Using split sample technique for quality control in a clinical laboratory

Elvar Theodorsson

IKE/Clinical Chemistry

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Health-care in processes of major change

- Aging population
- Higher costs
- The number of hospital beads decreases rapidly
- Shorter periods of hospital stay
- Healthcare increasingly done in out- patient departments without hospitalization
- Increasing volumes of healthcare performed in primary care

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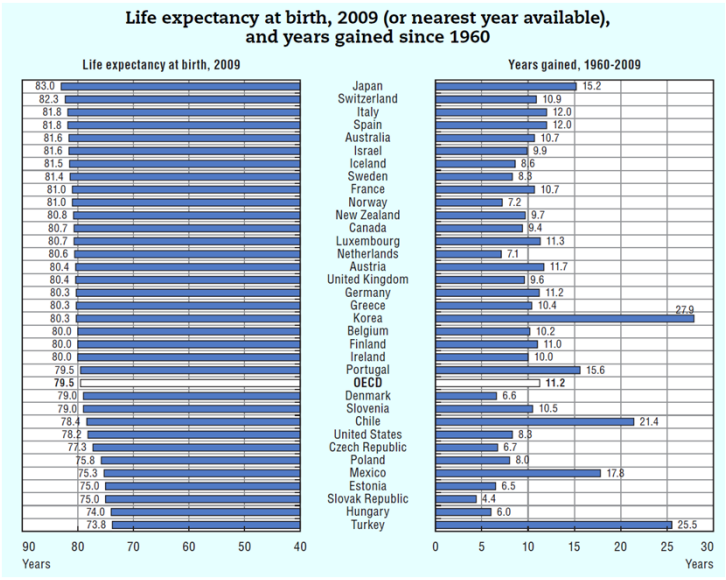

Source OECD http://www.oecd.org/els/healthpoliciesanddata/49105858.pdf

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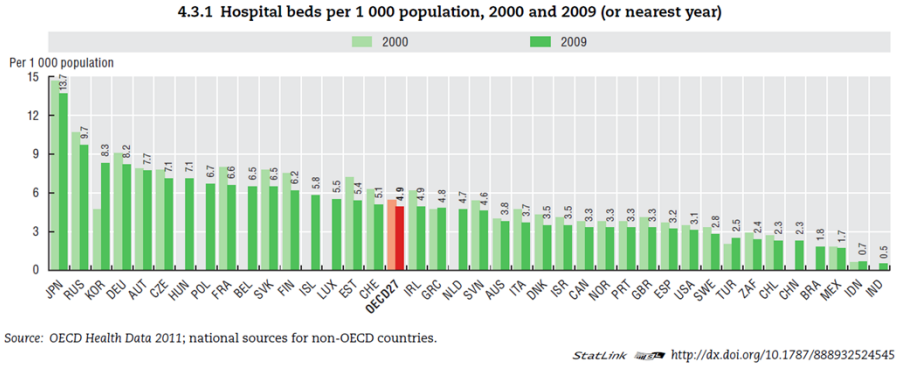

Source OECD http://www.oecd.org/els/healthpoliciesanddata/49105858.pdf

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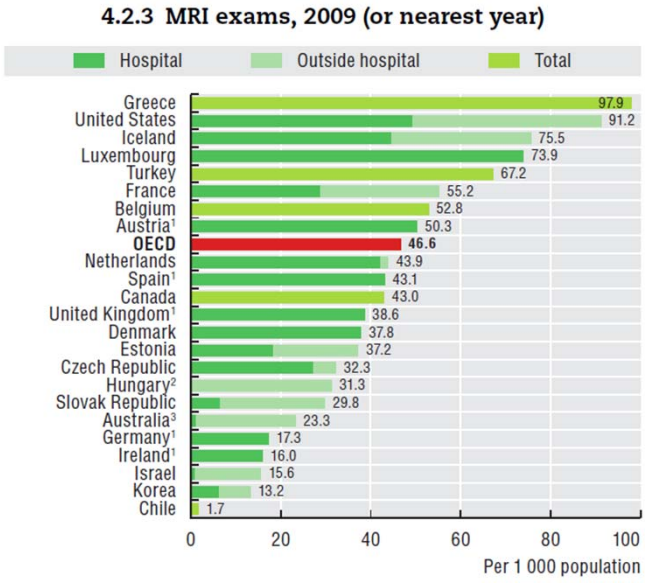

MRI= Magnetic Resonance Imaging

Source OECD http://www.oecd.org/els/healthpoliciesanddata/49105858.pdf

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5

Changes in Laboratory Medicine

- Development of measurement techniques and instrumentation
  - Better techniques for point-of-care testing
  - Increased automation in centralized laboratories
- Development of information technologies
- Development/changes of organizational structures and creation of large laboratory organizational structures
- Increased use of point-of-care measurements
- Development of calibration and quality control techniques appropriate for the new situation

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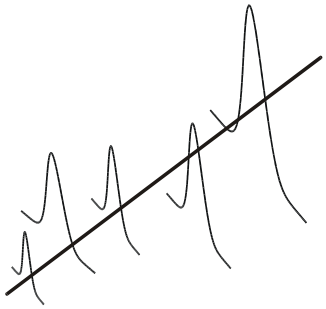
Consequences for Laboratory Medicine

- Increased demands for short response time
- Increased use of point of care techniques
  - The number of measurement instruments increases
  - The number of measurement techniques increases
  - Increased risks for bias
  - Increased risks for increased measurement uncertainty
  - Increased risk for diagnostic uncertainty



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Diagnostic uncertainty when using laboratory results

- The **collected uncertainty** when interpreting analytical results due to all causes of uncertainty taken together
- Includes **all** causes of uncertainty, even those caused by factors that the laboratory does not normally control
- Only a fraction of the diagnostic uncertainty is caused by measurement uncertainty
  - **Bias-** the major part of the measurement uncertainty in laboratory medicine
  - **Random error**
- The largest part of uncertainty is biological variation

