HBM4EU

science and policy
for a healthy future

Validation of analytical methods

Institut "Jožef Stefan", Ljubljana, Slovenija

Milena Horvat, Janja Snoj Tratnik

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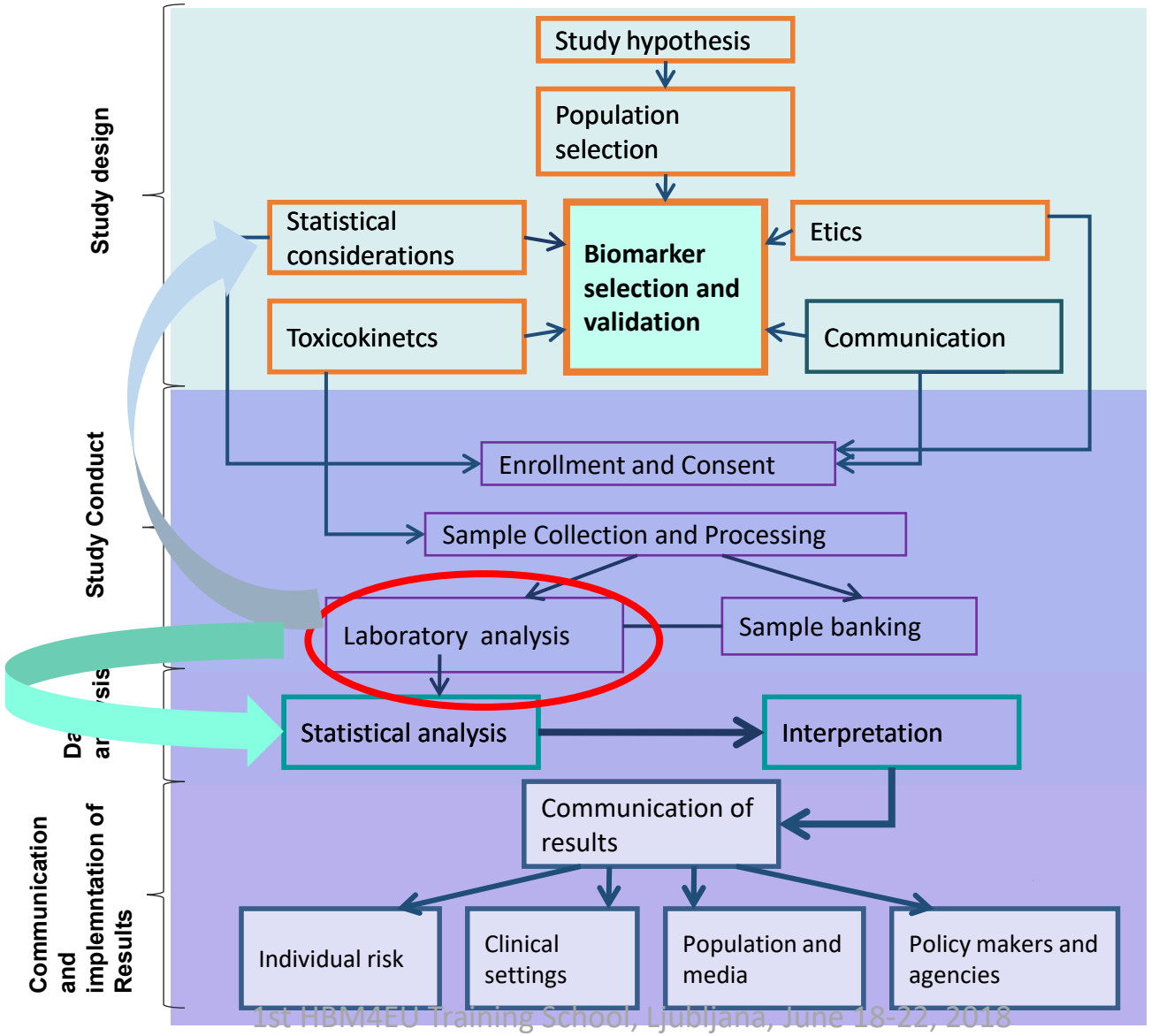
Outline

Demonstration case

Estimation of analytical performance characteristics

Use of (certified) reference materials

Analytical quality objectives and planning of HBM



Quality Assurance in HBM laboratory measurement results

"Metrology support is of great importance to allow sustainable implementation of the programme(s) and provide quality data to assess time and spatial trends."

De Bièvre et al., Metrological traceability of measurement results in chemistry: Concepts and implementation (IUPAC Technical Report), Pure Appl. Chem., 15 June 2011*

Appropriate design of protocols and recruitment strategies

Metrological comparability of the measurement results obtained in laboratories

Methodological approach

Laboratory variability (SD_{lab})

- Inter-laboratory excercise
- Variability within single laboratory (measurement uncertainty)

Population variability (SD_{pop})

- Exposure assessment in 6-11 years old children

→ Sample size calculation

Combined variability (SD_{comb})

$$SD_{comb} = \sqrt{SD_{lab}{}^2 + SD_{pop}{}^2}$$

Case study

To demonstrate the **equivalence of the measurement results** obtained by different laboratories

Project PHIME (*6th FP, Public health impact of long-term, low-level mixed element exposure in susceptible population strata*, FOOD-CT-2006-016253, 2006-2011)

- **Inter-lab excercise: total Hg**, **Cd** and **lead (Pb)** in freeze dried and fresh blood human samples
- **Study population: total Hg**, **Cd** and **lead (Pb) in blood of** 6-11 years old children

Inter-lab variability: intercomparison excercises

Trace elements (Hg, Cd, Pb) in lyophilised whole human blood

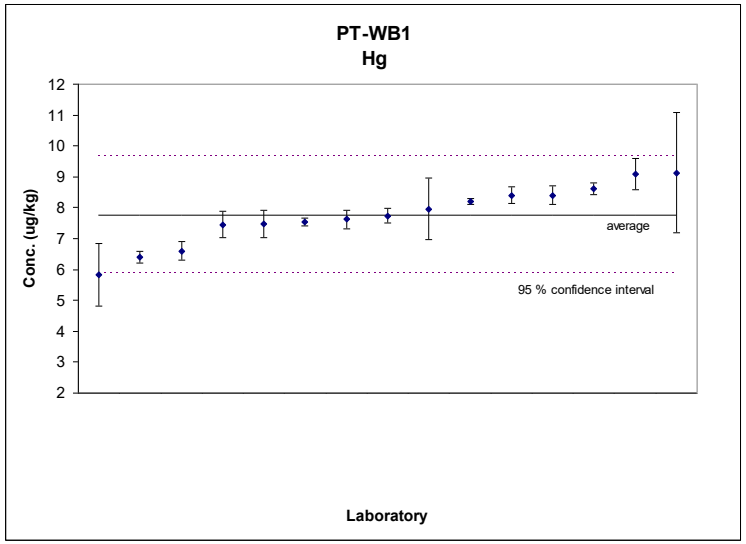
- **PT-WB1 (low Hg)**
- **PT-WB2 (occupationally exposed to Hg(0))**
- **PT-WB3 (fish eating population))**

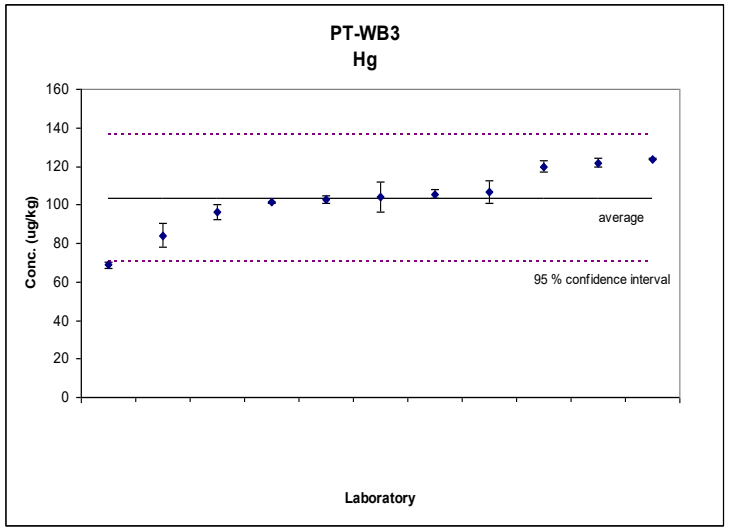
Trace elements (Hg, Cd, Pb) in whole human blood

