	67	68	68	68	69	69
	8	67	66	66	68	68	69
	9	67	67	66	69	68	69
	10	66	65	67	68	69	68
	11	67	67	69	68	68	70
	12	67	68	69	69	68	69
	13	67	67	68	69	68	68
	14	67	68	68	69	68	69
	15	65	66	65	68	68	67



SUMMARY						
Groups	Count	Sum	Average	Variance		
1	6	409	68.2	2.2		
2	6	406	67.7	1.1		
3	6	413	68.8	2.2		
4	6	406	67.7	1.1		
5	6	406	67.7	1.5		
6	6	405	67.5	2.3		
7	6	409	68.2	0.6		
8	6	404	67.3	1.5		
9	6	406	67.7	1.5		
10	6	403	67.2	2.2		
11	6	409	68.2	1.4		
12	6	410	68.3	0.7		
13	6	407	67.8	0.6		
14	6	409	68.2	0.6		
15	6	399	66.5	1.9		

Sum of squares

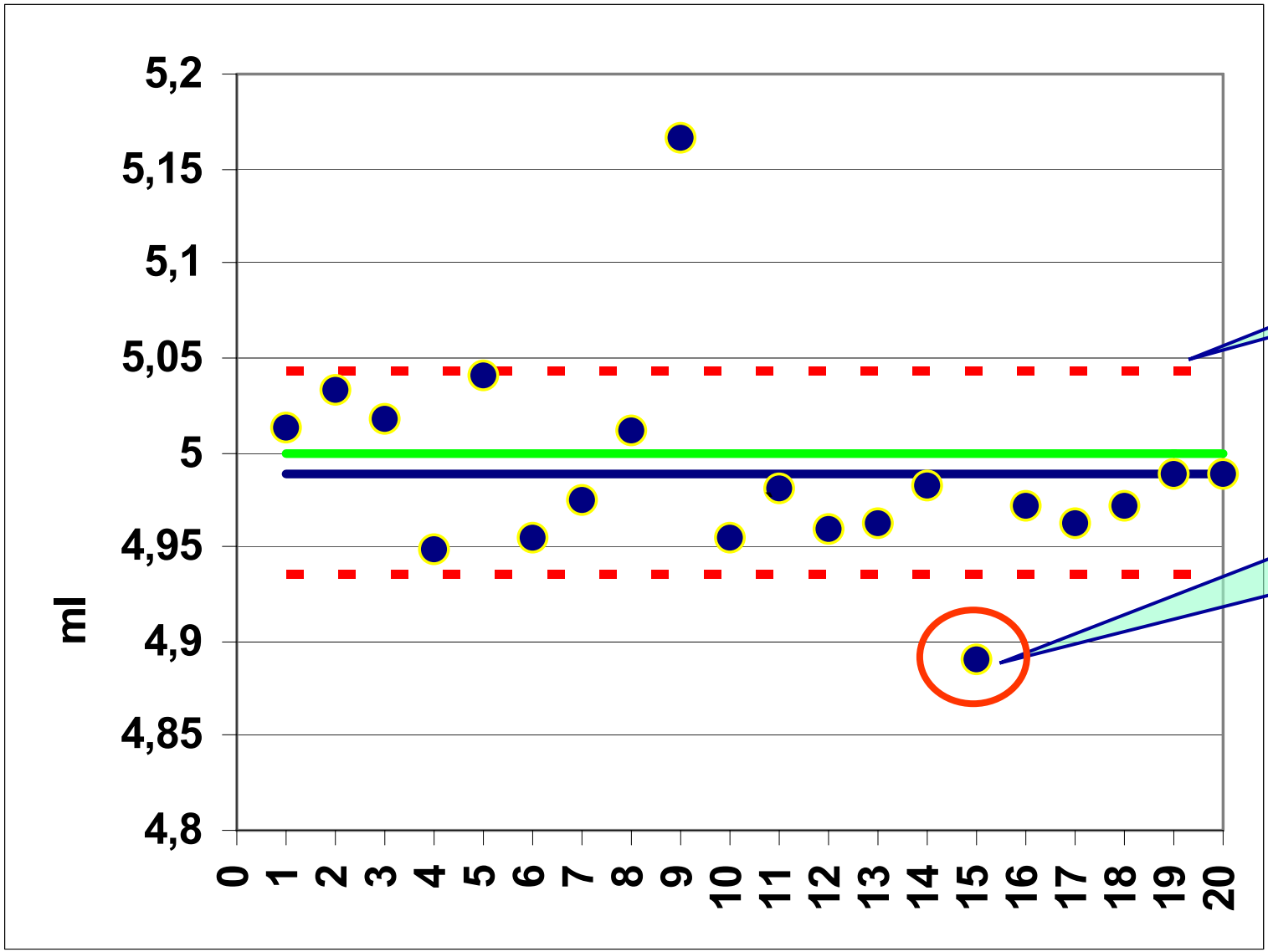
Degree of freedom

Mean squeres

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	26.2	14	1.87	1.34	0.207	1.83
Within Groups	104.8	75	1.40			
Total	131.0	89				

repeatability stdev	s_r	1.18	=sqrt(MSW)
reproducibility stdev	s_R	1.21	=sqrt(MSW+(MSB-MSW)/N)
			(n replicates)

Control charts



How to determine the interval ?

Is this an outlier ?

Outliers tests

Dixon's test – Q test

$$Q = \frac{|suspected - nearst|}{\max - \min}$$

Ако Q > Q_{tab} ⇒ OUTLIER

Grubbs' test – G test

$$G = \frac{suspected - \bar{X}}{S}$$

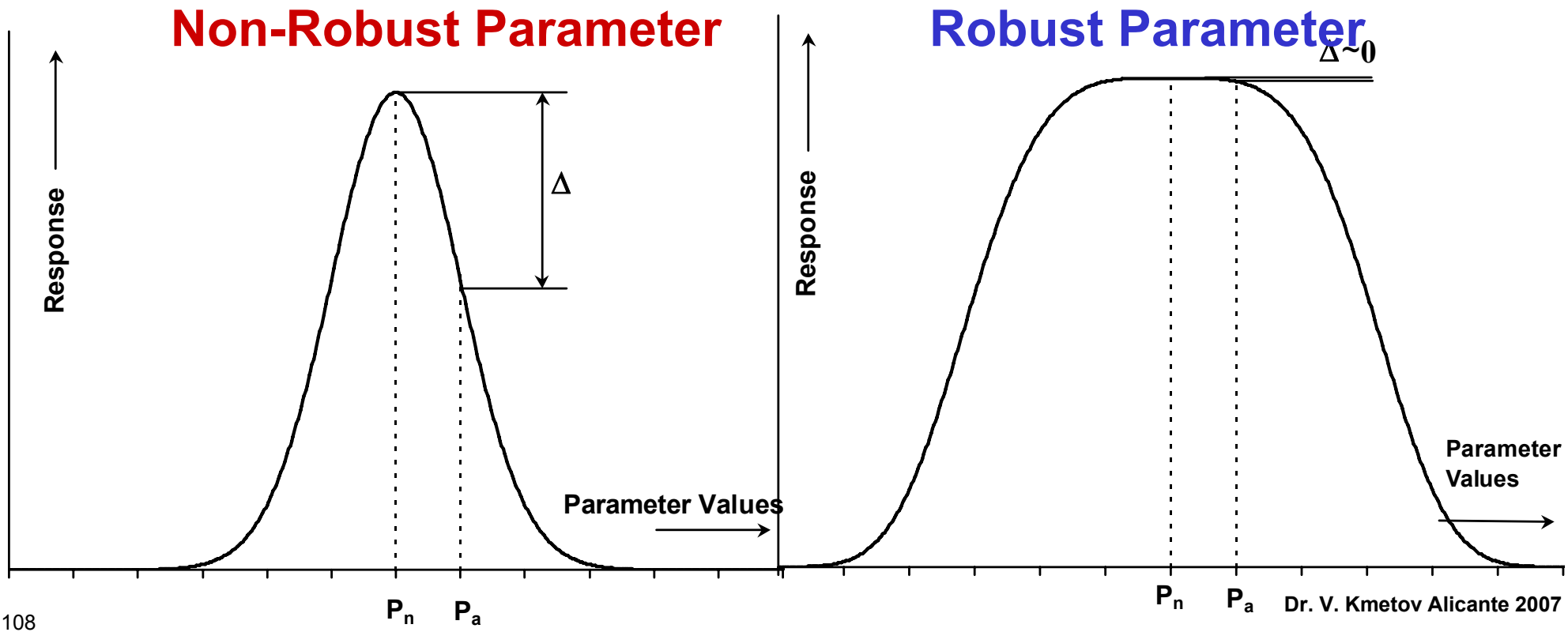
Ако G > G_{tab} ⇒ OUTLIER

Robustness (1)

The robustness (ruggedness) of the measurement procedure is the resistance to change in the result when minor deviations are made from the experimental conditions described in the procedure

Procedure prescribes the limits for experimental parameters

Examples: pH, temperature, concn. of reagent, operator, ...



Robustness (2)

- Identify variables of method: A,B, C, D etc
- Set-up experiments (Youden/Steiner)
- By systematic changing of one variable, determine effects on result (see table Y/S)
- Review the results to determine optimal conditions
- Procedure improvement from results obtained (gives also information on influence quantities)

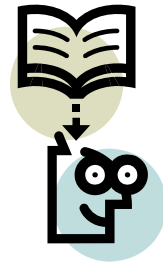
Experiment no.:		1	2	3	4	5	6	7	8
Parameters									
A	pH	5	5	5	5	7	7	7	7
B	temp.	25	25	35	35	25	25	35	35
C	reaction time	30	60	30	60	30	60	30	60
D	reagent 1	1	1	2	2	2	2	1	1
E	reagent 2	1	2	1	2	2	1	2	1
F	age of column	old	new	new	old	old	new	new	old
G	personnel	xx	yy	yy	xx	yy	xx	xx	yy
	Response	r	t	u	v	w	x	y	z

ANOVA:

A, B, and D are non-robust

- provide basic/preliminary information
- evaluate whether the model equation is valid
- better instructions for operators

Accuracy



Closeness of agreement between a test result of a measurement and the true value of the measurand (accepted reference value)

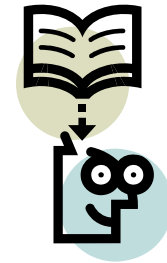
(ISO 3534-1)

Accuracy is not given by the spread of a normal distribution, but by the deviation of the arithmetic mean of a series of results from accepted reference value

Accuracy ↗ ⇒ Deviation ↘ (zero)

Where to obtain the reference value?