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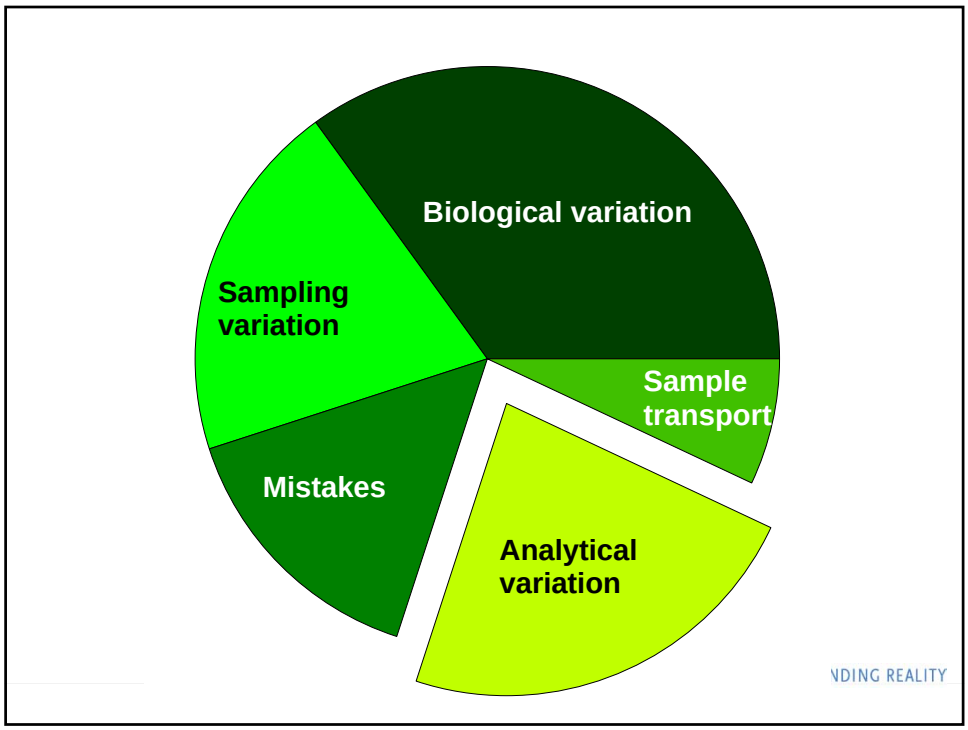

NDING REALITY

Biological variation

ROGER J. WILLIAMS

Professor of Chemistry
Director, Biochemical Institute
University of Texas

1956

Biochemical Individuality

THE BASIS FOR THE GENETOTROPHIC CONCEPT

New York · John Wiley & Sons, Inc.
London · Chapman & Hall Limited

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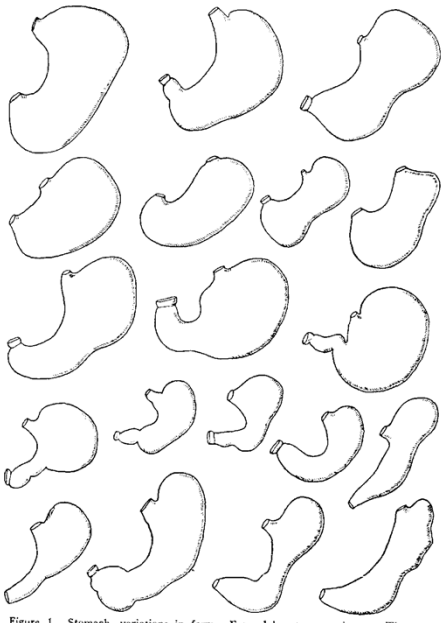

Figure 1. Stomach, variations in form. From laboratory specimens. The author is deeply indebted to Dr. Barry J. Anson of Northwestern University, who has kindly allowed him to reproduce illustrative material from his valuable *Atlas of Human Anatomy* (W. B. Saunders Co., Philadelphia, Pa., 1951). This illustration is on page 287.

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Database on biological variation

http://www.westgard.com/intra-inter.htm

J Chron Dis 1970, Vol. 23, pp. 469–480. Pergamon Press. Printed in Great Britain

DISTINGUISHING PHYSIOLOGIC VARIATION FROM ANALYTIC VARIATION

EUGENE K. HARRIS

Laboratory of Applied Studies, Division of Computer Research and Technology, National Institutes of Health, Public Health Service, Department of Health, Education and Welfare, Bethesda, Maryland 20014, U.S.A.

(*Received* 5 *January* 1970)

WHEN REPEATED measurements of an individual's blood chemistry or other physiologic variable are made over an extended period of time, the problem arises of separating long-term analytic or 'laboratory' deviation from the truly physiologic component of the total observed variation. In a normal person, the latter component represents deviations from homeostasis which may arise from short-term trends, cyclic changes, or simply transient events. This paper discusses the pros and cons of several experimental strategies for quantitatively estimating the physiologic component of variation in a series of results from a single individual. In addition, mathematical methods are proposed for inferring the statistical characteristics of this component over a population of individuals. This requires, of course, that repeated samples be taken from each of a set of N individuals, preferably a random sample from some pre-defined population.

Scand J Clin Lab Invest 1999; 59: 491–500

Current databases on biological variation: pros, cons and progress

C. RICÓS, V. ALVAREZ, F. CAVA, J. V. GARCÍA-LARIO, A. HERNÁNDEZ, C. V. JIMÉNEZ, J. MINCHINELA, C. PERICH & M. SIMÓN

Analytical Quality Commission from the Spanish Society of Clinical Chemistry and Molecular Pathology (SEQC), Spain

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Biological variation

- In medical practice the biological variation is commonly twice the measurement variation

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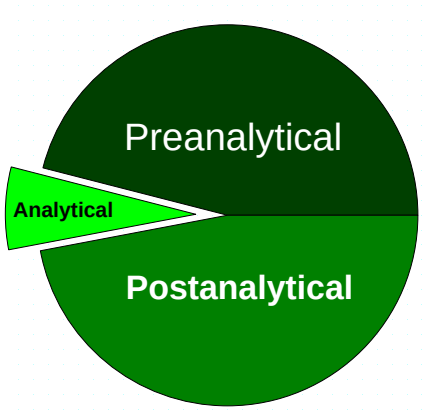
Mistakes in laboratory medicine



Ross J W, Boone D J (1989)
In: Martin L, Wagner W, Essien JDK (eds.) Institute of critical issues in health laboratory practice. DuPont Minneapolis, Minn., p 92

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All clinically known and relevant information should be made known to the users of the measurement results – **diagnostic uncertainty**

- Biological variation
- Sampling variation
- Variation caused by sample transport
- Measurement variation
- Effects of therapeutic drugs
- Effects of substances of abuse

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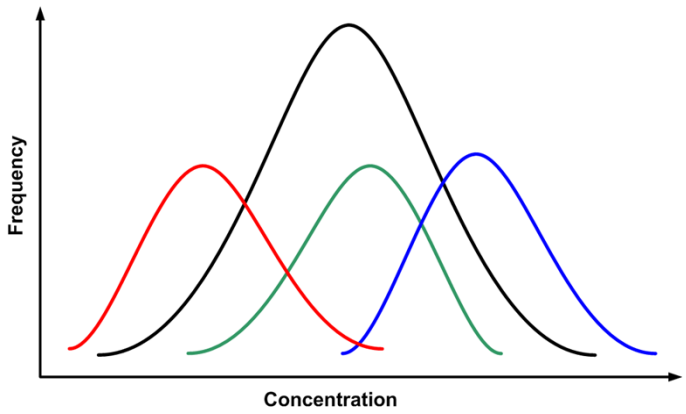
Bias – common in immunochemical measurement methods