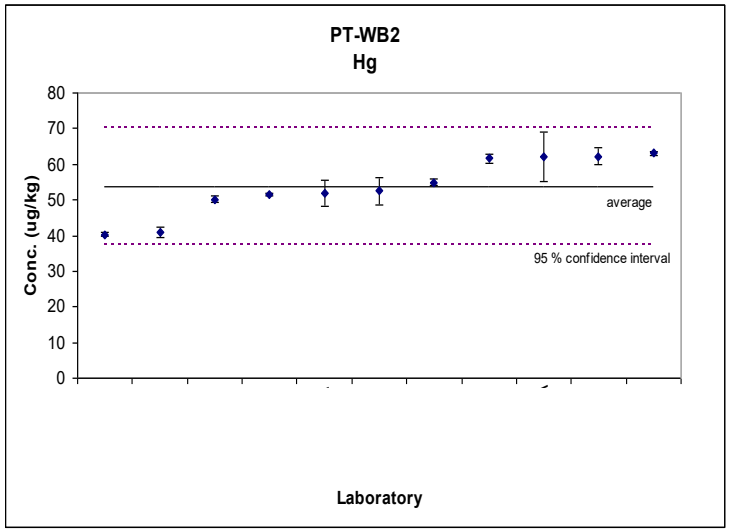
- **FF 3613 (low Hg)**
- **FF 3614 (low Hg)**
- **GG 0461 (low Hg)**

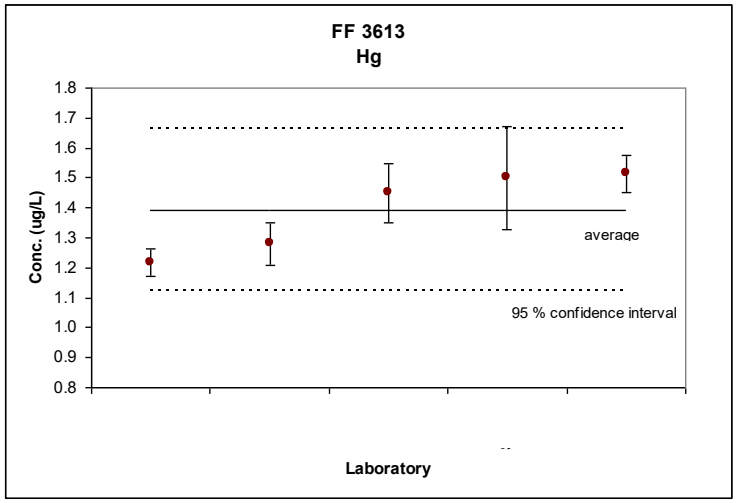
Source: Mazej et al., 2010; Snoj Tratnik et al, submitted

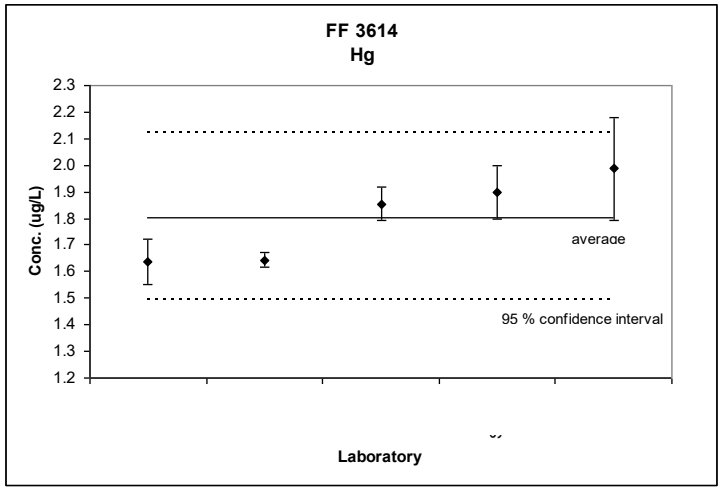
*Inter-lab variability: **Hg** in freeze dried and fresh blood*