Definitions



Inter-Laboratory Comparison (ILC) -
Organisation, performance and evaluation of tests on the same (or similar) items by two or more laboratories in accordance of predetermined conditions

Proficiency Testing (PT) -
Determination of laboratory testing performance by means of inter-laboratory test comparison.

[ISO/IEC Guide 43:1997]

Goals of ILC

- ILC to demonstrate competence and establish degree of equivalence between results and of the participating laboratories
- ILC used for assign certified value to RMs
- ILC to standardise/impove a method *(determine repeatability, reproducibility ...)*
- ILC as a training exercise to improve skills

Why participate?

It is needed from the ISO/IE 17025

'trust is nice, proof is better"

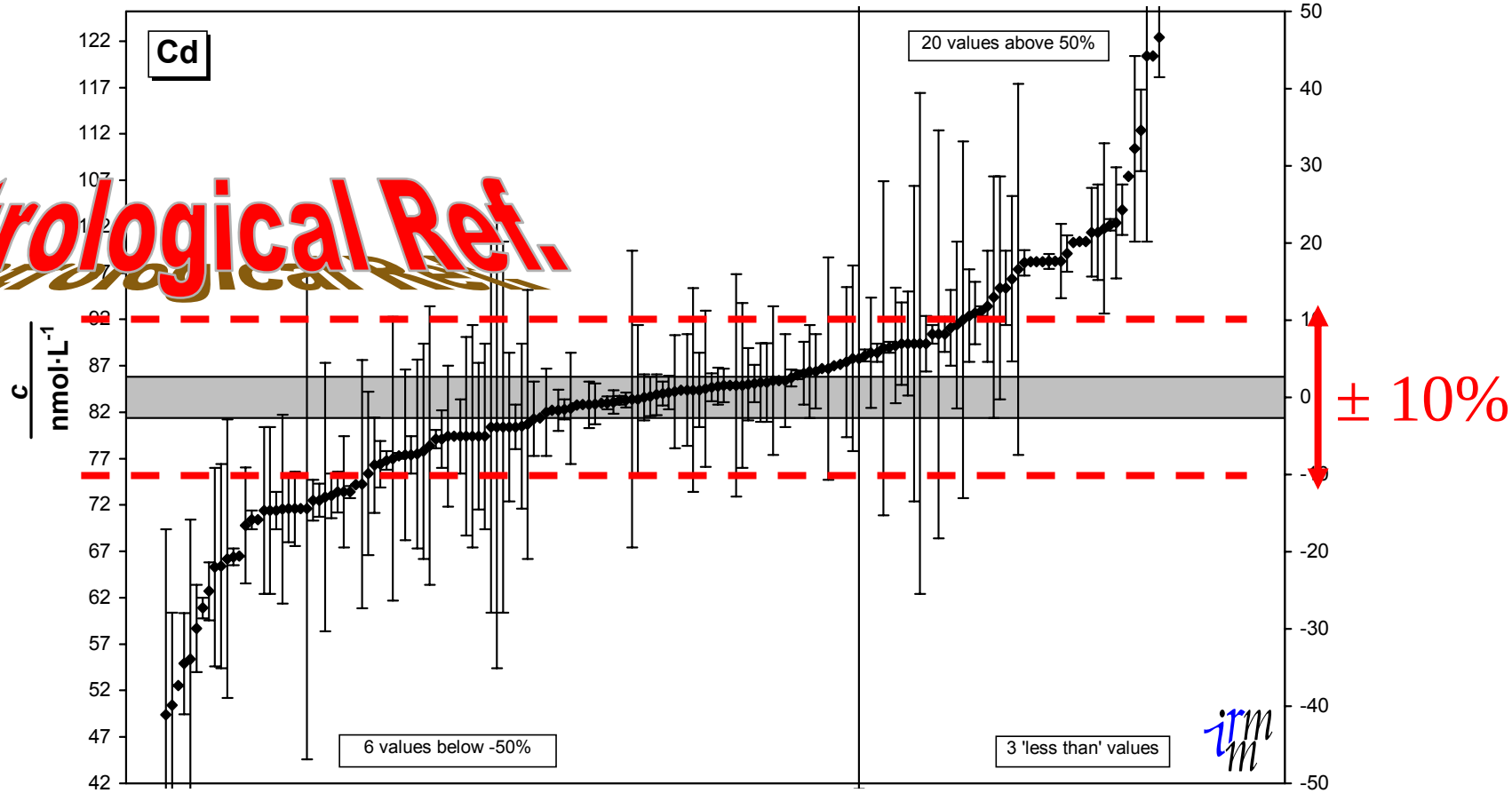
- To demonstrate competence :
  - to yourself (inside the lab)
  - to your direct customers
  - to 3rd parties (e.g. accreditation)
- To improve measurement skills (education aspect)

just a snapshot

Guide to the Accreditation Bodies (EN 45003:1995, § 6.8.1):
"Laboratories shall be encouraged by accreditation bodies to participate in PT or other ILC."

IMEP- 9 : Trace elements in Water

Certified range ($\pm U = 2u_c$): 81.0 - 85.4 nmol·L^{-1}

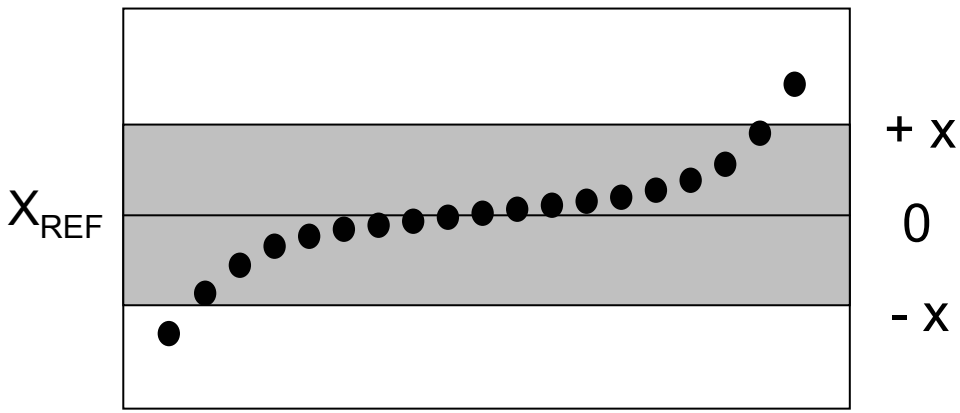


Metrological Ref.

Results from all laboratories.

Performance Indicators

- **Percent Error**; $\%E = (x_{lab}-x_{ref})/x_{ref}$
- **Z-scores**; $Z = (x_{lab}-x_{ref})/s$
- **En numbers;** $En = (x_{lab}-x_{ref})/(u_{lab}^2+u_{ref}^2)^{½}$



Performance evaluation, P:

P ≤ x, Satisfactory

P >x,Unsatisfactory

(Traditional) Z-score

$$Z = \frac{x_{lab} - x_{ref}}{"s"}$$

Difference ➔ distance ➔ accuracy

"Normalized" versus ...

- Target performance *(i.e. 5%)*
- Reference uncertainty (nominal value)
- Inter-Laboratory Comparison reproducibility

Performance evaluation:

0 <|Z|< 2 : good

2 <|Z|< 3 : warning ➔ preventive action

|Z|> 3 : unsatisfactory ➔ corrective action

En-score according to GUM

$$En = \frac{x_{lab} - x_{ref}}{\sqrt{(u_{lab}^2 + u_{ref}^2)}}$$

"Normalized" versus ...

propagated combined uncertainties

Performance evaluation:

0 <|En|< 2 : good

2 <|En|< 3 : warning ➔ preventive action

|En|> 3 : unsatisfactory ➔ corrective action

Improving the infrastructure for metrology in chemistry in the candidate and New Member States Qua-NAS

The ultimate goal of validation

A well-defined and documented validation process provides regulatory agencies with evidence that the system and method are suitable for their intended use.

LOGO

Validation Report

- Procedure: Cadmium determination by GF-AAS
- Measurand: Cadmium concentration in food products
- Source of the Method: Developed in-house
- Intended Use: Screening of food samples
- Matrix: food and feed products
- Analytical protocol: Microwave digestion, followed by GF-AAS
- Calibration: with solution standards from Supplier-ZZZ
- Working Range: up to 20 ng/g
- LOD: 1 ng/g
- LOQ: 3.5 ng/g

Selectivity: Free from interference up to 1000 ng/g of Chloride

- Traceability: to SI.
  Established by calibration
  Demonstrated by measurement of certified reference material-YYY
- Typical Uncertainty: 10%, see Uncertainty budget (Annex)

Name		
Function	*Laboratory Assistant*	*Head of Laboratory*
Signature		
Date		

Traceability of the results

Validation *(method fit-for-purpose)*

Traceability

(my result is comparable - common reference)

Uncertainty Budget

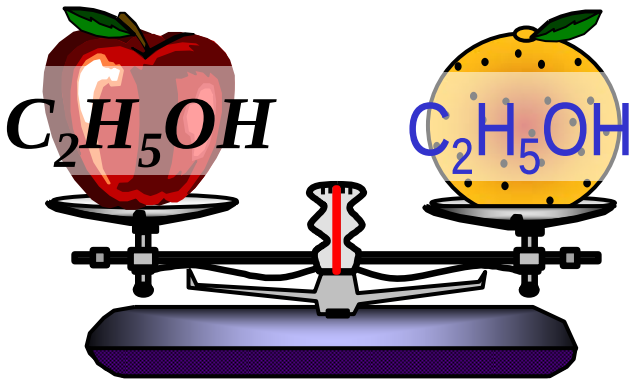
(How well I know the result)

Scope of the lecture

- What is Traceability?
- What is it needed for?
- How to establish Traceability?
- How to demonstrate Traceability?
- How CRM should be used?