Different colors depict different measurement methods

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Differences in the epitopes that the antibodies react with

- Proteins are complex macromolecules containing several **epitopes**
- **Chance** determines which epitopes induce the production of antibodies
- The **specificity of the epitopes** determines the concentration measured
- International **calibrators** usually constitute a **mixture** of different epitopes

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Matrix effects

- Effects on the final analytical results on **all other factors/substances** in the sample and in the sample container except those you intend to measure, e.g.
  - Sample container
  - Anticoagulants
  - Plasma proteins
  - Lipids

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Control materials

- **Modified** to increase their stability during storage, e.g. by delipidation, addition of analytes and lyophilization– causes for matrix effects
- **Matrix effects** result in lack of modified control materials with addition of analytes to result in identical or comparable concentrations using all available techniques

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Why use natural patient samples as control materials?

- The control materials are **modified** and the concentrations of the analytes in them adjusted by **addition**. Different instruments and methods may react differently to the consequent matrix effects
- Methods used for analysing patient samples should ideally not differ since normal patient sample is the sample matrix the methods were/are optimized for
- The most important issue is that the measurement instrument should report the **correct/optimally fit for purpose** results for patient samples.
- Therefore we should – if at all possible – use **patient samples** to monitor the quality of the analytical results for instruments and methods

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Matrix effects and minimizing bias between analytical methods

- **Matrix effects** are of major importance for the calibration and quality control of analytical methods
- To minimize bias and measurement uncertainty it is imperative to establish routines for **secondary calibration** of analytical methods by means of natural patient samples
- Diagnoses are base on measurements in **natural patient samples** and measurement methods should therefore show **identical** results using this sample matrix

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The ISO document Guide to the expression of uncertainty in measurement (GUM), 1993

- The essence is to improve the way to work in order to **eliminate bias** and thus minimize the total variation and the analytical uncertainty
- Advanced statistics and mathematics only play a **secondary and supporting role** in this work, even if they have a prominent place in later documents, e.g. in the "Eurachem document"

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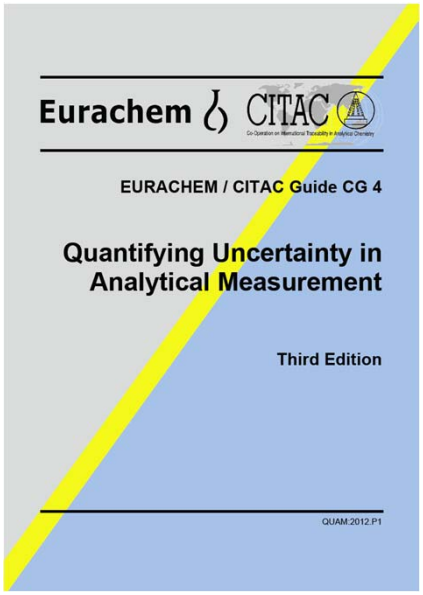

Eurachem/CITAC Guide CG 4

http://eurachem.org/images/stories/Guides/pdf/QUAM2012_P1.pdf

LiU EXPANDING REALITY

The ISO document Guide to the expression of uncertainty in measurement (GUM), 1993

1. To establish, maintain and monitor analytical methods with optimal techniques and calibrators
2. When all parts in #1 are successfully accomplished, **secondary calibration** (e.g. factorizing) should be performed to minimize and if at all possible – **eliminate bias**

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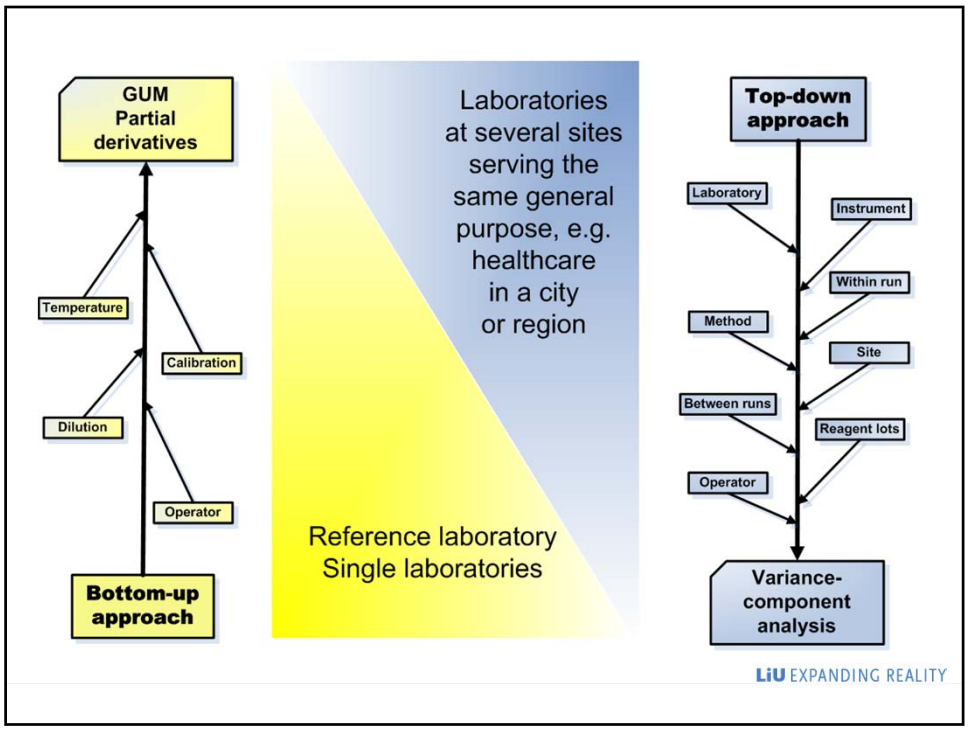
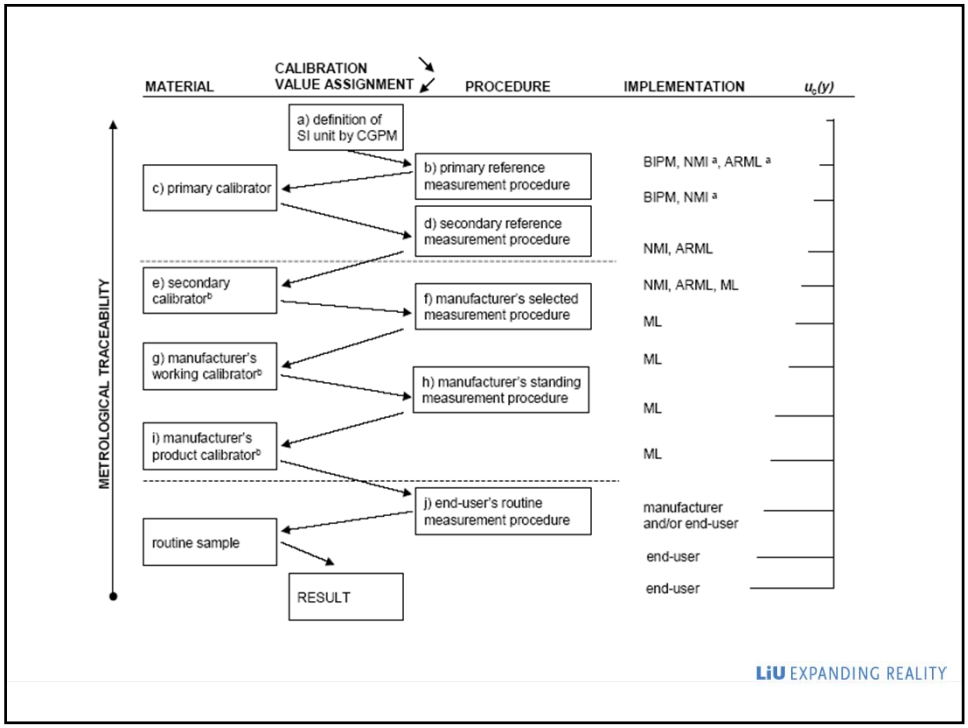