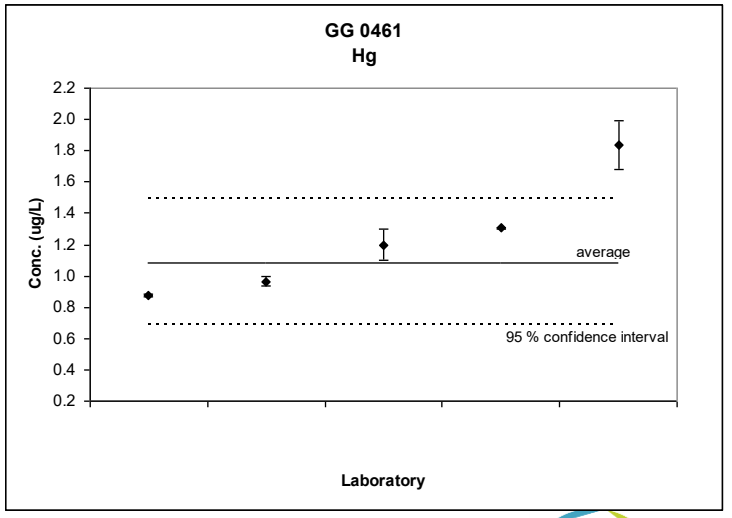




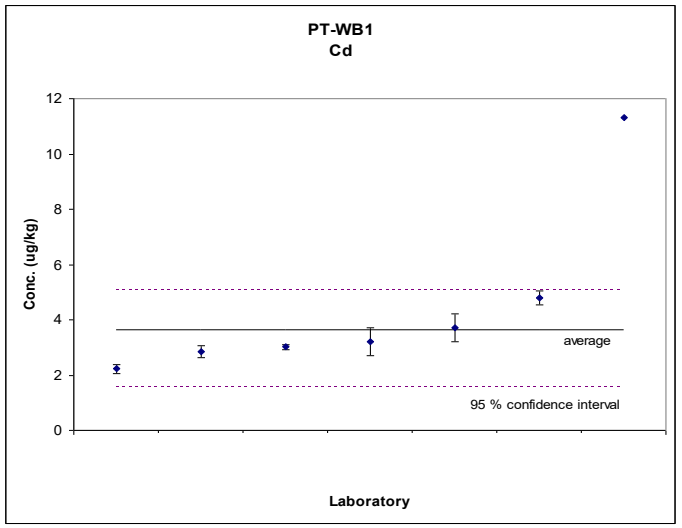


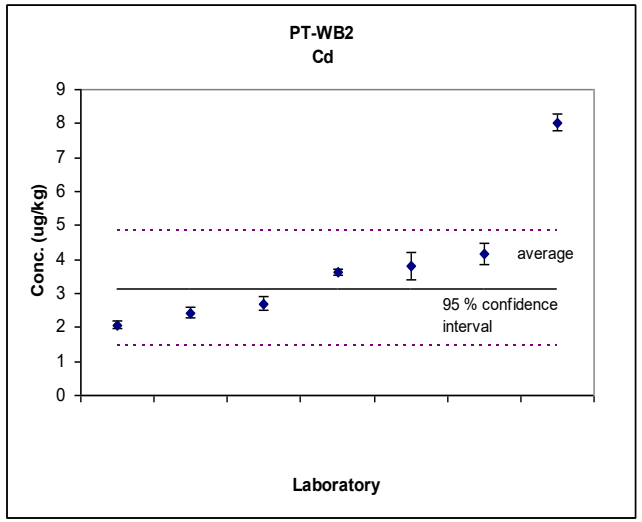


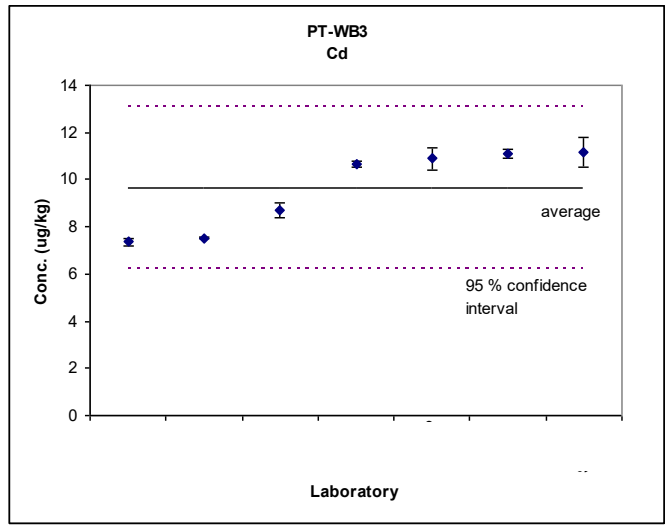


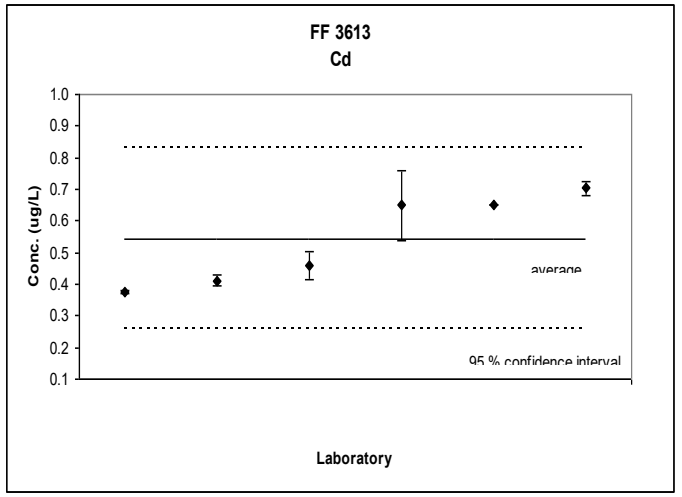


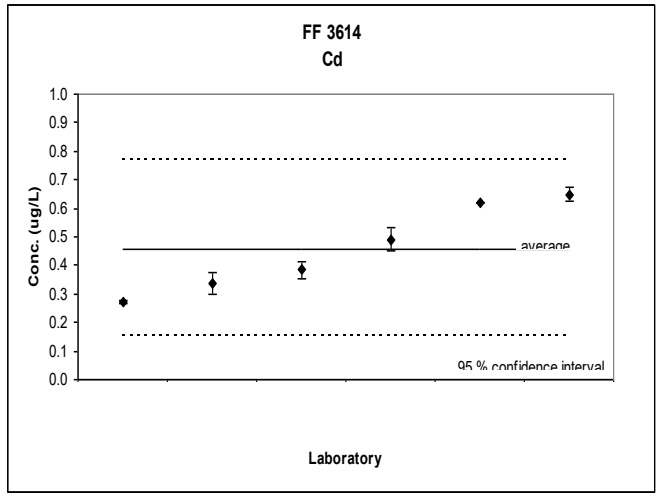
*Inter-lab variability: **Cd** in freeze dried and fresh blood*