The goal of the measurement is to assign a value to an unknown quantity

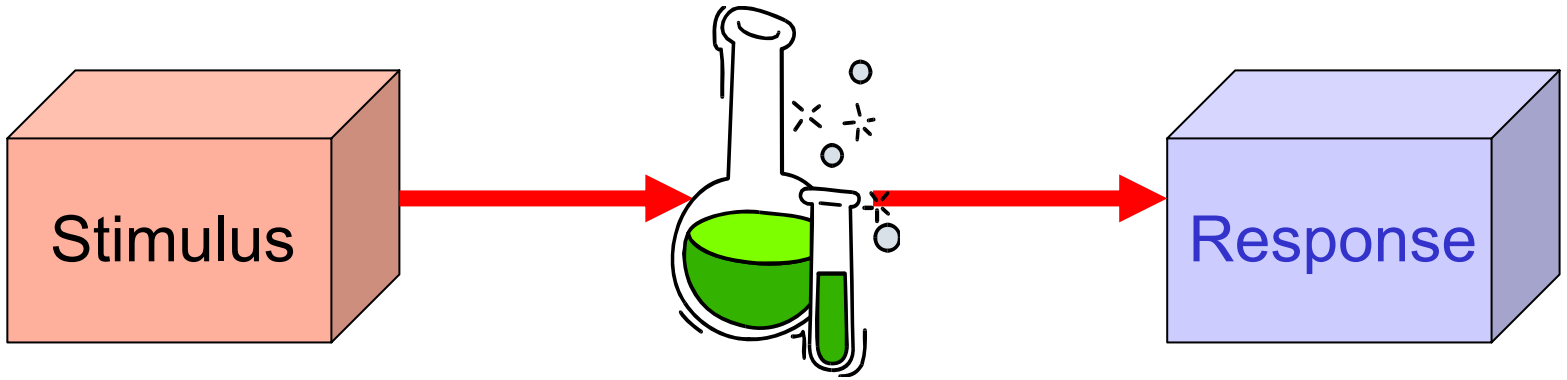
THE ANALYSIS IS COMPARISON !



The result of the chemical measurement is little more than a DECLARATION of an isolated figure

until /or someone does not prove the contrary !

The analysis concept



Energy

Object

Analytical information

MEASURAND

=

STANDARD

Variable

SIGNAL

=

SIGNAL

Variable

Traceability of ...

Not concerned by:

- ☒ … sample in the lab
- ☒ … documents in a filing system

trackability

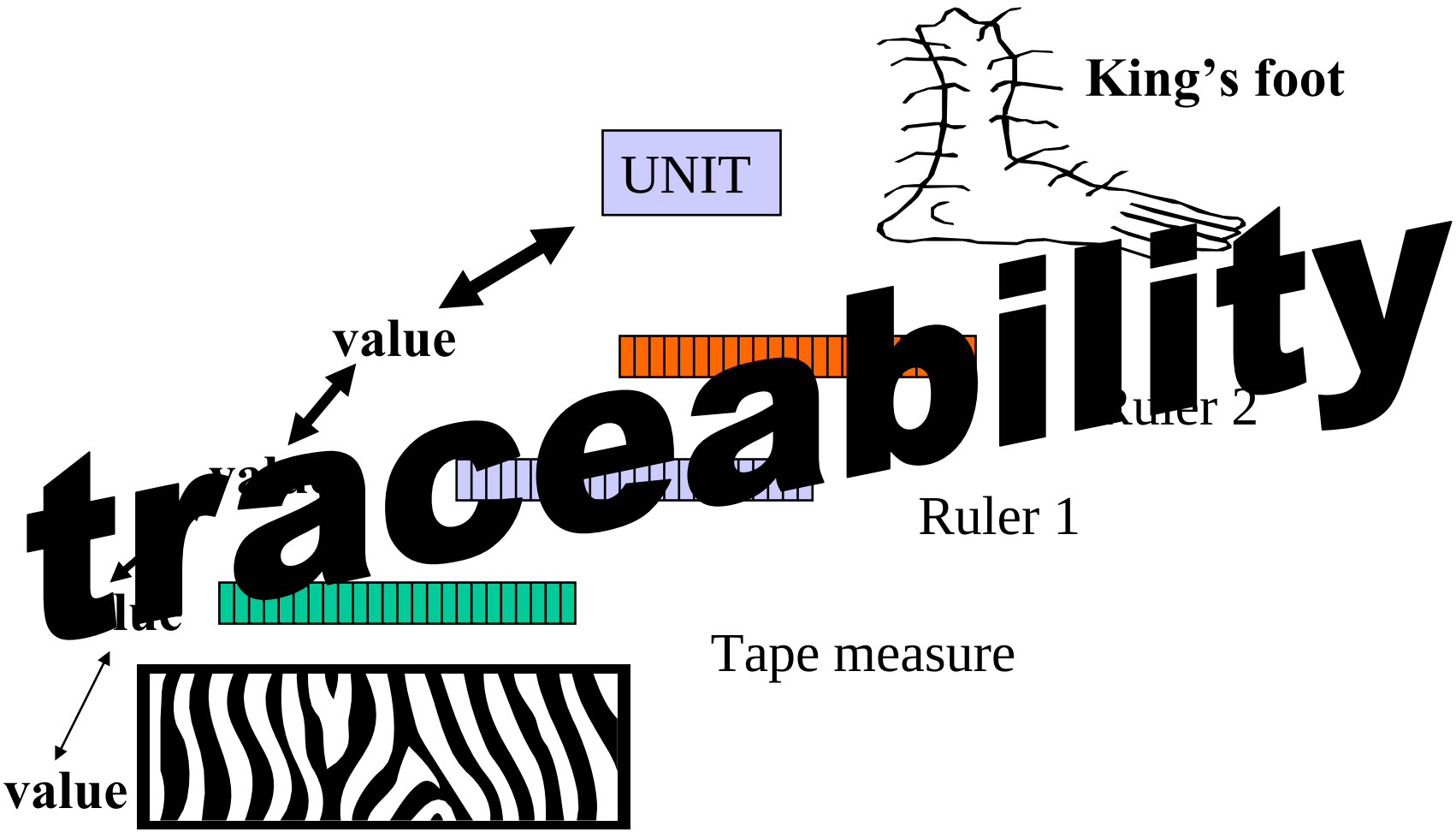
Not applicable to:

- ☒ … institution
- ☒ … method

Relevant for

- ☑ … measurement results
- ☑ … reference values

LENGTH MEASUREMENT



Definition

A posteriori

Traceability is a property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties.

[VIM, 6.10]

A priori

1
2
3
4

compare your result to stated reference

LINKED TO THE OUTSIDE WORLD

traceability !

Associativity of Comparability

The comparison is always done locally

C_1

St1

Le Système international d'unités
The International System of Units
SI

C_2

St2

NS_0

PS_0

SI

Certificate 3
$Y \pm U$

Certificate 2
$Y \pm U$

Certificate 1
$Y \pm U$

Are C_1 & C_2 Comparable?

Stated reference units

Meaningful comparisons between measurements are only possible if the results are expressed in the same units (measurement scale)

- SI units *(m, kg, s, A, K, mol, cd) or combination*
- to best internationally agreed reference (if no SI), such as:
  - ✓delta scale for isotopic measurements
  - ✓ pH scale
  - ✓ the scale of octane numbers for petroleum fuel

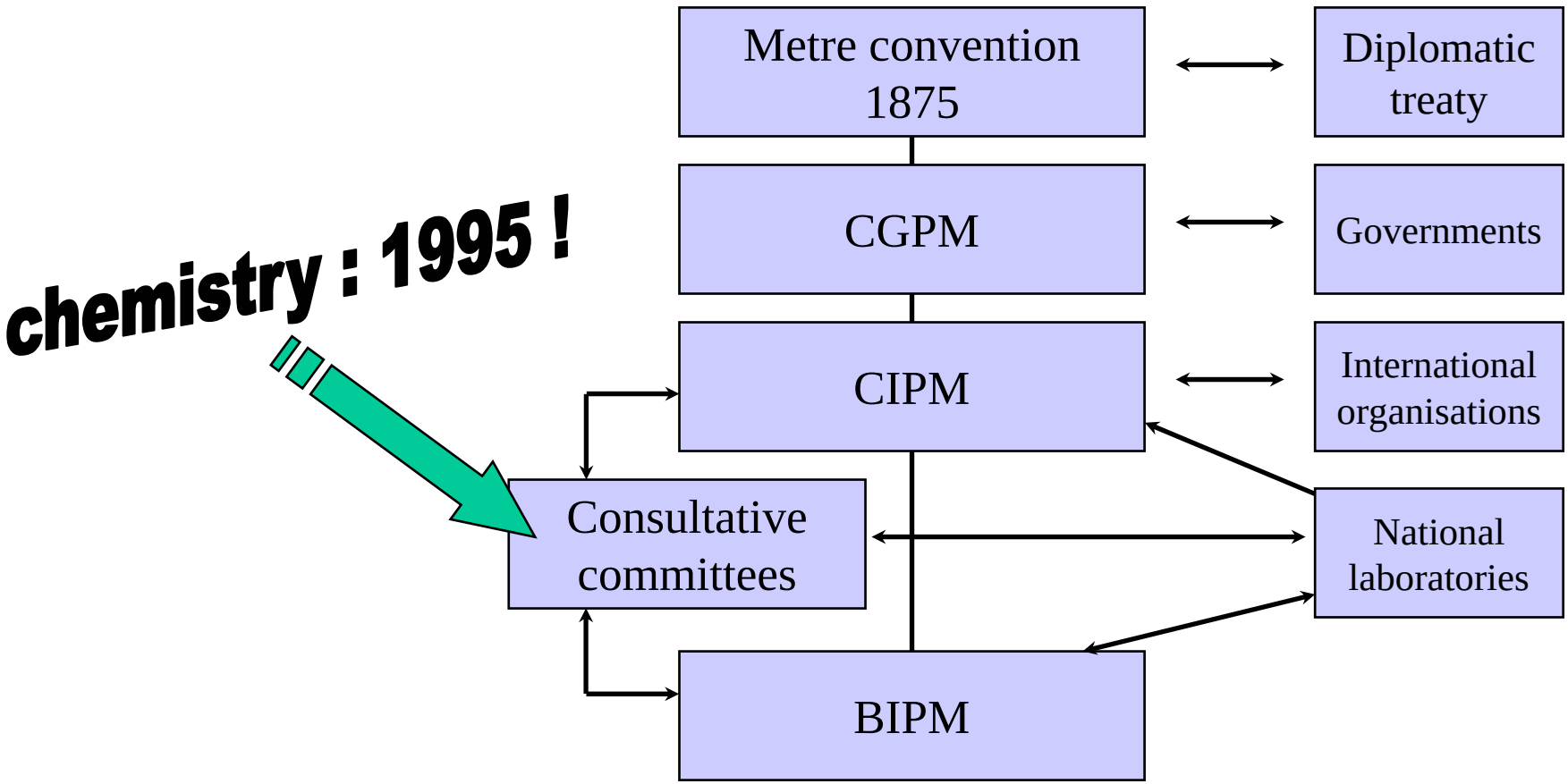
International System of Measurements : the SI

The system was created because of ‘compelling’ forces in trade & economy
It is valid worldwide and is constantly maintained and improved by the BIPM to ensure that results are compatible and the values are anchored.