GUM
Partial
derivatives
Temperature
Calibration
Dilution
Operator
Bottom-up
approach
Laboratories
at several sites
serving the
same general
purpose, e.g.
healthcare
in a city
or region
Reference laboratory
Single laboratories
Top-down
approach
Laboratory
Instrument
Within run
Method
Site
Between runs
Reagent lots
Operator
Variance-
component
analysis
LiU EXPANDING REALITY


MATERIAL
CALIBRATION
VALUE ASSIGNMENT
PROCEDURE
IMPLEMENTATION
$u_c(y)$
METROLOGICAL TRACEABILITY
a) definition of SI unit by CGPM
b) primary reference measurement procedure
c) primary calibrator
d) secondary reference measurement procedure
e) secondary calibrator[b]
f) manufacturer's selected measurement procedure
g) manufacturer's working calibrator[b]
h) manufacturer's standing measurement procedure
i) manufacturer's product calibrator[b]
j) end-user's routine measurement procedure
routine sample
RESULT
BIPM, NMI [a], ARML [a]
BIPM, NMI [a]
NMI, ARML
NMI, ARML, ML
ML
ML
ML
ML
manufacturer and/or end-user
end-user
end-user
LiU EXPANDING REALITY

Accred Qual Assur (1999) 4:401–405

GENERAL PAPER

René Dybkaer

From total allowable error via metrological traceability to uncertainty of measurement of the unbiased result

Received: 5 March 1999
Accepted: 21 April 1999

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Abstract The concept of "total allowable error", investigated by Westgard and co-workers over a quarter of a century for use in laboratory medicine, comprises bias as well as random elements. Yet, to minimize diagnostic misclassifications, it is necessary to have spatio-temporal comparability of results. This requires trueness obtained through metrological traceability based on a calibration hierarchy. Hereby, the result is associated with a final uncertainty of measurement purged of known biases of procedure and laboratory. The sources of bias are discussed and the importance of commutability of calibrators and analytical specificity of the measurement procedure is stressed. The practicability of traceability to various levels and the advantages of the GUM approach for estimating uncertainty are shown.

Key words Metrological traceability · Total allowable error · Trueness · Unbiased result · Uncertainty of measurement

TY

Monitoring quality using patient samples

- Repeating measurements of a patient sample using many different instruments/methods enables you to accomplish several goals:
  - Estimate the **bias** between different analytical methods/instruments
  - Estimate the **random variation** when measuring individual analytes using the methods and instruments
  - Estimate **the total measurement variation** using several instruments/methods within the organisation to measure the same analyte