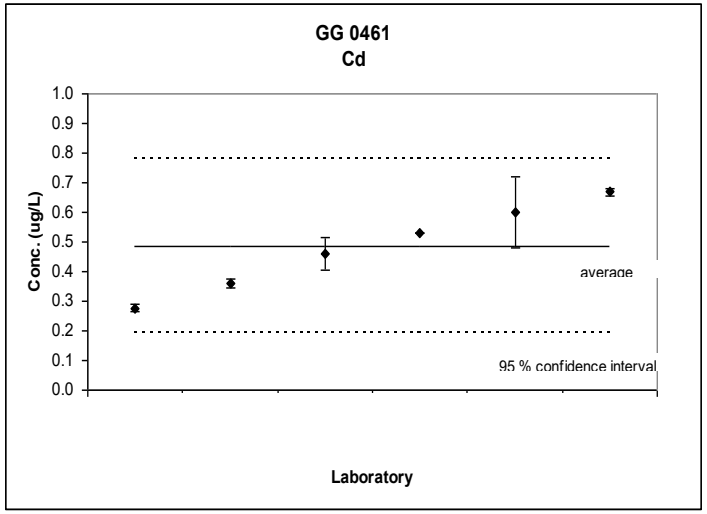




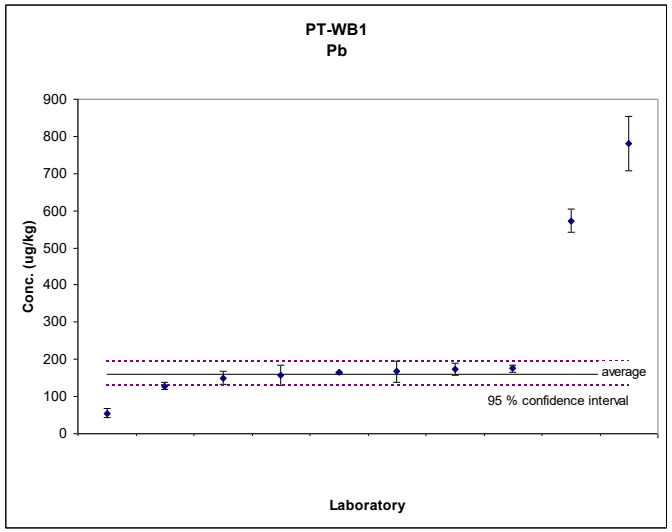


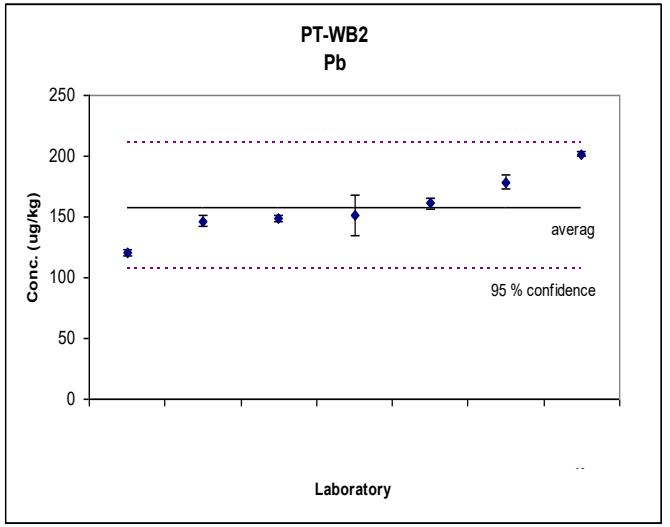


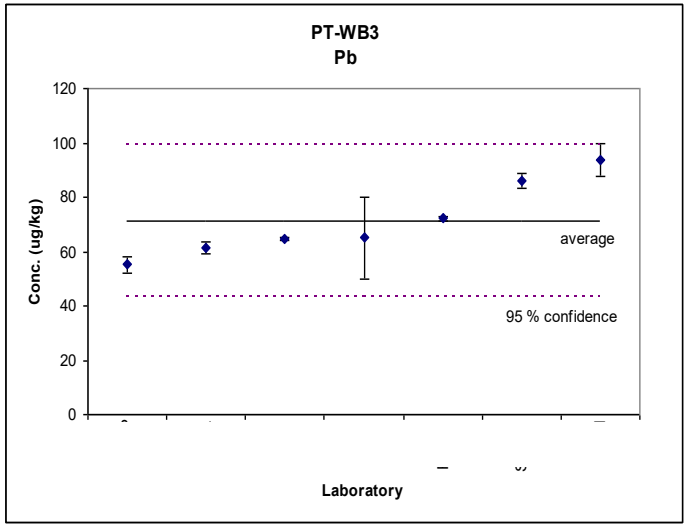


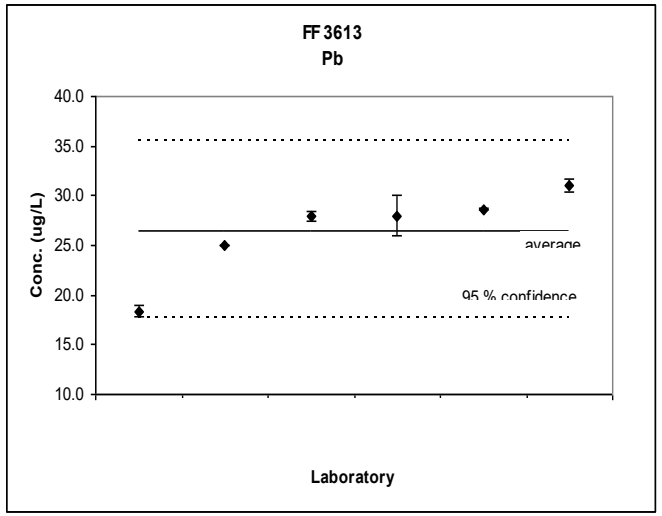


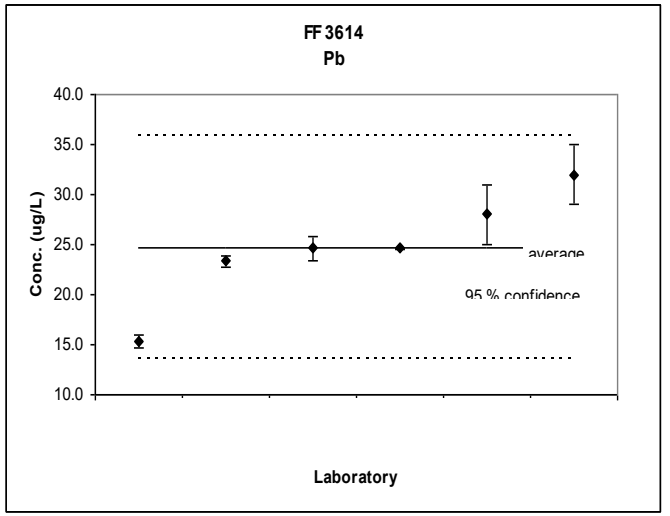
*Inter-lab variability: **Pb** in freeze dried and fresh blood*

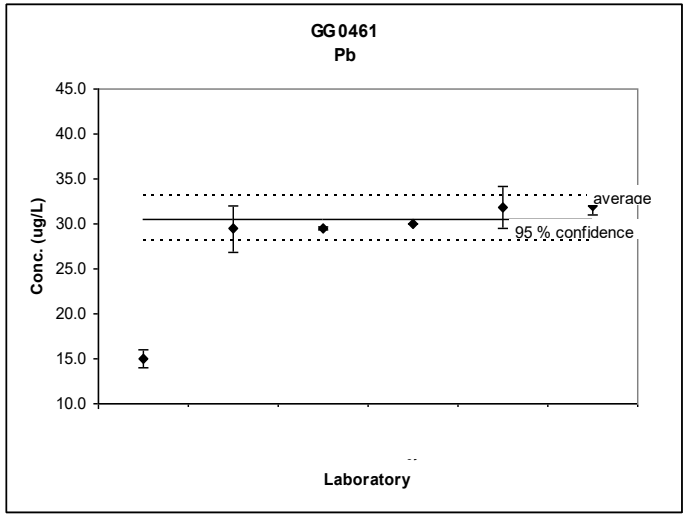












Population variability

- population group of 6-11 years old school-children
- equally representing both genders and all ages within the selected age range
- participating children were socio-economically from different backgrounds
- results of the urban study group published in *Hrubá et al., 2012*

Analyte / Study area	n	min-max	Geom. mean	Geom. SD	95% CI
B-THg	**174**	**0.35 - 4.39**	**0.84**	**0.55**	**0.78 – 0.91**
urban	45	0.35 – 3.05	0.94	0.54	0.82 – 1.08
rural	66	0.35 – 3.72	0.71	0.44	0.63 – 0.80
Idrija	63	0.41 – 4.39	0.92	0.63	0.81 – 1.05
B-Cd	**150**	**<0.13 - 0.69**	**0.20**	**0.12**	**0.19 – 0.22**
urban	42	0.09 – 0.28	0.14	0.04	0.13 – 0.16
rural	65	0.13 – 0.54	0.23	0.12	0.20 – 0.25
Idrija	43	0.13 – 0.69	0.24	0.15	0.20 – 0.28
B-Pb	**165**	**5.33 - 56.8**	**15.4**	**7.15**	**14.6 – 16.4**
urban	42	6.9 – 23.7	13.4	4.68	12.3 – 14.8
rural	64	5.33 – 56.8	16.1	8.84	14.4 – 16.5
Idrija	59	7.38 – 36.9	16.3	6.81	14.9 – 17.9

Power analysis

Sample size required to detect significant difference in total Hg, Cd and Pb blood concentration *between population groups*

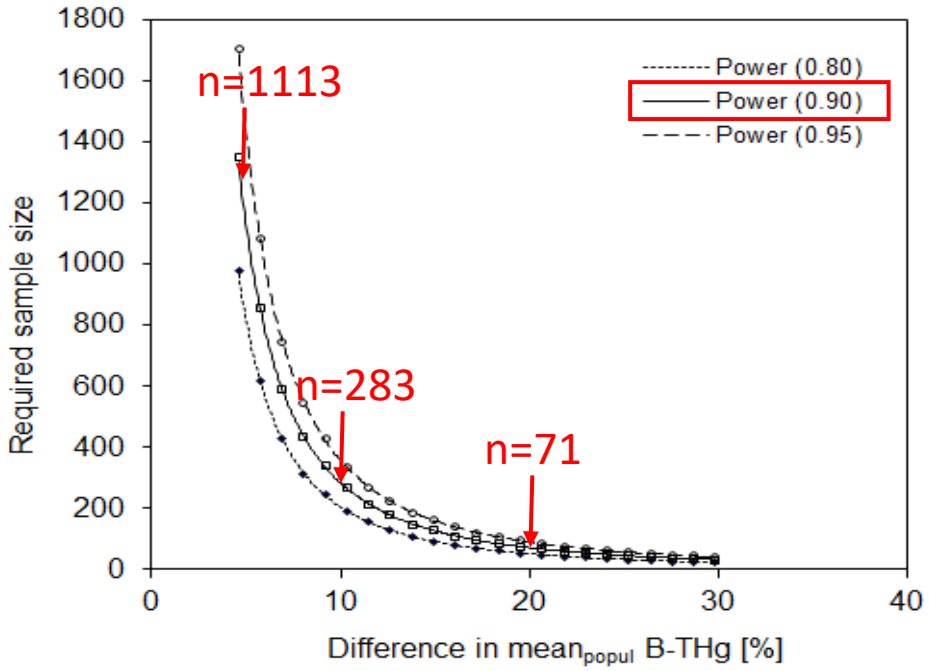
T-test (H0: Mean1 = Mean2)

statistical power = 0.80, 0.90 and 0.95; α = 0.05

Relative differences in means: 5, 10, 20, 30 %

Required sample size – Hg in blood

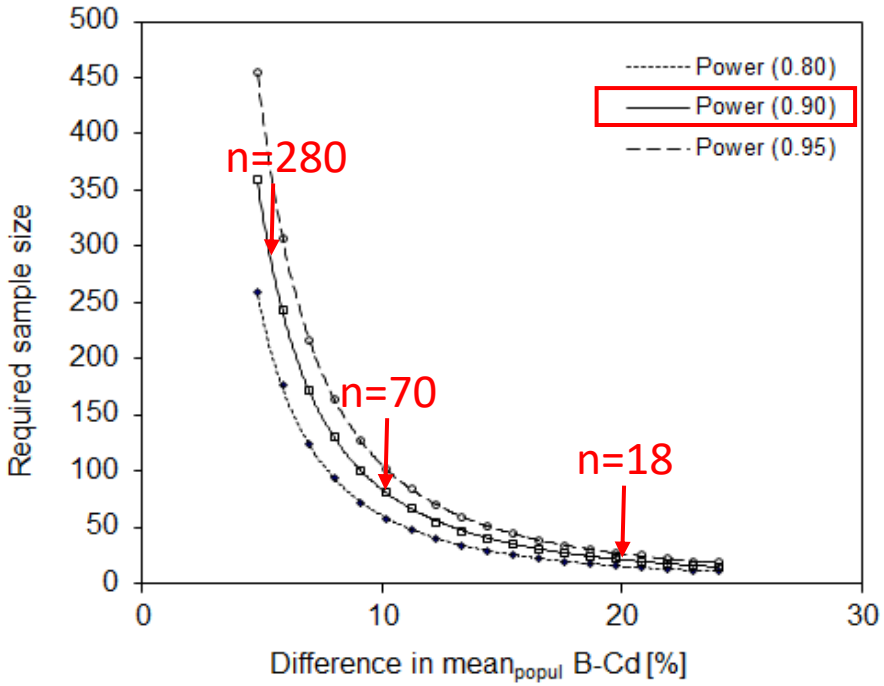
Relative difference in means (power: 0.80 / **0.90** / 0.95)