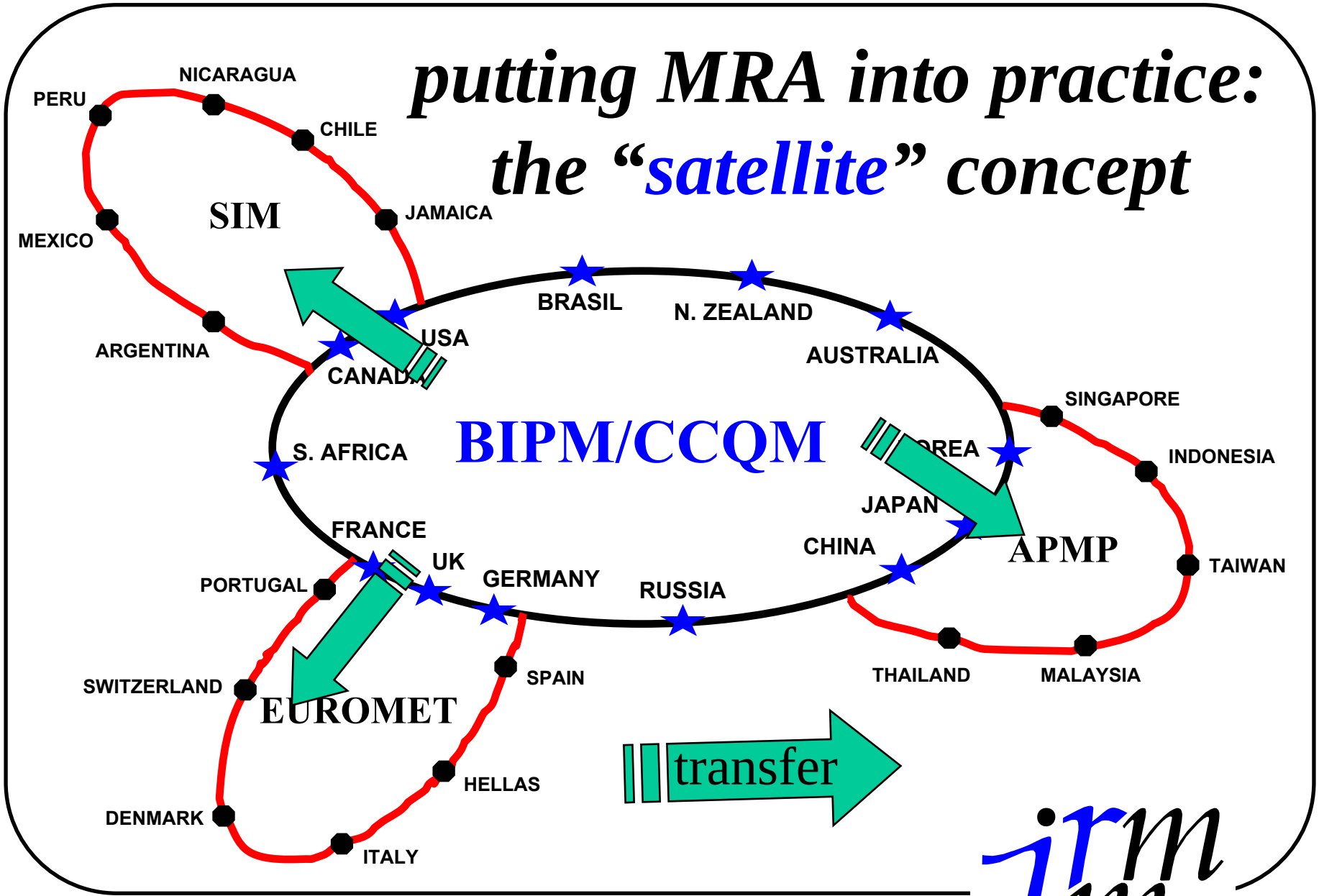
Advantage of SI traceability ?

- **traceability is needed to be able to compare, so the traceability acts as a tool to obtain equivalence**
- **values are independent of time**
- **values are independent of place**

Organising measurements on an international scale



putting MRA into practice: the "satellite" concept



Consultative committee for amount of substances

Stating ... is a claim

&

Establishing ... is what I do in my lab

&

Demonstrating ... and I can show it

... Traceability

Establishing traceability

① Specifying the measurand

② Choosing a suitable

- measurement procedure
- model equation

③ Demonstrating (validation) that:

- the model equation is adequate (all significant influence quantities have been taken into account)
- the measurement conditions are adequate

④ Establishing traceability for each influence quantity:

- Choosing appropriate reference standards
- Calibrating using the standards that are traceable

⑤ Evaluating uncertainty

[EURACHEM/CITAC Guide, 2002]

Demonstrating Traceability

① measurand: content of nitrate in plants

② suitable model equation

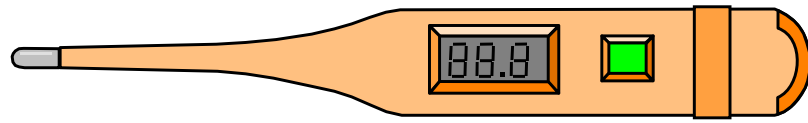
③ validation (ILC, CRM)

④ traceability

⑤ evaluate uncertainty

Wageningen Agricultural University/Department of
Soil Science and Plant Nutrition

certificates of manufacturer



$$w_{NO_3^-} = C_{st} \frac{A_{NO_3^-} \cdot V_{NO_3^-}}{A_{st} \cdot m} \times f_{di} \times \frac{1}{R}$$

calibration (solution standard)

gravimetry

using matrix CRM (determining R)

Establishing Traceability procedure ④

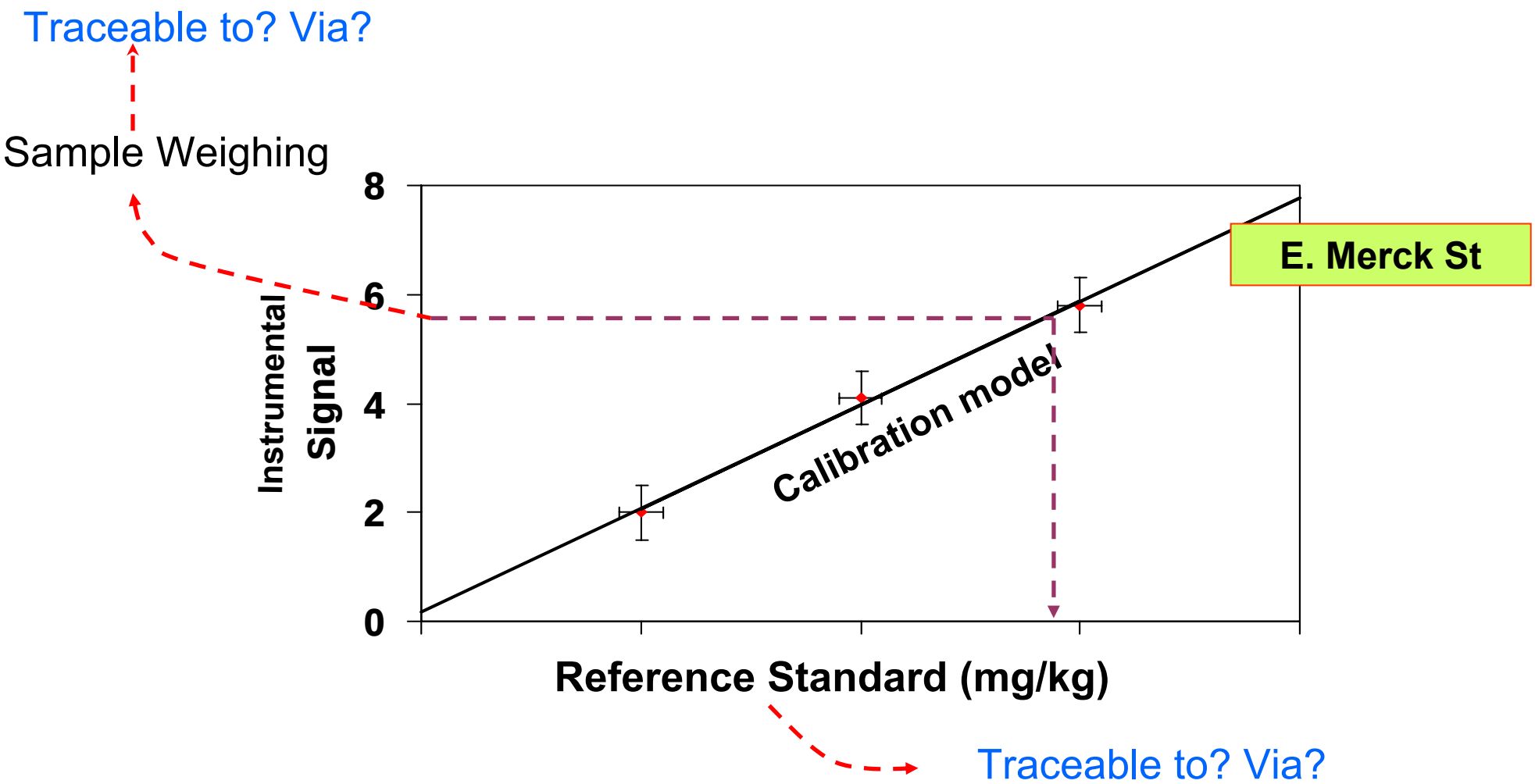
Traceability to be established for each input quantity specified in the procedure / model equation

- established by calibration using appropriate standards

Calibration:
Set of operations which establish, under specified conditions, the **relationship between values indicated** by a measuring instrument - (including chemical steps) **and** the corresponding **known values** of the **measurand**.

Must be performed by reference standards with demonstrated traceability and adequately small uncertainty.

Result Traceable to Value of Reference Standard



You certainly heard about...

✓Standards
- Primary and Secondary standard
- International (measurement) standard
- National standard
- Calibration standard
- Measurement standard

✓Reference Materials (CRM, SRM, ...)
- Primary and Secondary RM
- Laboratory RM
- Internal, “in-house” RM
- Matrix RM

Traceability should be established by:

1. Use of traceable standards to calibrate the measuring equipment

2. By using, or by comparison to the results of, a primary method

3. By using a pure substance RM.

4. By using an appropriate matrix Certified Reference Material (CRM)

5. By using an accepted, closely defined procedure.

QUAM (3.3.4)

primary method is a method having the highest metrological qualities, whose operation is completely described and understood in terms of SI units and whose results are accepted **without reference to a standard** of the same quantity.