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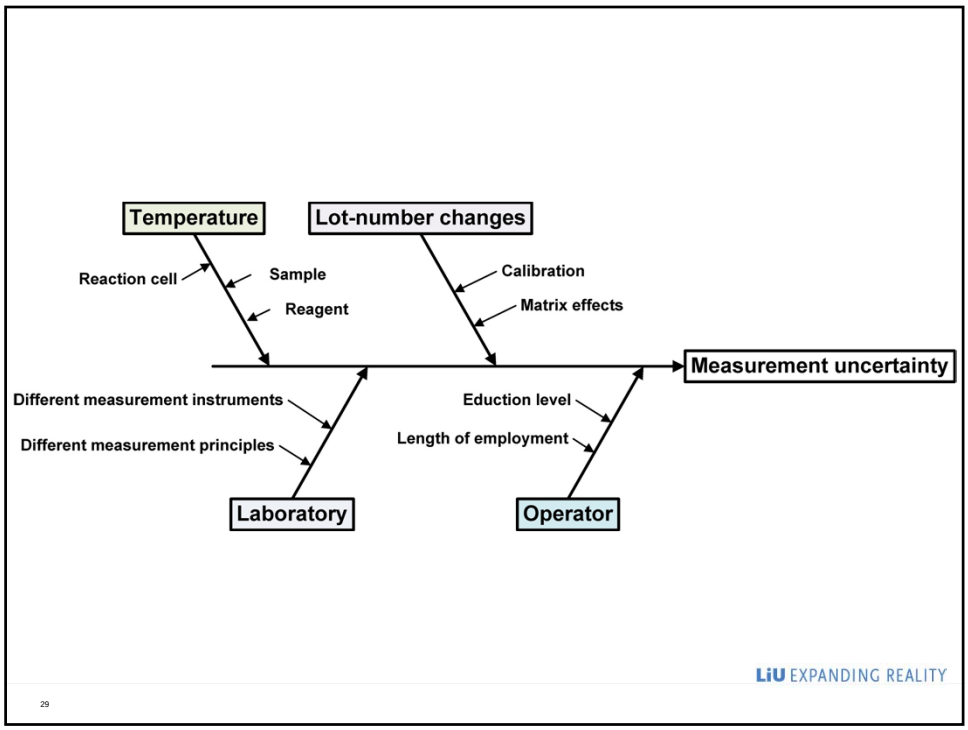
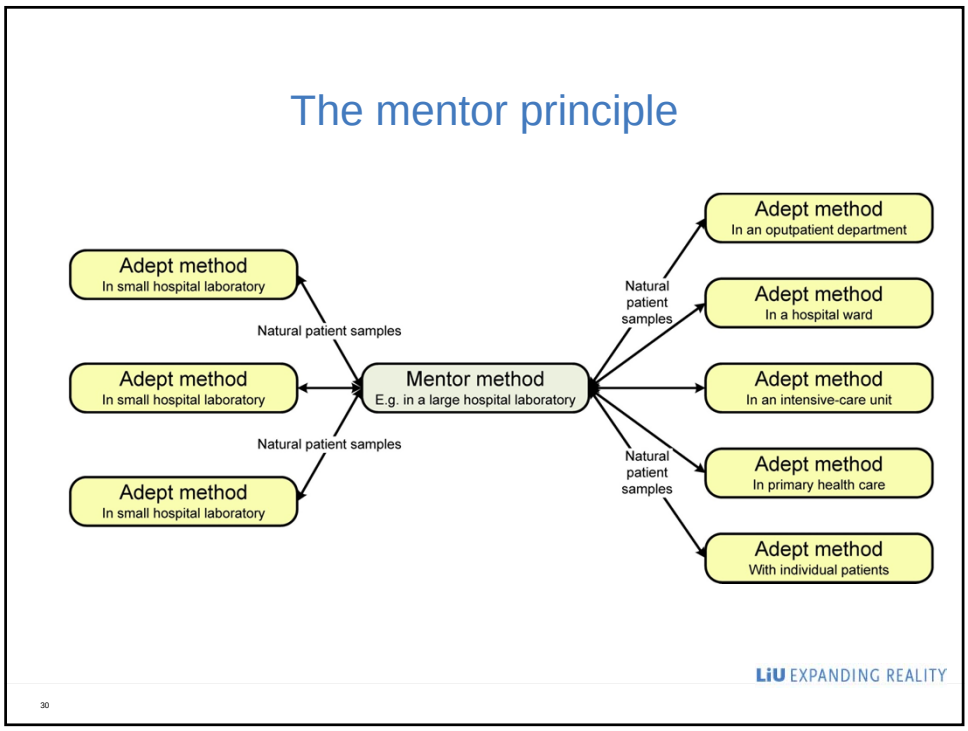

Temperature
Lot-number changes
Reaction cell
Sample
Reagent
Calibration
Matrix effects
Measurement uncertainty
Different measurement instruments
Different measurement principles
Laboratory
Eduction level
Length of employment
Operator
LiU EXPANDING REALITY
29


The mentor principle
Adept method
In small hospital laboratory
Adept method
In small hospital laboratory
Adept method
In small hospital laboratory
Natural patient samples
Natural patient samples
Mentor method
E.g. in a large hospital laboratory
Natural patient samples
Natural patient samples
Adept method
In an oputpatient department
Adept method
In a hospital ward
Adept method
In an intensive-care unit
Adept method
In primary health care
Adept method
With individual patients
LiU EXPANDING REALITY
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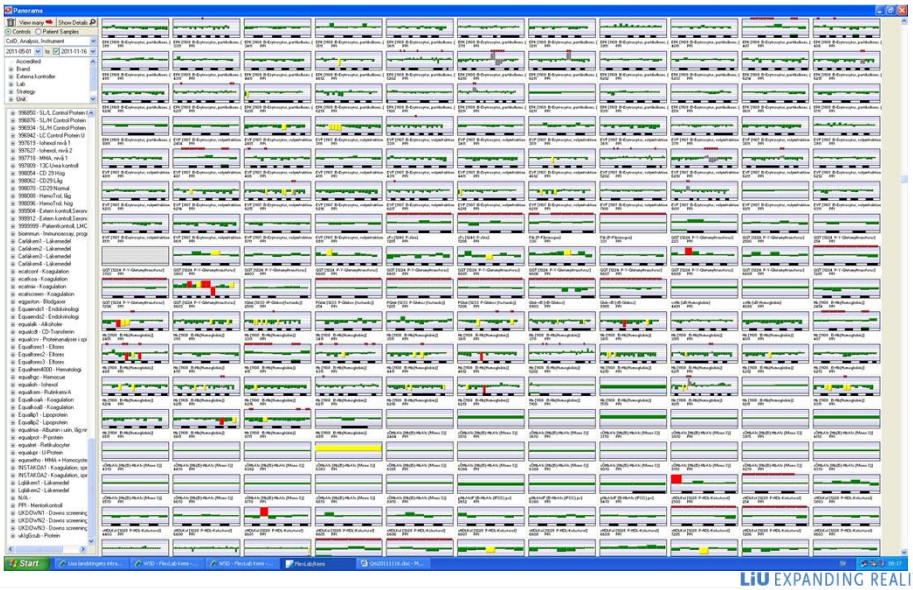

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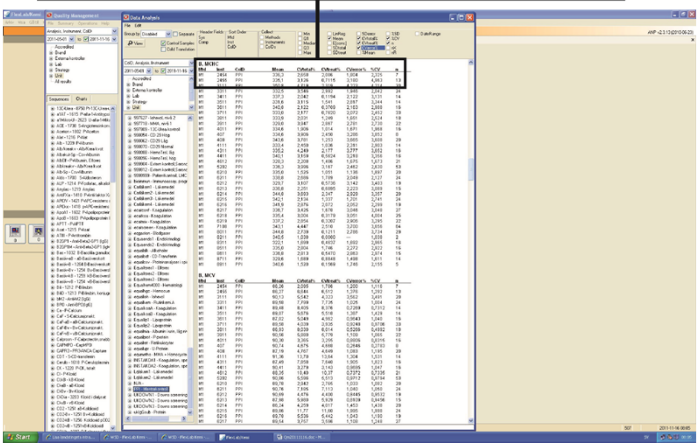
B, MCHC

Mtd	Inst	ColD	Mean	CVtotal%	CVtreat%	CVerror%	%CV	n
M1	2454	PPI	336,3	2,658	2,086	1,804	2,325	7
M1	2455	PPI	335,1	3,126	0,7115	3,180	4,963	13
M1	3111	PPI	350,8	4,719	2,319	4,222	4,214	20
M1	3311	PPI	332,5	3,546	2,992	1,946	2,042	24