Analyte in blood	Population mean ± SD [µg/L]	Combined SD [µg/L]: mean (range)	N in relation to the difference [%] in mean blood levels: mean (range)		
			5 %	**10 %**	**20 %**
Total Hg	0.94 ± 0.54	0.554 (0.546-0.567)	1190 (1156-1247)	**298** (289-312)	75 (73-78)

Required sample size – Cd in blood

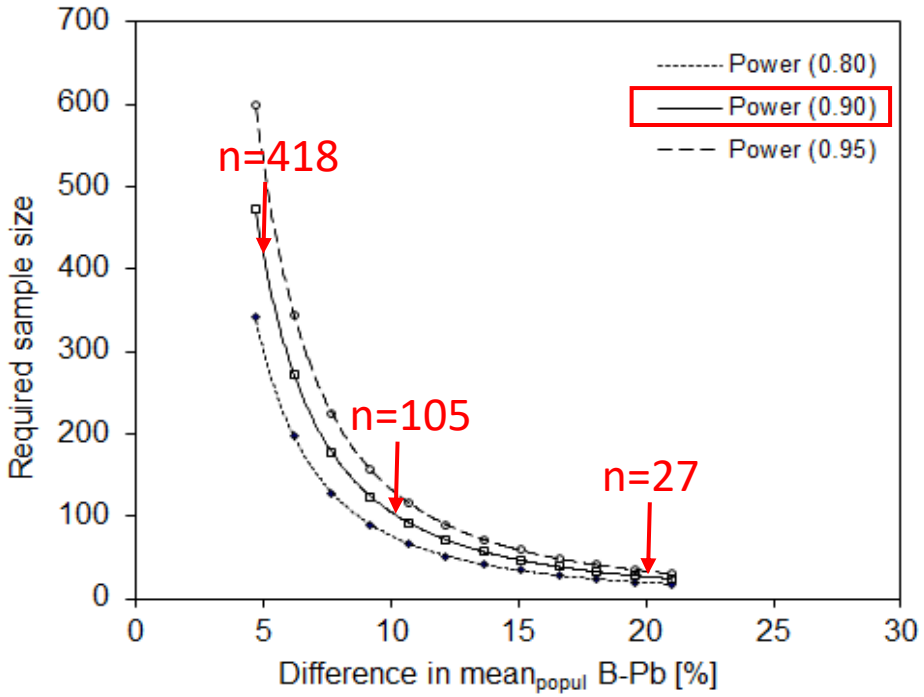
Relative difference in means (power: 0.80 / **0.90** / 0.95)



Analyte in blood	Population mean ± SD [µg/L]	Combined SD [µg/L]: mean (range)	N in relation to the difference [%] in mean blood levels: mean (range)		
			5 %	**10 %**	**20 %**
Cd	0.14 ± 0.04	0.059 (0.052 – 0.066)	609 (473 - 762)	**153** (119 - 191)	39 (30 - 48)

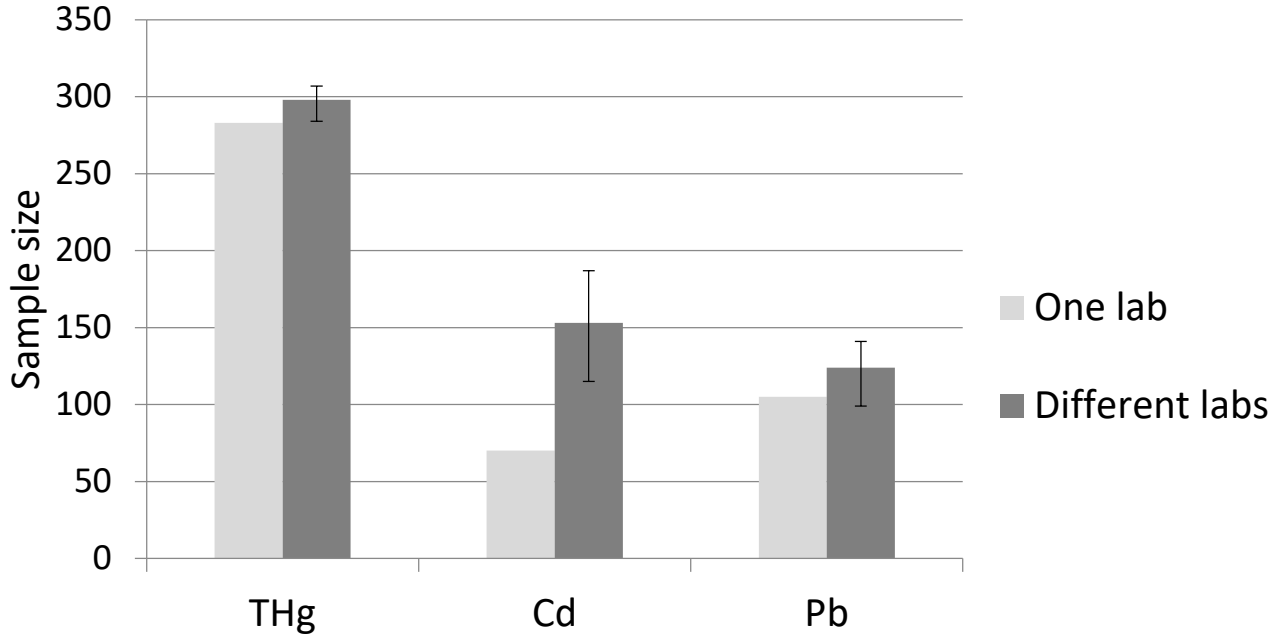
Required sample size – Pb in blood

Relative difference in means (power: 0.80 / **0.90** / 0.95)



Analyte in blood	Population mean ± SD [µg/L]	Combined SD [µg/L]: mean (range)	N in relation to the difference [%] in mean blood levels: mean (range)		
			5 %	**10 %**	**20 %**
Pb	13.4 ± 4.68	5.096 (4. 721– 5.572)	496 (426 - 593)	**124** (107 - 149)	31 (27 - 38)

Significance of analytical method performance: Required sample size



$SD_{popul} + SD_{interlab}$

Sample size increase:

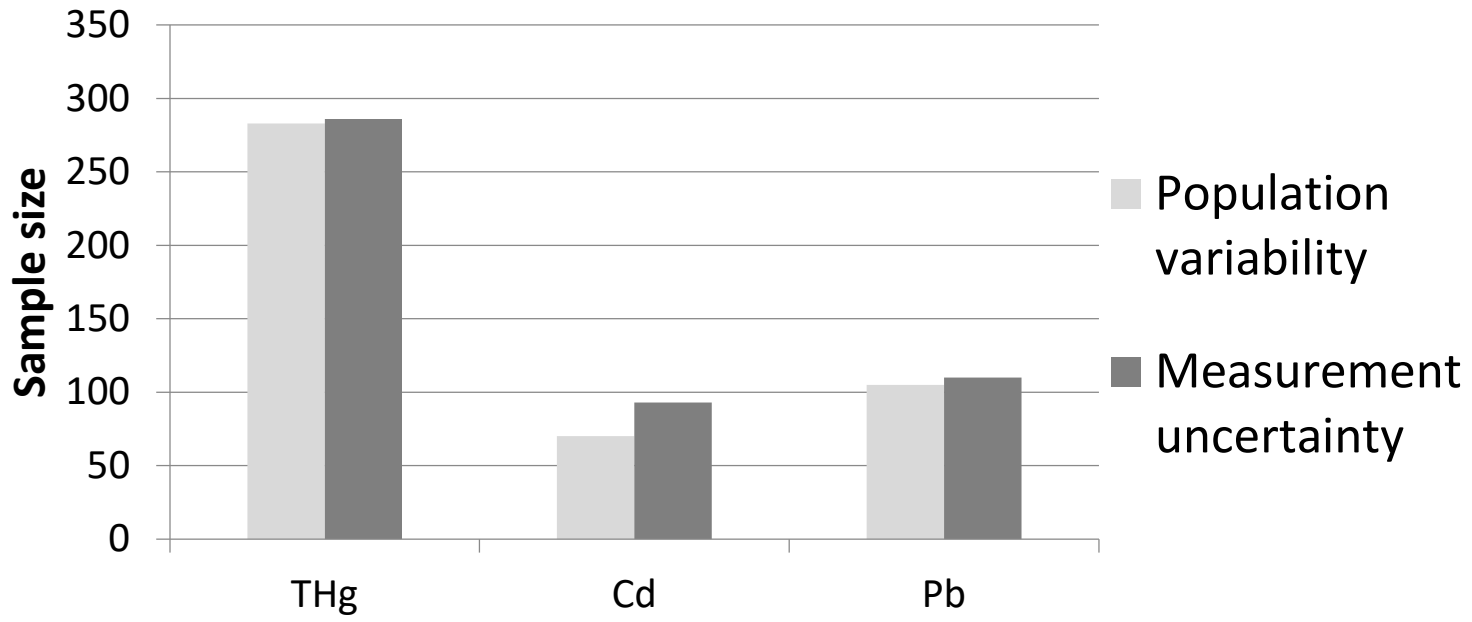
Total Hg	30 % - 60 %
Cd	2.5x – 4.5x
Pb	20 % - 100 %