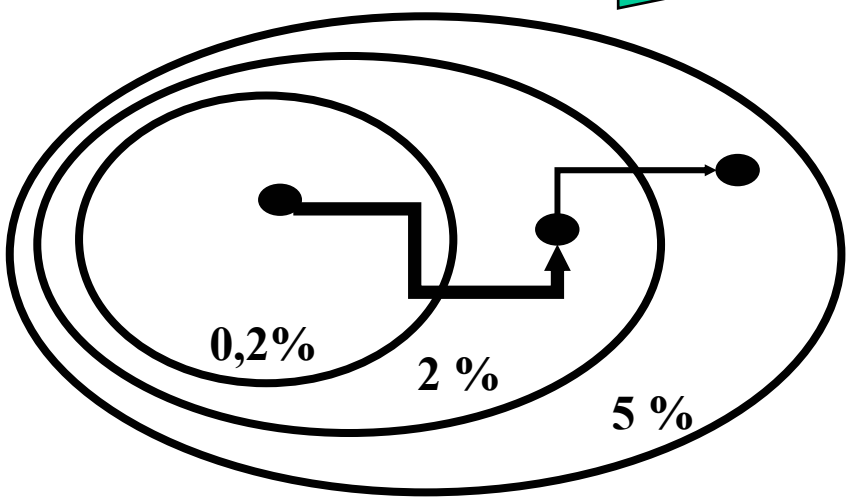
Calibration Hierarchy

Tools

Metrological Traceability

- Primary Std
- International Std
- National Std
- Reference Std
- Transfer Std
- Travelling Std
- Working Std

uncertainry



Service Providers

- BIPM
- Nat. Metrology Institutes
- Accredited Calib. Labs
- Company (in-house)
  - calibration centre
  - test laboratory

Uncertainty

[ILAC-G2:1994]
Traceability of Measurements

Commare your results with CRM conected with the outside world!

Definition *According to VIM*

Reference Materials (RM),

material or substance one or more of whose properties are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials

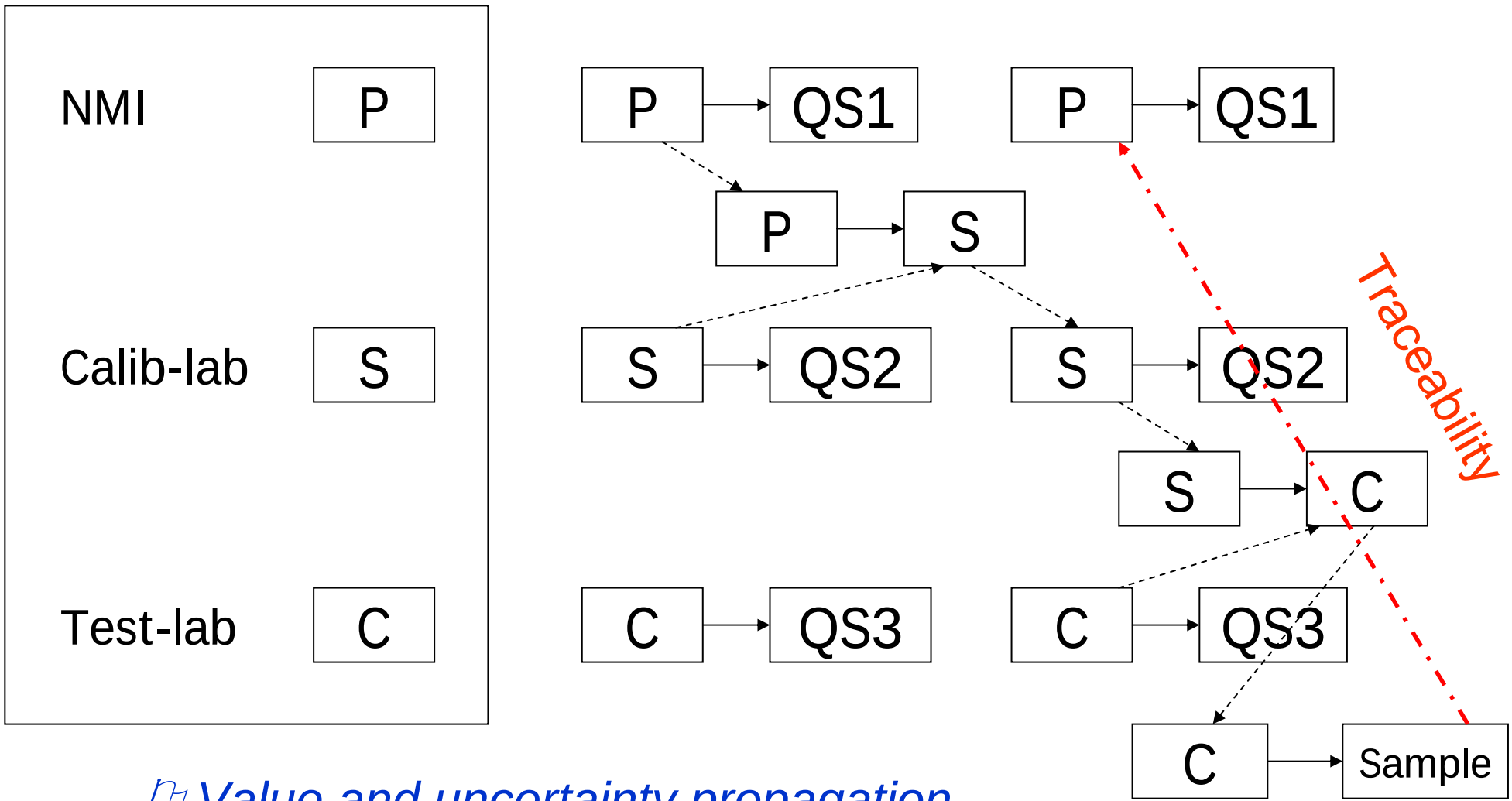
Certified Reference Materials (CRM)

reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence

Matrix (compositional) Reference Material (CRM)

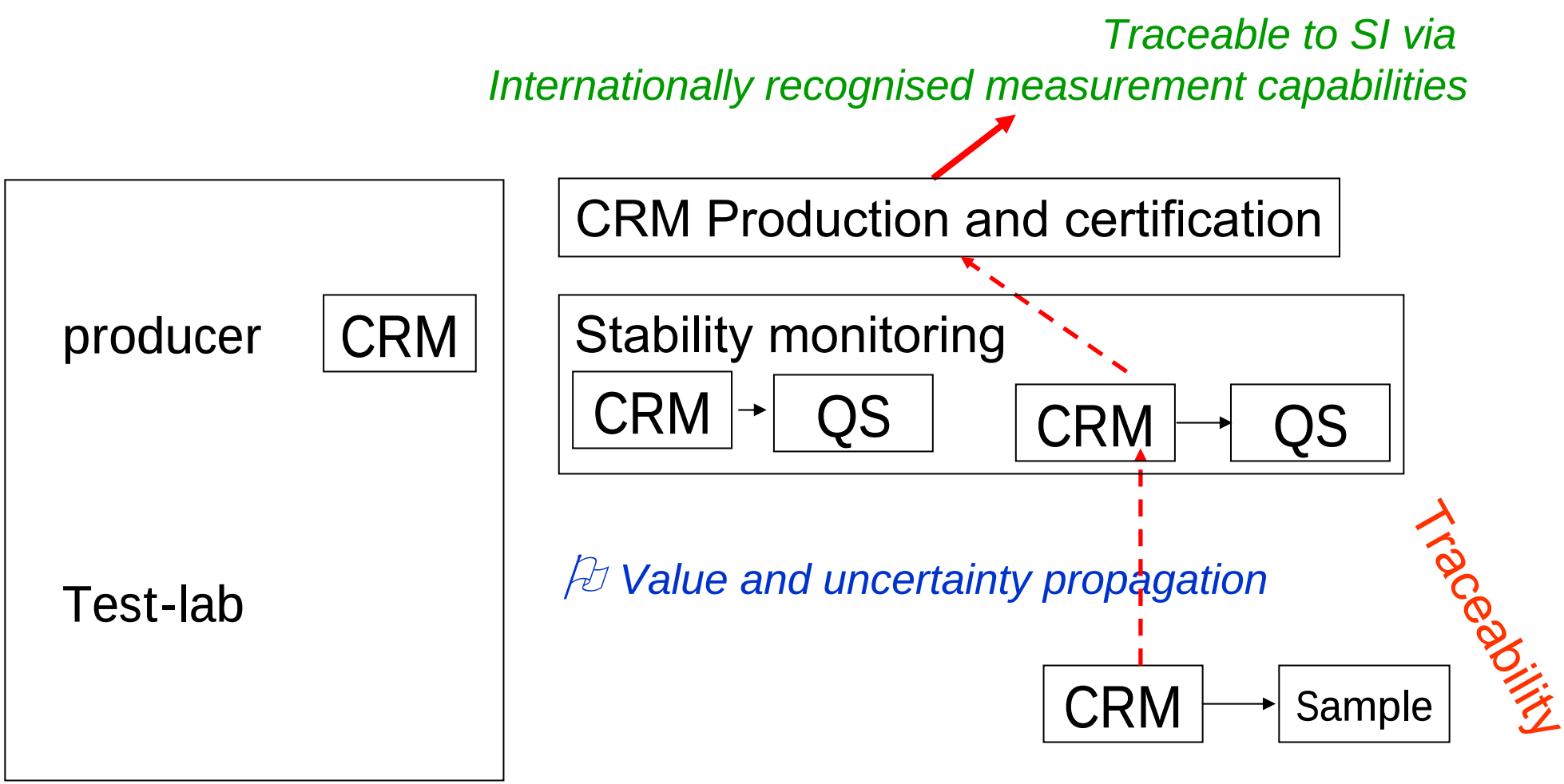
A “natural” substance more representative of laboratory samples that has been chemically characterised for one or more elements, constituents etc. with a known uncertainty