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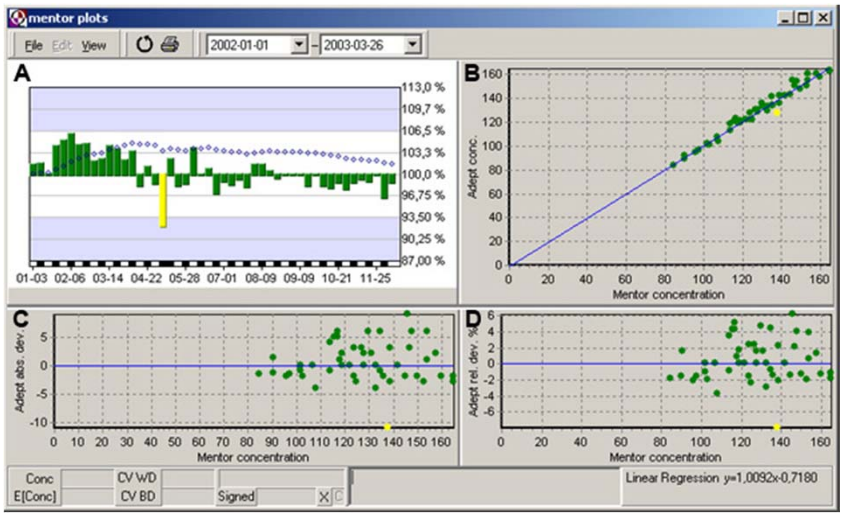

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33

Automatic pairing of the data

- If you are sending three patient control samples simultaneously – how can you know which samples should be paired?

Adept		Mentor	
99092581	130	131	99092580
99092580	133	128	99092581
99092582	155	154	99092582

The (bar)code used to identify the control sample plays a crucial role in identifying the samples

99092580 99092581
99092582
99092583

99999999

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Automatic pairing of data

- Two analytical results are considered stemming from the same sample if…
  - …the results stem from the same (bar) code uniquely identifying the sample
  - …the time stamp on the samples does not differ more than 72 hours
- The combined result (“the two become one”) is entered into the database using a common (bar)code identity

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Automatic pairing of results

- The combined results has the adept result as **measured** concentration and the mentor instruments result as the **determined** concentration for the actual control sample

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Example; controlling hemoglobin

- The **adept** instrument
  - Hemocue, e.g. in primary health care
- The **mentor** instrument
  - Celldyn 4000 on the central laboratory

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Using the data in the laboratory information system (LIMS)

- Four (bar)codes are (arbitrarily) defined in the LIMS for – in this case - haemoglobin:
  - 99092580
  - 99092581
  - 99092582
  - 99092583
- When the software gets results under these (bar)codes it recognizes them as control samples and automatically pairs them
- The paired control samples are stored in the LIMS under a predefined unique control sample identity – e.g. 99999999

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HB

Tidsstämpel	Instrument	Adept	Mentor
2002-07-01 12:00	925	163,0	165,0
2002-07-09 09:40	925	96,0	97,6
2002-07-15 07:30	925	101,0	102,0
2002-07-24 10:00	925	94,0	96,0
2002-07-29 09:40	925	130,0	128,0
2002-08-09 10:00	925	133,0	131,0
2002-08-15 09:29	925	155,0	154,0
2002-08-21 10:09	925	134,0	135,0
2002-08-30 10:30	925	119,0	119,0
2002-09-02 12:49	925	102,0	102,0
2002-09-09 11:10	925	122,0	122,0
2002-09-16 07:59	925	150,0	153,0
2002-09-23 10:50	925	128,0	128,0
2002-10-02 09:00	925	83,0	84,6
2002-10-08 10:00	925	136,0	139,0
2002-10-15 09:35	925	143,0	145,0
2002-10-21 10:02	925	143,0	145,0
2002-10-28 10:30	925	122,0	125,0
2002-11-04 11:39	925	134,0	136,0
2002-11-12 14:35	925	113,0	114,0
2002-11-19 08:50	925	158,0	160,0
2002-11-25 10:20	925	142,0	142,0
2002-12-02 10:50	925	104,0	108,0
2002-12-09 11:10	925	148,0	150,0

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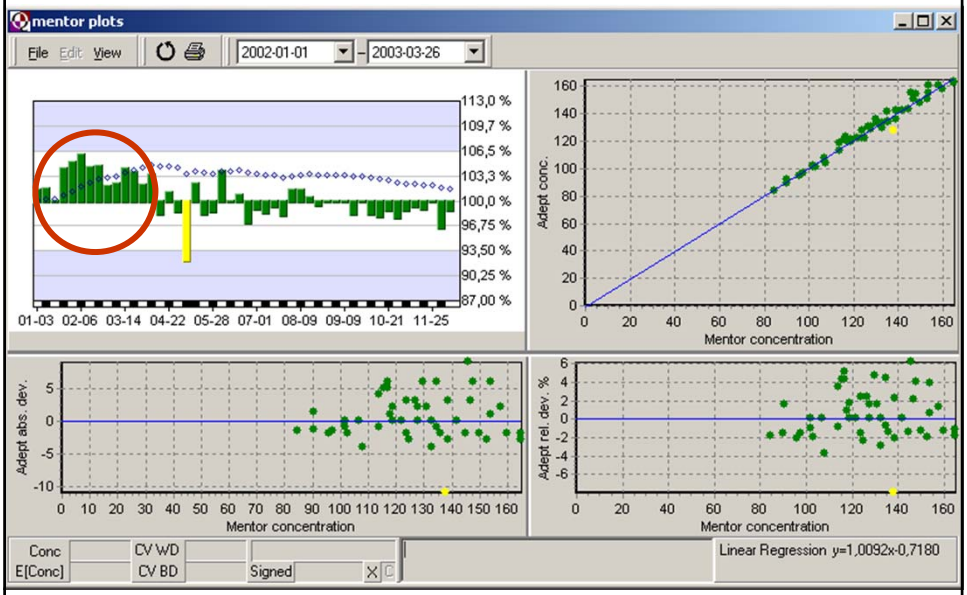

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Norming results

$$\text{Normed result} = \frac{\text{Adept} - \text{Mentor}}{\text{Mentor}} * 100$$

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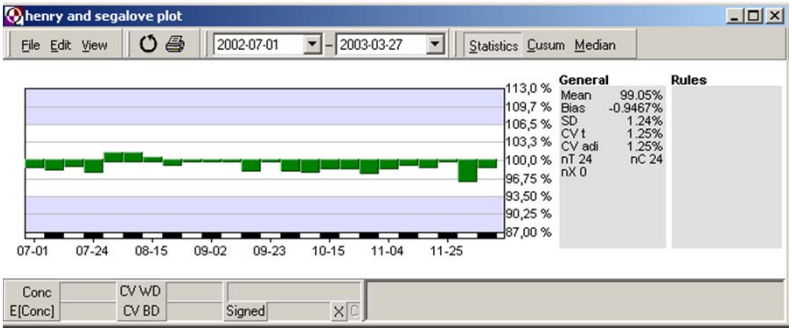
Norming the concentrations

Express each of the adept values as a percent of the corresponding mentor value.