Sample size calculation based on t-test: two mean B-THg values of 10 % difference, statistical power = 0.90; α = 0.05.

Significance of analytical method performance

Measurement uncertainty

$SD_{popul} + SD_{lab(uncertainty)}$



Summary of findings

Analytical requirements for sample size in HBM studies depend on the:

- Analyte and its variability within study groups,
- Magnitude of a difference in exposure level,
- Number of laboratories involved in chemical analysis and their analytical performance

Significance of measurement variability in cases of small population variability (e.g. Cd).

Analytical quality performance in HBM

Single laboratory vs. multiple laboratory

Importance of regular analytical quality control

Participation in ICIs

Expression of measurement uncertainty

Analytical quality performance characteristics

LOD, LOQ

Repeatability/Reproducibility

Use of RMs and CRMs

Interlaboratory comparisons

Validation

Definitions

Why measurement procedure must be validated?

Approach to validation procedure

Realization of validation

What is method validation

Method validation is the process of proving that an analytical method is acceptable for its intended purpose.

Ludwig Huber 1998, Validation and Qualification in Analytical Laboratories.

Definition

Validation

(ISO/IEC 17025)

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

Difference in terminology

ISO/IEC 17025

- Method: **method validation**

VIM

- Measurement procedure: **procedure validation**

GLP

- Standard operation procedure: **SOP validation**

Why validation?

Method validation is an important requirement in the practice of chemical analysis.

Why validation?

Importance of analytical measurement

- Provides information on procedure

The professional duty of the analytical chemist

- For analyst (the user of the procedure)
- For customer (the user of the results)

Regulatory requests

ISO 17025 requirement

Validation

Why do we need it?

ISO/IEC 17025

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

- Method (procedure) validation
- Traceability of results
- Uncertainty of results

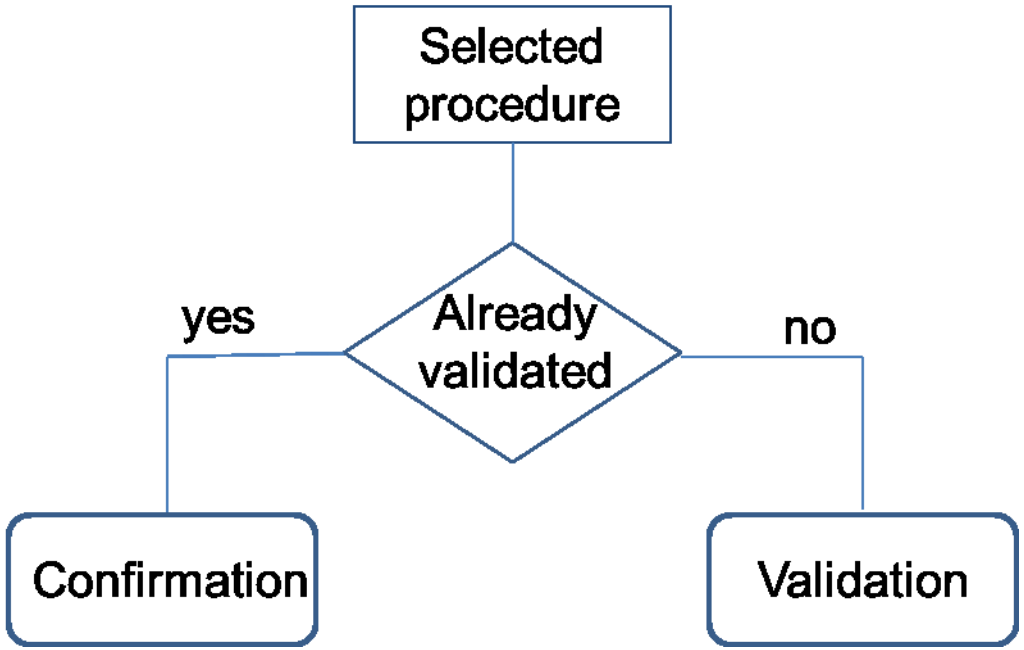
Validation

Full validation

- **All procedures** used in the lab must be validated

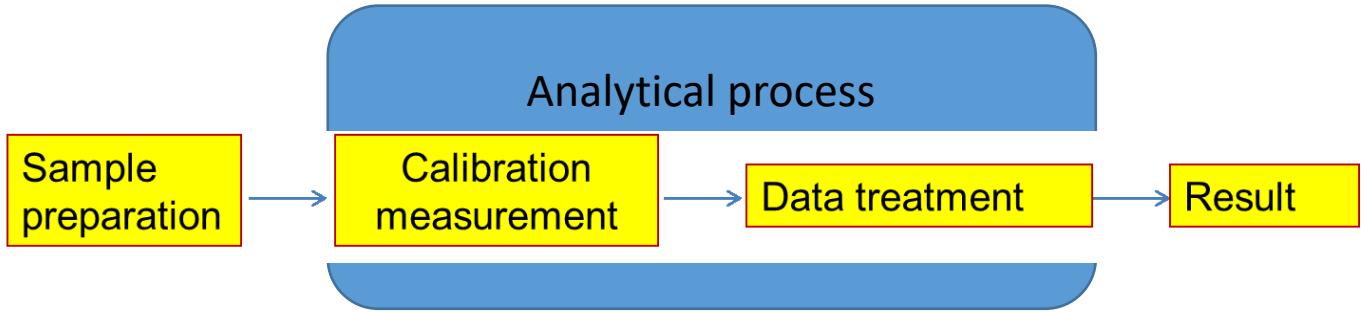
Confirmation

- Procedures published as international, regional or national standards are considered to be validated



Procedures published in scientific journal are not standard procedures

Validation



Whole procedure

Full concentration range

All intended types of matrices

What must be considered in Method validation

Precision

Trueness

Accuracy

Limit of detection, LOD

Limit of quantification, LOQ

Selectivity/specificity

Linearity and range

Ruggedness (or Robustness)

Sensitivity

Recovery

Precision

It is the measure of the degree of repeatability of an analytical method under normal operation

Precision shows how close results are to one another

Trueness

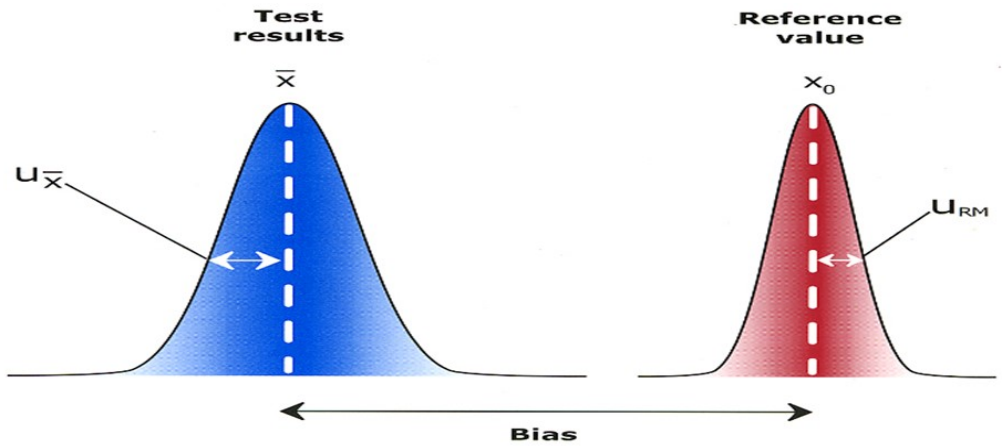
Closeness of agreement between the average of an infinite number of replicate **measured quantity values** and a **reference quantity value.**