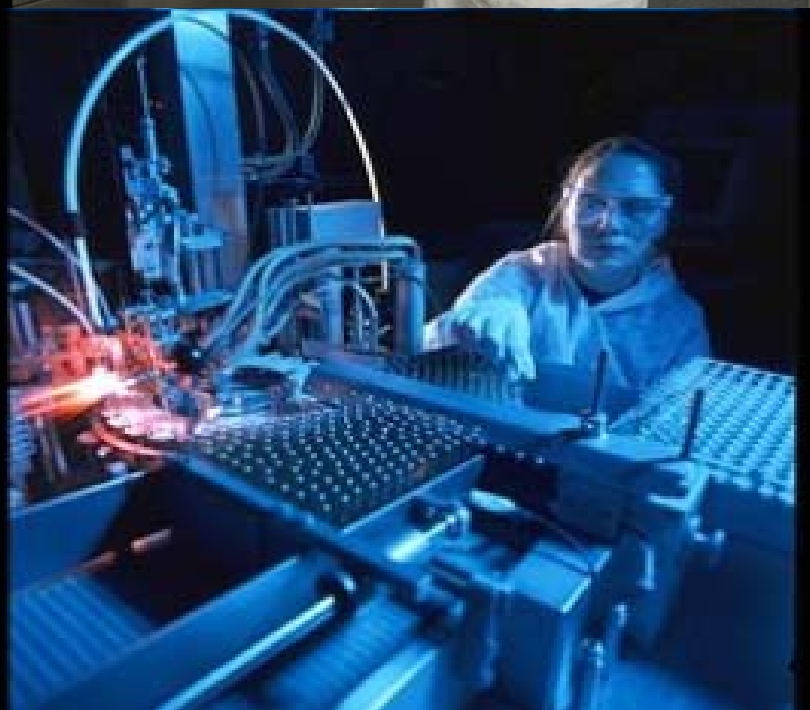
Traceability Timeline



- *Value and uncertainty propagation*

Traceability Timeline





Sli

A high quality (C)RM should :

- State traceability of certified value
  (e.g. traceability to S.I., or to values obtained with method XYZ)
- State an ISO-GUM uncertainty of certified value
- Demonstrate traceability & uncertainty of certified value *(e.g. in a certification report ; experimental evidence of demonstrated capability from participation to international intercomparisons such as those from BIPM)*
- Produced according to ISO-35 and ISO-34 (preferably)

OK, we can rely on (C)RMs

inorganic ventures / iv labs

195 lehigh avenue, suite 4, lakewood, nj 08701 usa
phone: 800-669-6799 • 732-901-1900 • fax: 732-901-1903
e-mail: ivsales@ivstandards.com • website: www.ivstandards.com

certificate of analysis

1.0 **Inorganic Ventures / IV Labs is an ISO Guide 34-2000 Certified Reference Material (CRM) Manufacturer: Certificate #883-02.** *The certificate is designed and the certified value(s) and uncertainty(ies) are determined in accordance with ISO Guide 31-2000 (Reference Materials - Contents of certificates and label(s), ISO Guide 34-2000 "Quality System Guidelines for the Production of Reference Materials," and ISO Guide 35-1989 "Certification of Reference Materials - General and Statisical Principles."*

2.0 DESCRIPTION OF CRM Custom-Grade **10000 µg/mL Lead in 0.35% (abs) HNO_3**

Catalog Number:	CGPB10-1 and CGPB10-5
Lot Number:	**T-PB02113**
Starting Material:	Pb(NO3)2
Starting Material Purity (%):	99.99997
Starting Material Lot No	22150
Matrix:	0.35% (abs) HNO_3

3.0 CERTIFIED VALUES AND UNCERTAINTIES

Certified Concentration: 10,009 ± 22 µg/mL

Certified Density: 1.014 g/mL (measured at 22° C)

The Certified Value is based upon the most precise method used to analyze this CRM. The following equations are used in the calculation of the certified value and the uncertainty:

Certified Value ($\bar{x}$) = $\frac{\sum x_i}{n}$

Uncertainty (±) = $\frac{2[(\sum s_i)^2]^{1/2}}{(n)^{1/2}}$

($\bar{x}$) = mean
x_i = individual results
n = number of measurements
$\sum s_i$ = The summation of all significant estimated errors. (Most common are the errors from instrumental measurement, weighing, dilution to volume, and the fixed error reported on the NIST SRM certificate of analysis.)

The independent samples t-test was used to determine if there is agreement between the above assay methods at the 95% confidence interval. Both methods were compared and showed agreement within the stated uncertainties. This agreement is a confirmation of the accuracy of this CRM.

4.0 TRACEABILITY TO NIST AND VALUES OBTAINED BY INDEPENDENT METHODS

structured measurement system

The new challenge in the global measuring world

THE MUTUAL RECOGNITION ARRANGEMEN

once measured

all measurements

accepted everywhere

Mutual recognition
of national measurement standards
and of calibration and measurement certificates
issued by national metrology institutes

Paris, 14 October 1999

Comité international des poids et mesures

Bureau international des poids et mesures	Organisation intergouvernementale de la Convention du Mètre

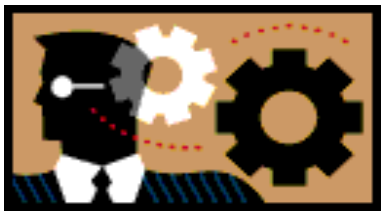
It is easy to say, but is difficult to be accomplished !

Analysis of Gold Alloys by FAAS

TrainMiC
Training in Metrology in Chemistry

Jamboree at JRC-IRMM 19-21 June 2006

Challenge of the best TrainMiC example



Let's do it together

Please, read the next slides and try to identify:

- What is the intended use?
- What are the parameters that must be considered for procedure validation?
- Which are the input quantities?
- How to build the uncertainty budget?

Introduction

- The jewellery gold alloys contain as major components Au, Ag, Cu and Zn, which precise determination is of great importance.
- The fineness of precious metal alloys are specified in **ISO 9202:1991**, according to the purity of gold as ‰: 375, 585, 750 and 916 (9, 14, 18 and 22 karats respectively).
  One karat is equal to 41,667‰
- The Au analysis has to keep <u>the expanded uncertainty (k=3)</u> **<u>less than 9 ‰</u>**
- The traditional internationally recognised method is based on cupellation (fire assay) ***ISO Standard 11426.***

Dr. V. Kmetov Alicante 2007

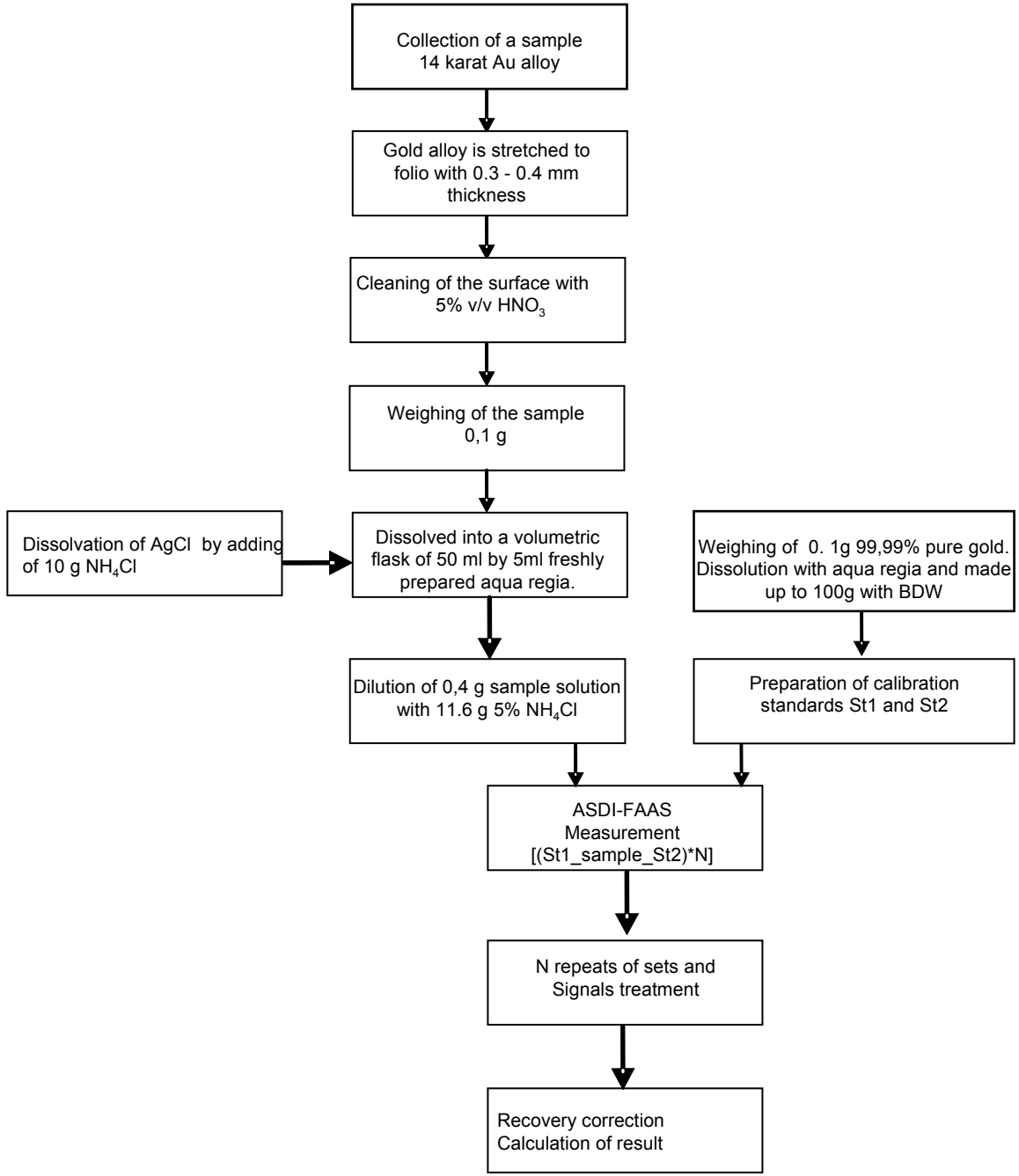
Procedure description

Gold alloy samples are stretched to folio with 0.3 - 0.4 mm thickness. The surface is washed by 5% v/v HNO_3. A dry piece of 0.1 g accurately weighted to $\pm$ 0,0001 g is directly dissolved into a volumetric flask of 50 ml with 5ml freshly prepared aqua regia.

The flask is heated on ceramic hot plate for 20 min. During this process Ag precipitates as AgCl. AgCl is dissolved by adding of 10 g NH_4Cl to the cooled solution and total weigh of solution is made up to 50 g with BDW at (20°C).