"The results of the adept method in this case is about 1% lower than the measurements performed on the adept instrument. This relative deviation varies with a standard deviation of 1,24%

Tidsstämpel	Instrument	Adept	Mentor	Normerat värde
2002-07-01 12:00	925	163,0	165,0	98,79%
2002-07-09 09:40	925	96,0	97,6	98,36%
2002-07-15 07:30	925	101,0	102,0	99,02%
2002-07-24 10:00	925	94,0	96,0	97,92%
2002-07-29 09:40	925	130,0	128,0	101,56%
2002-08-09 10:00	925	133,0	131,0	101,53%
2002-08-15 09:29	925	155,0	154,0	100,65%
2002-08-21 10:09	925	134,0	135,0	99,26%
2002-08-30 10:30	925	119,0	119,0	100,00%
2002-09-02 12:49	925	102,0	102,0	100,00%
2002-09-09 11:10	925	122,0	122,0	100,00%
2002-09-16 07:59	925	150,0	153,0	98,04%
2002-09-23 10:50	925	128,0	128,0	100,00%
2002-10-02 09:00	925	83,0	84,6	98,11%
2002-10-08 10:00	925	136,0	139,0	97,84%
2002-10-15 09:35	925	143,0	145,0	98,62%
2002-10-21 10:02	925	143,0	145,0	98,62%
2002-10-28 10:30	925	122,0	125,0	97,60%
2002-11-04 11:39	925	134,0	136,0	98,53%
2002-11-12 14:35	925	113,0	114,0	99,12%
2002-11-19 08:50	925	158,0	160,0	98,75%
2002-11-25 10:20	925	142,0	142,0	100,00%
2002-12-02 10:50	925	104,0	108,0	96,30%
2002-12-09 11:10	925	148,0	150,0	98,67%
			Medelvärde	99,05%
			SD	1,24%

Norming the results



The results from the adept instrument/method as a negative bias of about 1% compared to the mentor instrument. This bias varies with a standard deviation of 1.24%

Two ways of estimating variation and bias

- **Simple**: Norming the values and calculating as usual using analysis of variance
- More **difficult** and more **powerful**: Analysis of covariance with linear regression

Analysis of covariance

- The mentor instruments defined ”the truth” and is therefore devoid of bias. It however has random error
- A measurement result made by the adept method can be represented by:

$$y_i = cx_i + d + e_{yi} - ce_{xi}$$

- **xi** is the concentration measured by the mentor instrument/method while c and d is the slope and intercept, respectively, of the straight line relation
- **exi** and **eyi** is the random error for the measurement for the mentor and adept method respectively

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Principally there are two types of mentor methods

- Mentor method **sending out** (the classical split sample method)
- Mentor method **sending in**

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Important characteristics of the mentor instrument/method

- The mentor method should have clear relation to Internationally accepted **calibrators** or
  - Widely accepted **reference method** or
  - **Absolute method**
- Those practically responsible for the mentor method **must have** special knowledge and interest in quality control
- All **fundamental characteristics** (volyme, temperature, absorbance etc.) concerning the mentor method must be regularly **controlled**

LiU EXPANDING REALITY

Important characteristics of the mentor instrument/method

- The results of the mentor method should be controlled using **at least two different programs for external quality control**
- Optimal **quality culture** in the organisation around the mentor instrument/method, including that all relevant information around the mentor instrument/method is made available throughout the organisation

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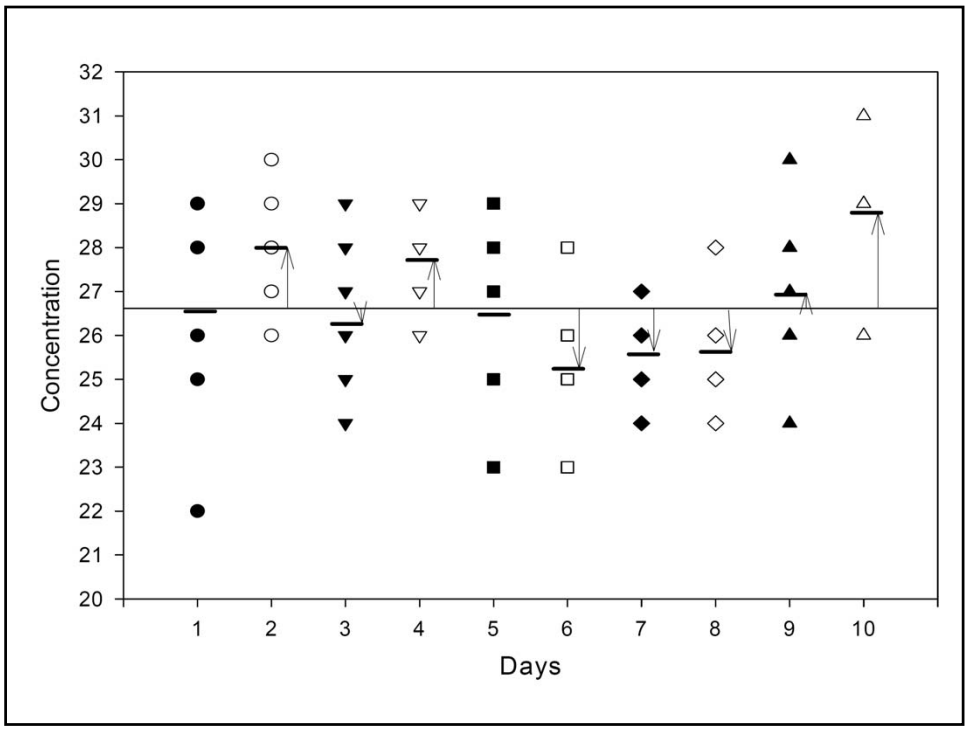