Estimation of trueness by:

- Using Certified Reference Materials
- Using RM or *in-house* materials
- Using Reference methods
- Results from proficiency testing
- Spiked samples

Bias

Bias is a quantitative expression of trueness



Picture outline from: In House Method Validation, LGC

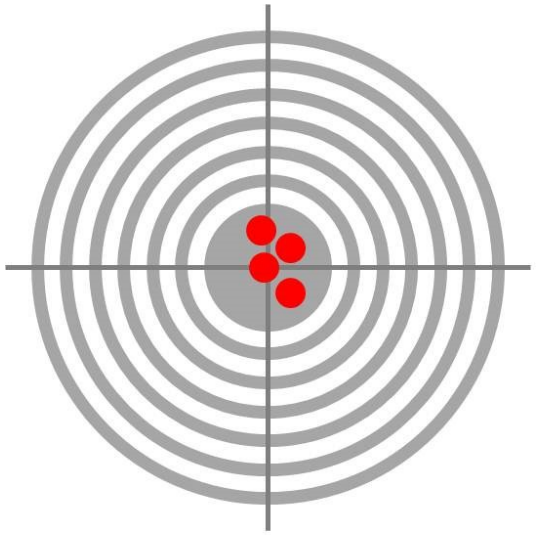
The trueness of result improves when bias decreases.

Accuracy

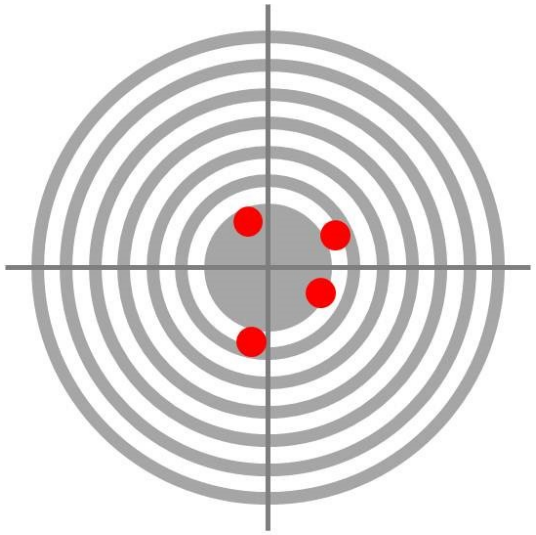
- Closeness of agreement between a measured quantity value and a “true” quantity value of a measurand.

- Describes the measure of exactness of an analytical method.

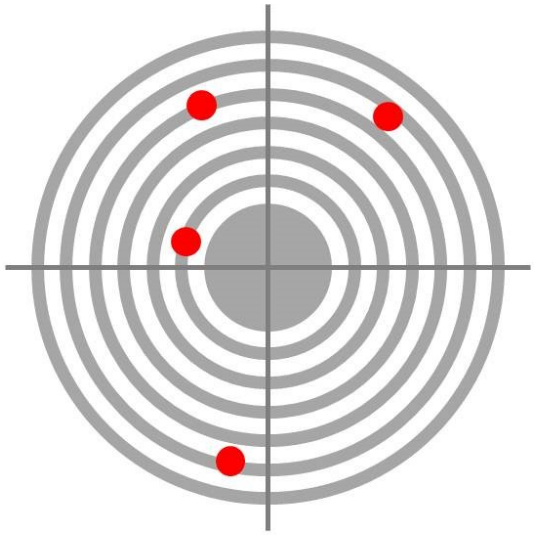
Precision and trueness = accuracy



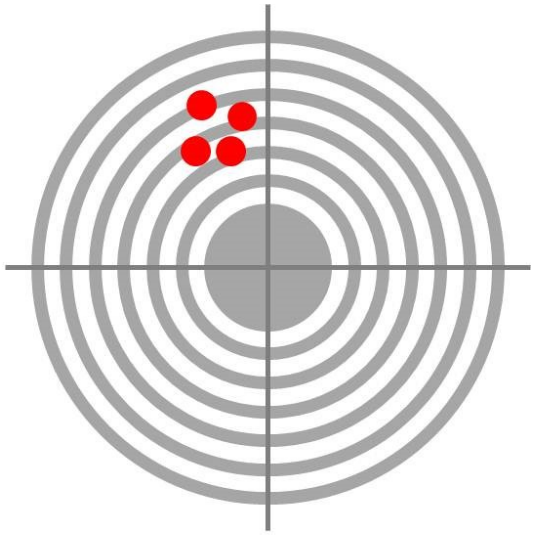
High accuracy, good precision, good trueness



Low accuracy, poor precision, good trueness



Low accuracy, low precision, poor trueness



Low accuracy, high precision, poor trueness

Recovery

A measure of the trueness of a measurement procedure

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

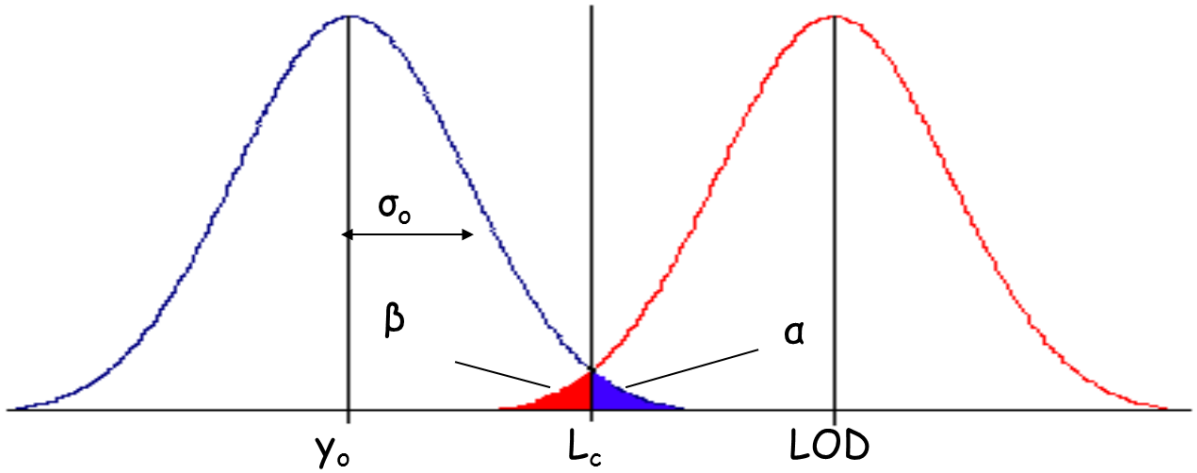
- CRM
- Spike of pure substance

Limit of detection

LOD is defined as the lowest concentration of an analyte in a sample that can be distinguished from a blank

$LOD = B+3S_0$ or $0+3S_0$

B=Blank
S_0=standard deviation of 10 measurements



Y_o -Signal equal to the blank signal
L_c- decision level
LOD- limit of detection