The solution is diluted additionally by transferring 0,400 g with micro-pipette to a conical vial adding 5% NH_4Cl in order to keep the solution homogeneous with final weight of 12,000 g gravimetrically controlled.

Procedural blank is subject to exactly the same sample preparation procedure as the analysed sample.


Collection of a sample
14 karat Au alloy
Gold alloy is stretched to folio with 0.3 - 0.4 mm thickness
Cleaning of the surface with 5% v/v HNO_3
Weighing of the sample 0,1 g
Dissolvation of AgCl by adding of 10 g NH_4Cl
Dissolved into a volumetric flask of 50 ml by 5ml freshly prepared aqua regia.
Weighing of 0. 1g 99,99% pure gold. Dissolution with aqua regia and made up to 100g with BDW
Dilution of 0,4 g sample solution with 11.6 g 5% NH_4Cl
Preparation of calibration standards St1 and St2
ASDI-FAAS Measurement [(St1_sample_St2)*N]
N repeats of sets and Signals treatment
Recovery correction Calculation of result

Uncertainty sources

1. Conversion of alloy to homogeneous solution and total amount of gold to be transferred into the solution
   - ***Use the trick with NH_4Cl***
2. Dilution factor
   - ***Use minimum steps***
   - ***Be sure that your (results) balances and glassware are traceable***
3. Standards for calibration
   - ***Provide standards with certificate (traceable)***
   - ***Make fresh standard from pure gold 99,99 certified***
4. Repeatability of absorption measurements
   - ***Work with the best S/N ratio according the scedastic curves***
   - ***Remove drift by standard-sample-standard sequence***
   - ***Apply signal smoothing and ensemble summation***

Instrumental conditions

Table 1 Instrumental parameters for ASDI-FAAS determination of Au

FAAS parameters	values	ASDI parameters
Au spectral line (nm)	242,8	Q_{l-} aspiration rate 6,4 ml/min checked by BDW
Au spectral slit	0,7	Injection time 5 s; Injection volume ≈ 0,530 µL
Au Hollow cathode lamp current	10 mA	Washing time 10 s; Total replicate time 15 s
Air/C_2H_2 units	50/18	Smoothing Savitzky-Golay 24 points
Observation high (mm)	6	Ensemble summation N signal profiles
Working range ppm	37 - 43	Pseudo plateau 3 s
Deuterium BG corrector	OFF	Sampling mode (St_1 _ sample _ St_2)* N
Readings – points/s	50	Total time for one set 66 s

1. Calibration standards

An initial Au standard solution C_Au_999,9 mg/L is prepared by dissolution of 0,1 g pure gold 99,99 % (certified from Non-Ferrous Metallurgical Plant Plovdiv) with 5 ml aqua regia filled up to 100 g with 5 % NH_4Cl.

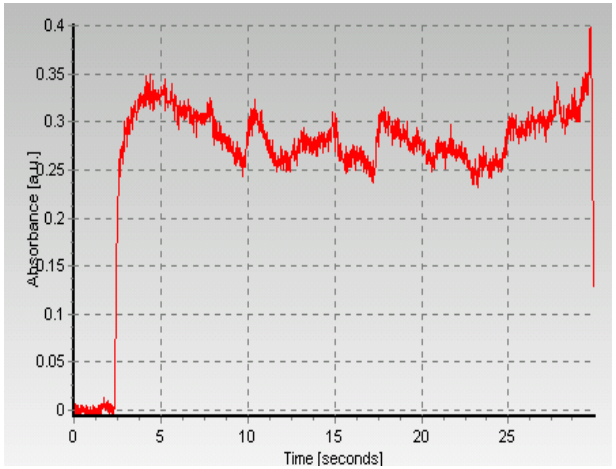
$$C_{Au\_999,9} = \frac{m\_pureAu * Au\_purity}{G\_100} * 10^4$$

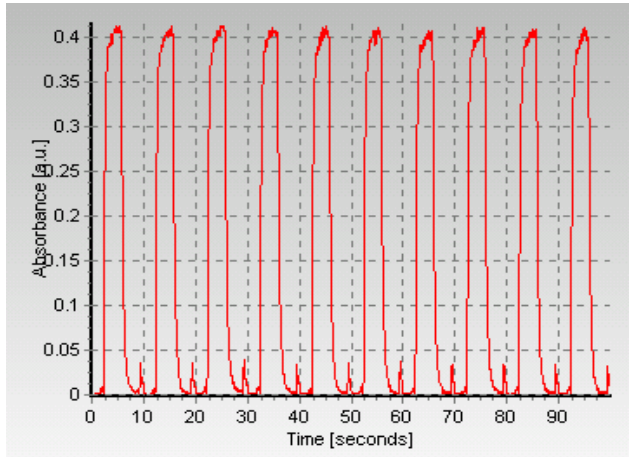
Two calibration standard solutions with concentration 37 and 43 µg/g respectively are prepared in 5 % NH_4Cl.Both calibration standards are obtained in polypropylene vials after further dilution of 0.370 g and 0.430 g from initial standard solution made up with procedural blank solution to 10.000 g (gravimetrically controlled).

$$C_{\_St1} = C_{Au\_999,9} * \frac{G_{p\_0,37}}{G_{\_10}}$$

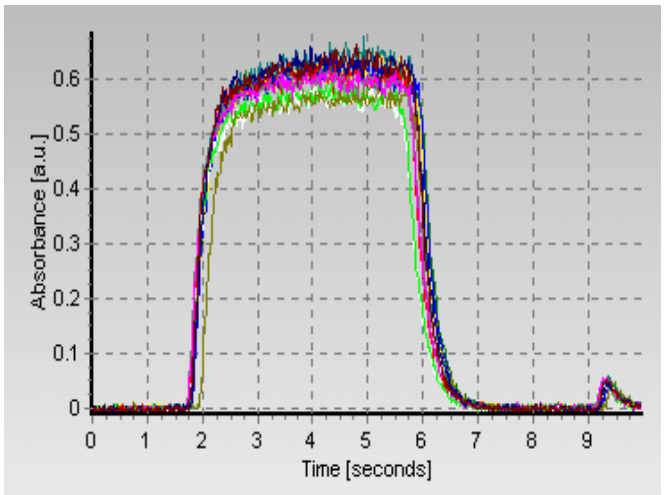
$$C_{\_St2} = C_{Au\_999,9} * \frac{G_{p\_0,43}}{G_{\_10}}$$

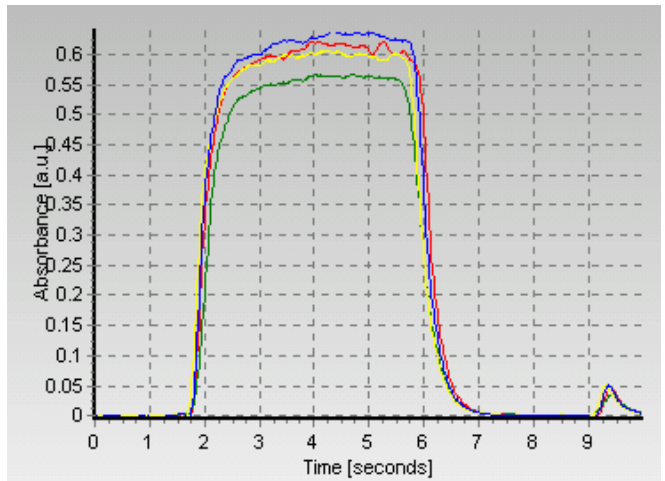
ASDI-FAAS



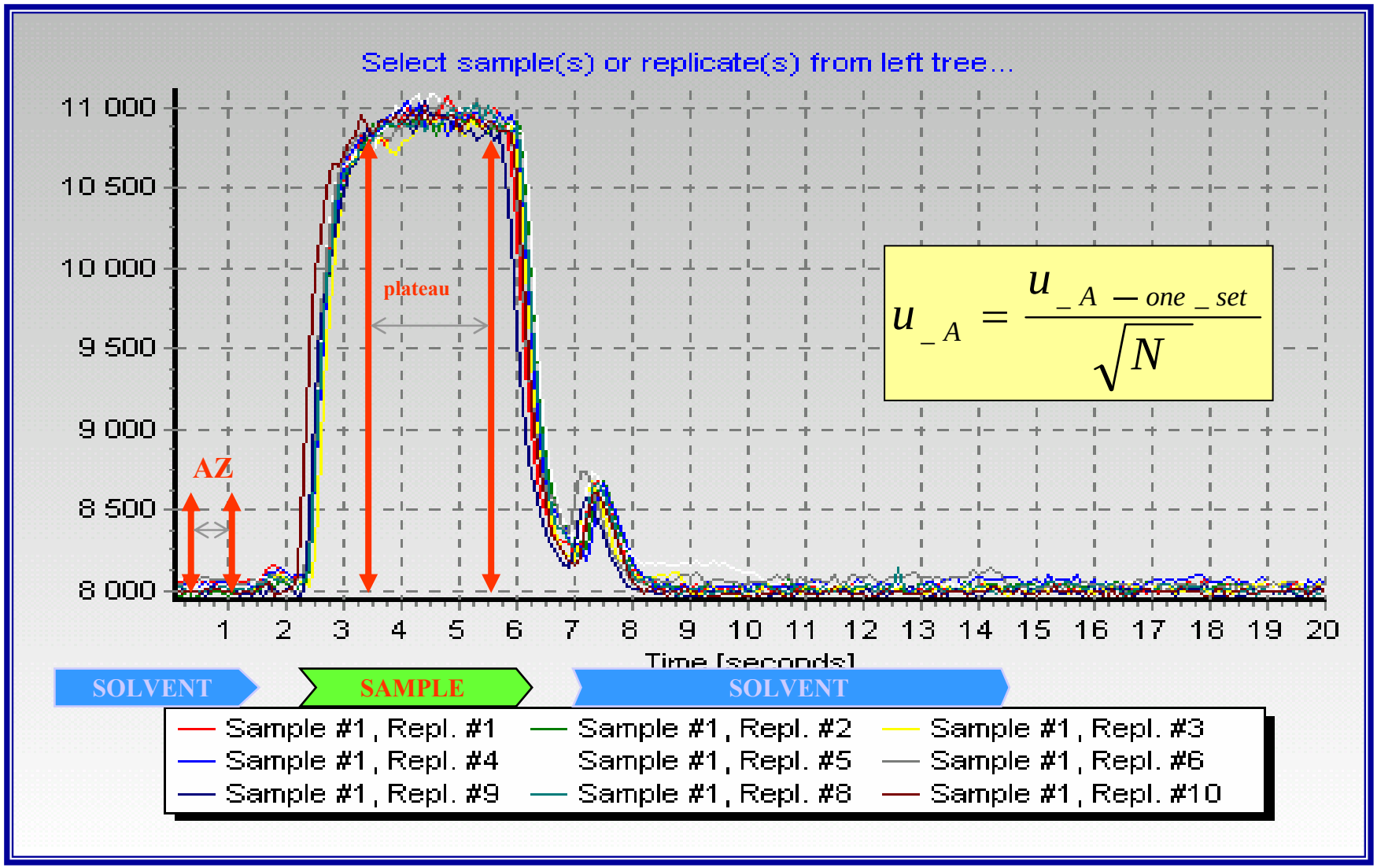


Air Segmented Discrete Introduction Flame Atomic Absorption Spectrometry (ASDI-FAAS)- way to improve signal-noise ratio