Balanced ANOVA

Source of variation	Sum of squares	Degrees of free-dom	Mean square	sd	Cv%
Within days	$ss_{wd} = \sum_{i=1}^{d}\sum_{j=1}^{n}\left(x_{ij} - \bar{x}_i\right)^2$	d(n-1)	$ms_{wd} = \frac{ss_{wd}}{d(n-1)}$	$sd_{wd} = \sqrt{ms_{wd}}$	$cv_{wd}\% = \frac{sd_{wd}}{\bar{\bar{x}}} * 100$
Between days	$ss_{bd} = \sum_{i=1}^{k} n\left(\bar{x}_i - \bar{\bar{x}}\right)^2$	d-1	$ms_{bd} = \frac{ss_{bd}}{(d-1)}$	$sd_{bd} = \sqrt{ms_{bd}}$	$cv_{bd}\% = \frac{sd_{bd}}{\bar{\bar{x}}} * 100$
Total	$ss_{tot} = \sum_{i=1}^{d}\sum_{j=1}^{n}\left(x_{ij} - \bar{\bar{x}}\right)^2$	dn-1	$ms_{tot} = \frac{ss_{tot}}{dn-1}$	$sd_{tot} = \sqrt{ms_{tot}}$	$cv_{tot}\% = \frac{sd_{tot}}{\bar{\bar{x}}} * 100$

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Unbalanced ANOVA

Source of variation	Sum of squares	Degrees of free-dom	Variance	sd	Cv%
Within days	$ss_{wd} = \sum_{i=1}^{d}\sum_{j=1}^{n_i} x_{ij}^2 - \sum_{i=1}^{d}\left(\frac{S_i^2}{n_i}\right)$	N-d	$\text{var}_{wd} = \frac{ss_{wd}}{N-d}$	$sd_{wd} = \sqrt{\text{var}_{wd}}$	$cv_{wd}\% = \frac{sd_{wd}}{\bar{\bar{x}}} * 100$
Between days	$ss_{bd} = \sum_{i=1}^{d}\left(\frac{s_i^2}{n_i}\right) - \frac{s^2}{N}$	d-1	$\text{var}_{bd} = \frac{ss_{bd}}{(d-1)}$	$sd_{bd} = \sqrt{\text{var}_{bd}}$	$cv_{bd}\% = \frac{sd_{bd}}{\bar{\bar{x}}} * 100$
Total	$ss_{tot} = \sum_{i=1}^{d}\sum_{j=1}^{n_i} x_{ij}^2 - \frac{S^2}{N}$	N-1	$\text{var}_{tot} = \frac{ss_{tot}}{N-1}$	$sd_{tot} = \sqrt{\text{var}_{tot}}$	$cv_{tot}\% = \frac{sd_{tot}}{\bar{\bar{x}}} * 100$

d=number of days/runs	N=total number of control samples
n_i =number of controls/day	S=total sum of observations
$\bar{\bar{x}}$ =mean of all observations	S_i=the sum of observations each day

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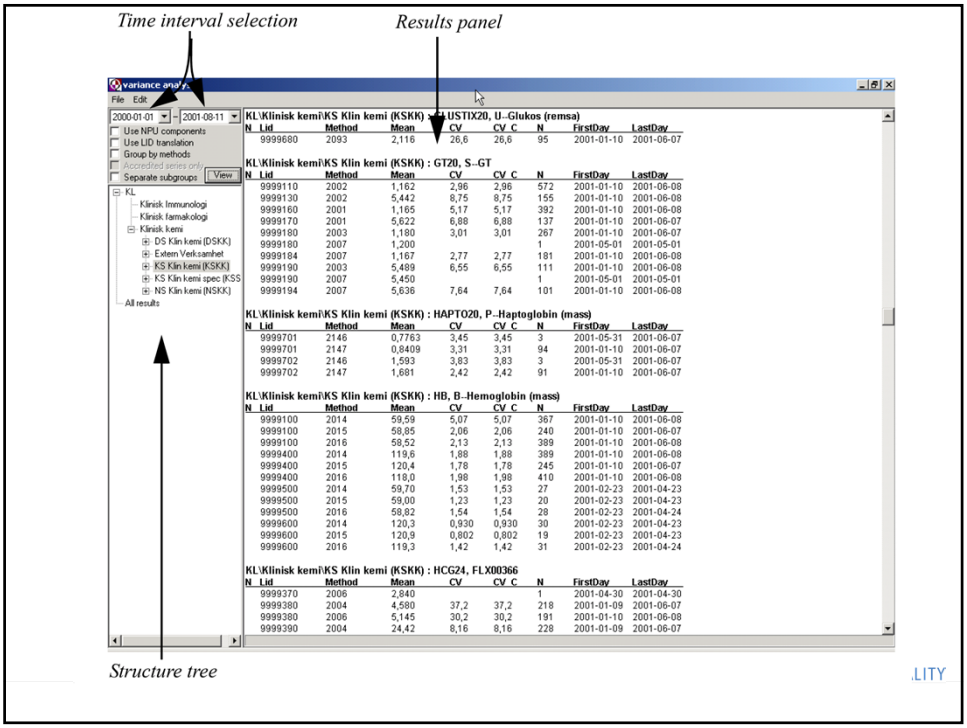

Procedures for control and calibration must be kept apart

- Natural patient samples can be used both for **secondary calibration** and for **quality control**
- Procedures, routines and series of defined numbers used for his **must not be mixed**
- **Secondary calibration** eliminates bias, reduces measurement uncertainty and the diagnostic uncertainty

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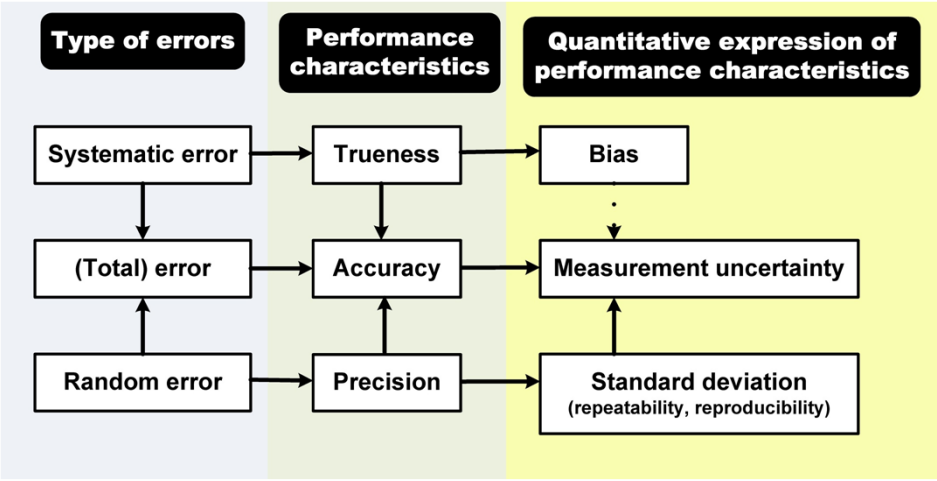

*Menditto, A., M. Patriarca, et al. (2007). "Understanding the meaning of accuracy, trueness and precision." Accred Qual Assur **12: 45-47.***

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