Limit of quantification

LOQ is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method

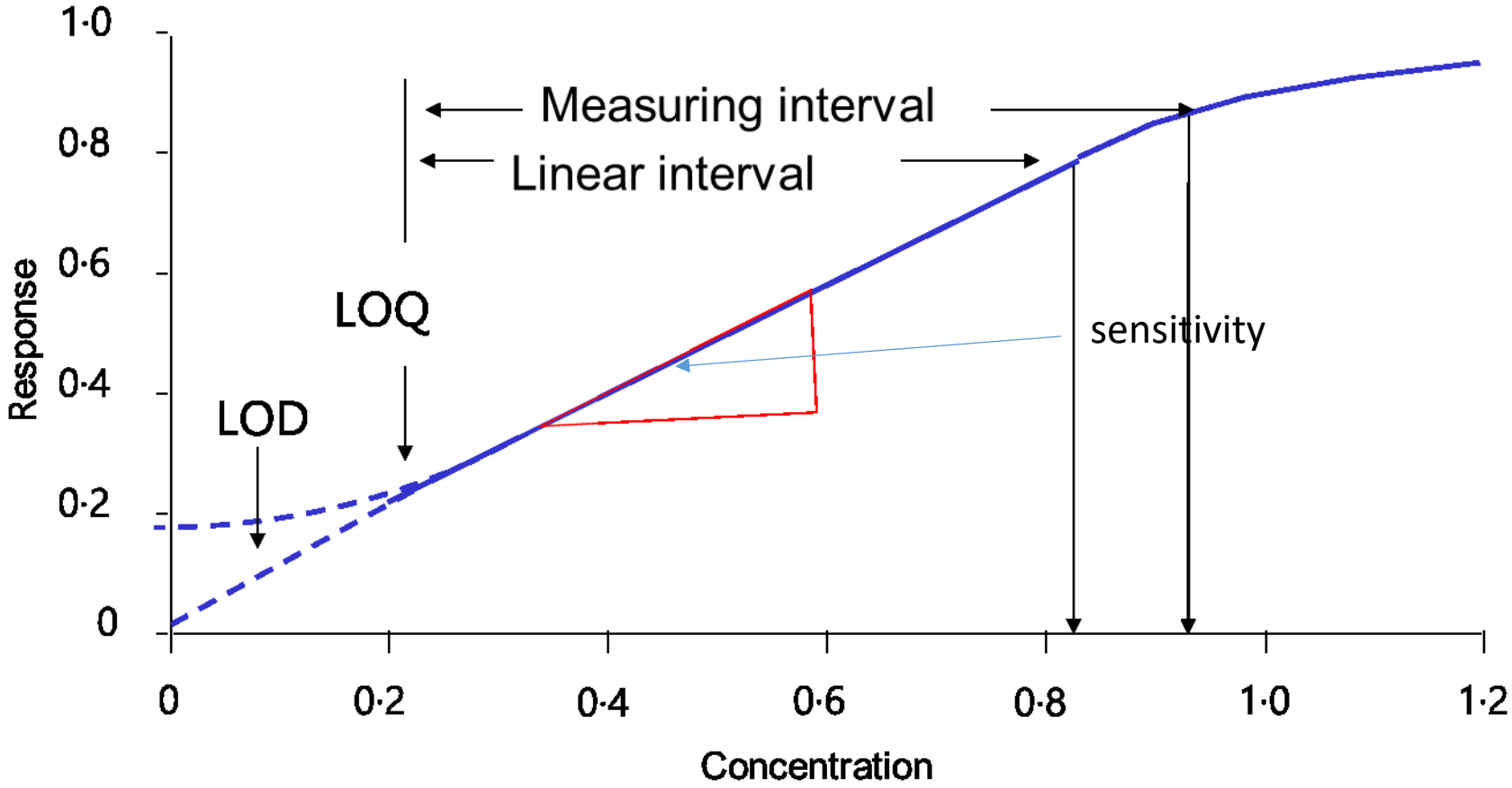
$$LOQ = B+10S_0$$

Selectivity / specificity

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

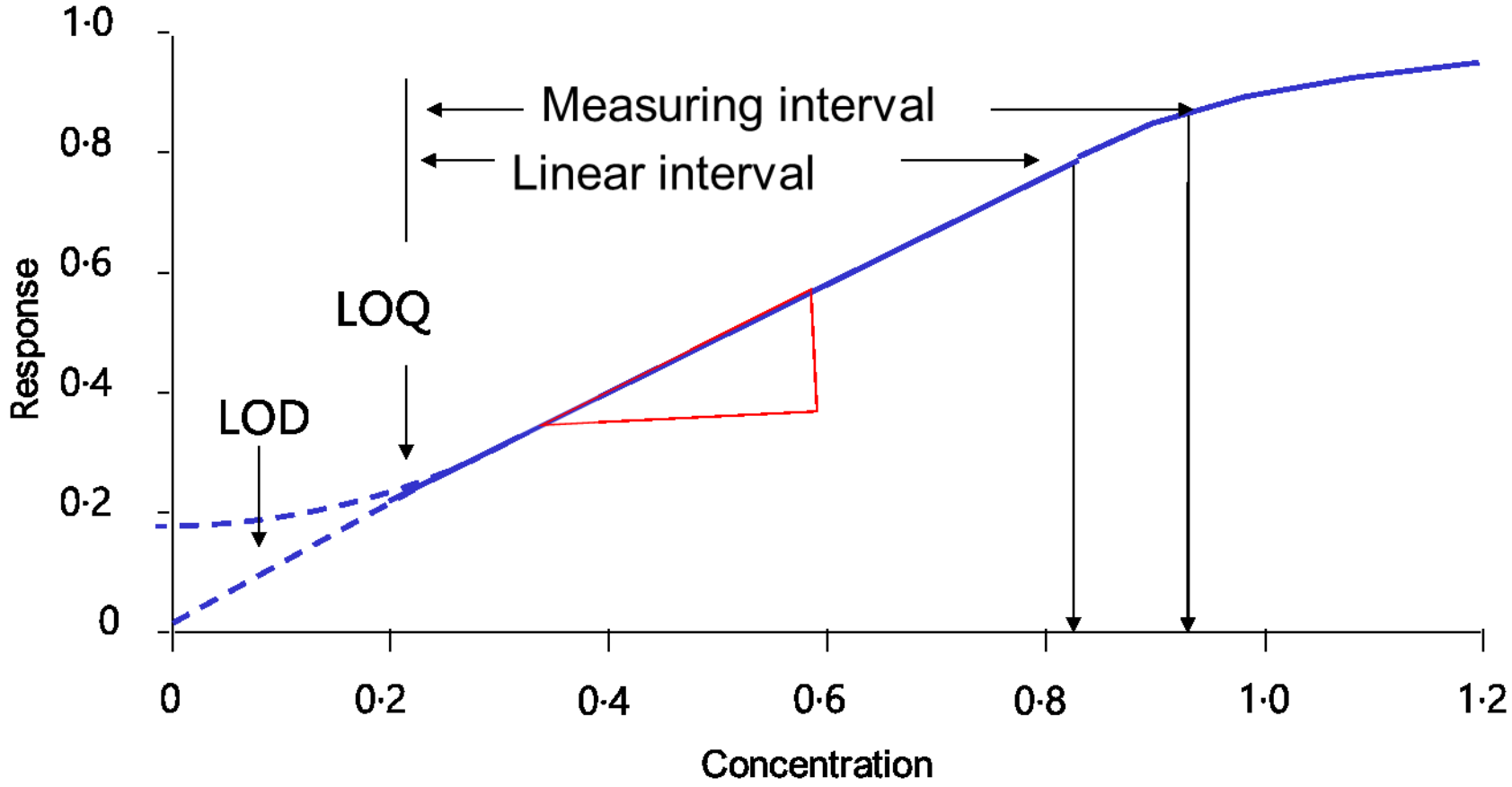
Sensitivity

The sensitivity of method is the rate of change of the measured response with change in the concentration of analyte



Linearity and range

The ability of the method to obtain test results which are proportional to the concentration of the analyte



Ruggedness (robustness)

The **ruggedness (robustness)** of an analytical method is the resistance to change in the result produced by an analytical method when minor deviations are made from the experimental conditions described in the procedure.

Validation of the method

Before validation you have to define:

- the requirements of the measurement procedure
- scope of experiments
- RM to be used
- Equipment
- Statistical tools to be used
- personnel for performing experiments and evaluating obtained results

Institut "Jožef Stefan", Ljubljana, Slovenija

Contacts:

janja.tratnik@ijs.si
milena.horvat@ijs.si

Speaker's information

Janja Snoj Tratnik works at the Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia. Her backround is in biology. She has 10 years experience in trace element analysis in biological samples, HBM recruitment, database management and statistical evaluation of the results. In HBM4EU she is part of the Cd chemical group.

Prof. Milena Horvat is the Head of the Department of Environmental Sciences at the Jožef Stefan Institute and a Dean of the International Postgraduate School Jožef Stefan. By basic training she is an analytical chemist. She coordinated the implementation of the Slovenian HBM and several research project in the domain of environment and health studies. Within the HBM4EU she is chemical group leader for Cd and involved in Training activities.

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