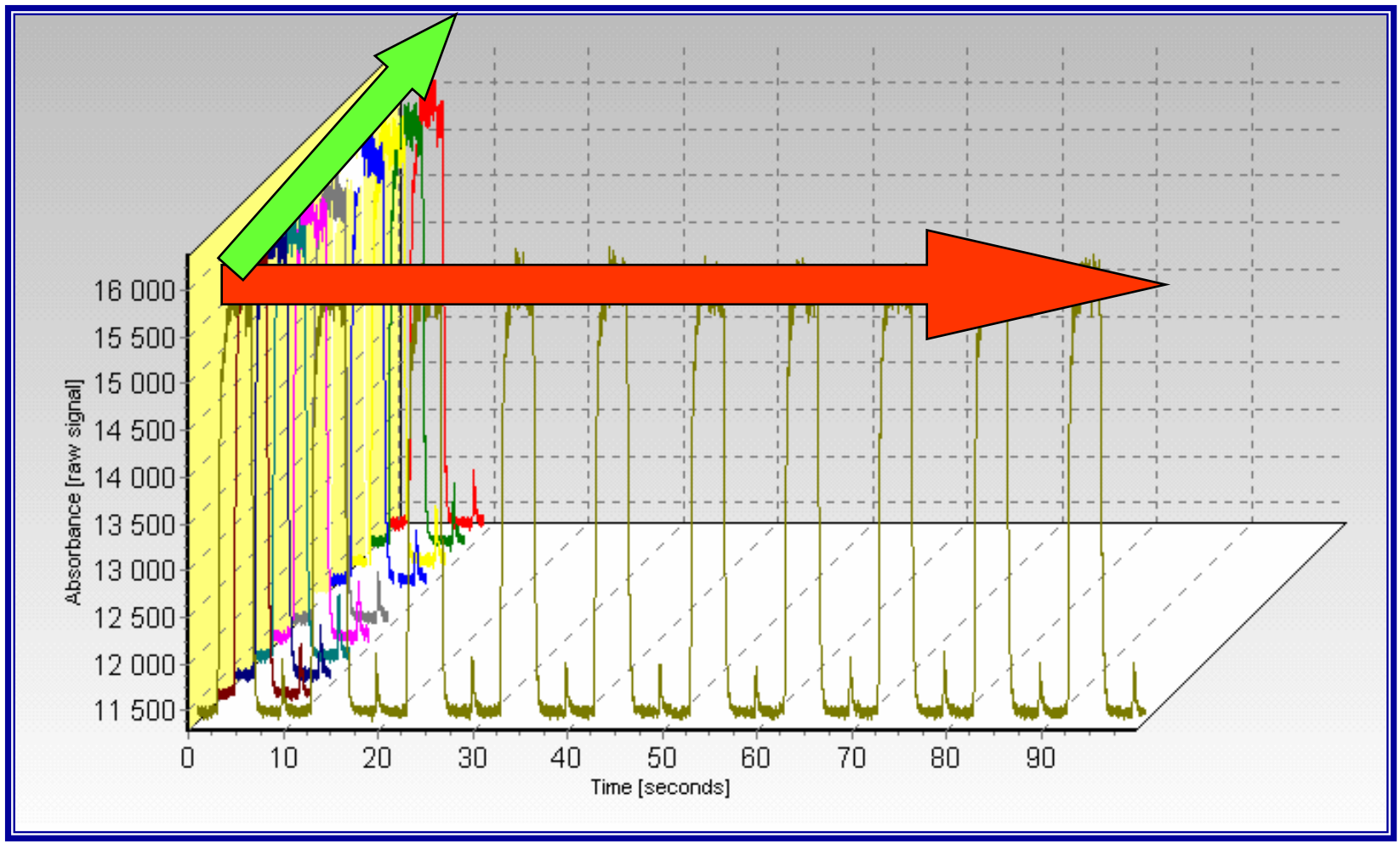


ASDI-FAAS
pseudo-steady state signals



$$u_{\_A} = \frac{u_{\_A\ \_one\ \_set}}{\sqrt{N}}$$

ASDI-FAAS
pseudo-steady state signals



2. Calculation of signal standard uncertainty

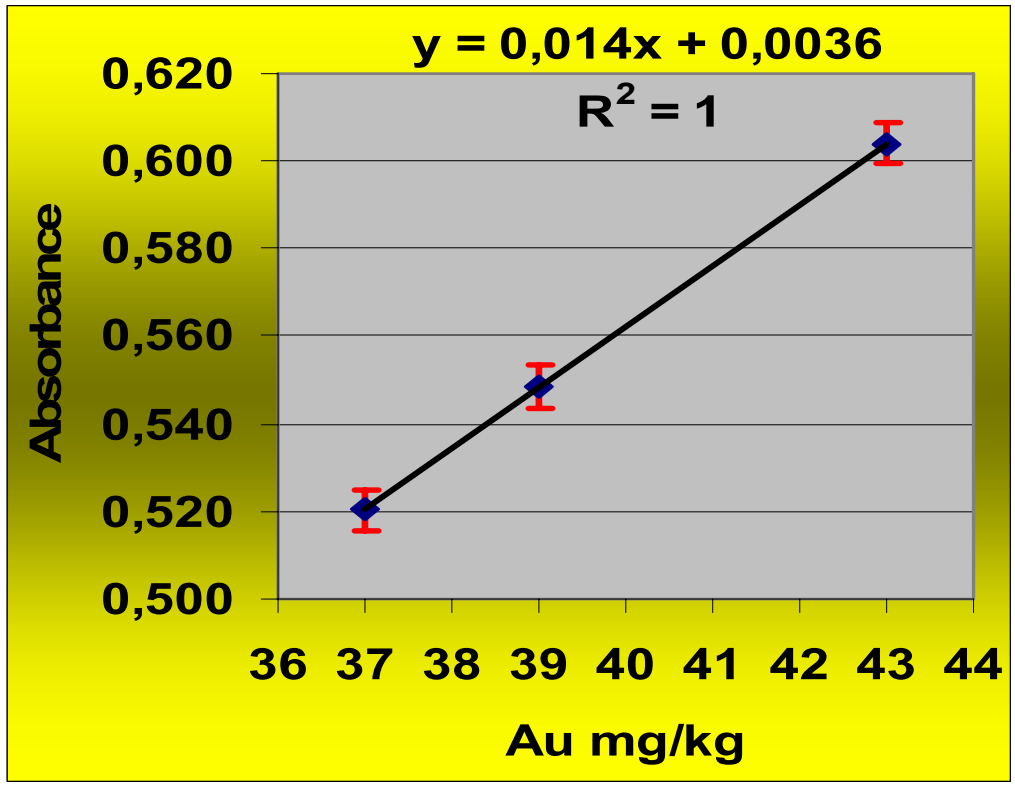
$$u_{\_A} = \frac{u_{\_A\ -one\ \_set}}{\sqrt{N}}$$

3. Bracketing calibration

- Two calibration standard solutions used are with Au concentration 37 and 43 mg/kg respectively in 5 % NH_4Cl

- The selected calibration range corresponds to the range 13.5 – 14.5 kt recalculated for analyzed alloy.

- It is proved that the S/N ration is minimum and the calibration interval belongs to the linear range



$$Cx = \frac{C_{-st1}(A_{\_St2} - A_X) + C_{-st2}(A_{-x} - A_{-St1})}{A_{-St2} - A_{-St1}}$$

5. Recovery correction

$$R = \frac{W\_\%}{W\_ref\_\%}$$

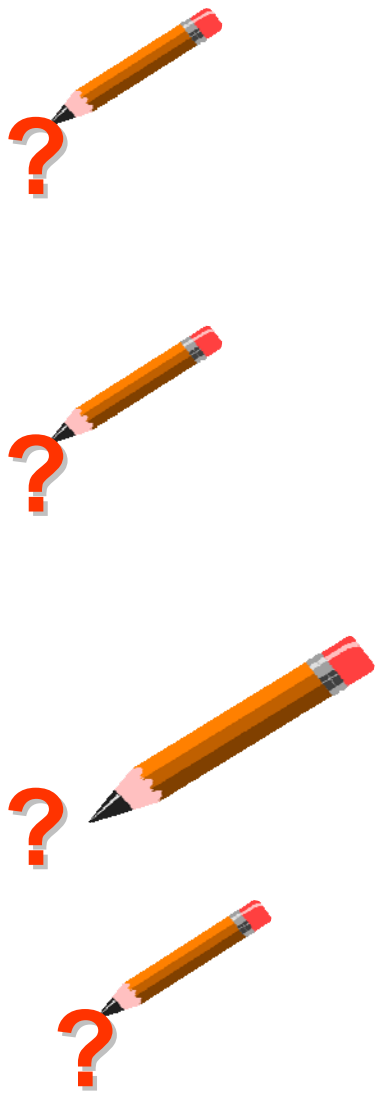
6. Gold content (‰) in jewellery gold alloys

$$W\_\%o = \frac{1}{1000} * \frac{V\_50}{m\_0,1} * \frac{G_{vials\ -12}}{G_P\_0,4}.C_x * \frac{1}{R}$$

The Validation Menu

The Validation Checklist

- ☒ selectivity/interference
- ☐ recovery
- ☒ linearity, working range
- ☒ LOD, LOQ
- ☐ repeatability,
- ☒ within-lab reproducibility
- ☒ robustness
- ☐ quality control
- ☐ exp uncertainty
- ☐ traceability



Comparative study of gold content in jewellery gold alloys

	W_ av	U (k=3)
	‰	‰
ASDI-FAAS	583,5	7,2
ICP-MS	584	58
Cupellation	585,1	

For all of you who succeed to understand our example…….

enjoy the picture!