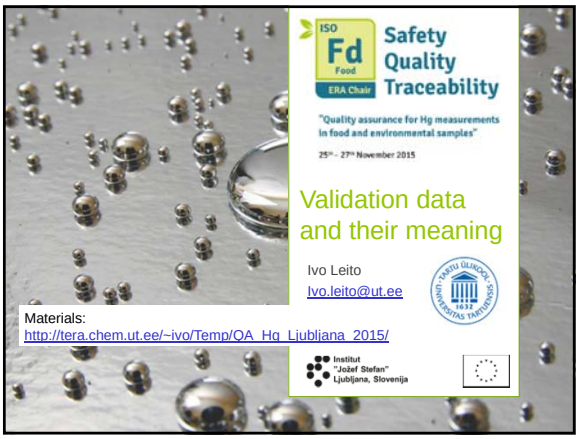
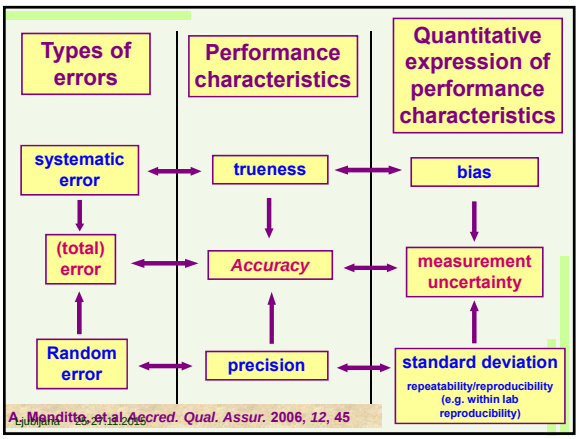


Overview

- We will look at measurement quality related concepts (precision, bias, …)
  - This discussion also serves as preparation for measurement uncertainty estimation
- We will try to **mimic the usual situations** in laboratories
- Slides contain **questions rather than answers**
  - We will find the answers in the course of the work



A. Mandiitto, et al. *Accred. Qual. Assur.* 2006, *12*, 45

Precision

What is the meaning of precision?

How is it usually expressed?

- Different precisions:
  - Repeatability (s_r)
  - Reproducibility
    - Within-lab reproducibility (s_{RW}), intermediate precision
    - Between-lab reproducibility (s_R)
    - …

Questions

What variations are taken into account by these standard deviations?

How do we know what type of precision we need?

Can precision be different at different concentrations?

How are the different types of precision related to measurement uncertainty?

How to determine precision?

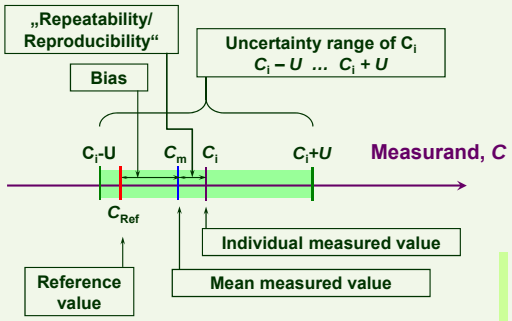
- Example:

An analyst analysed a food sample by HPLC. He carefully homogenized the sample in a blender and took a subsample. With the subsample he carried out sample preparation (consisting of extraction, precipitation and centrifugation). As a result he obtained a clear solution. He transferred it into a 100 ml volumetric flask and filled it up to the mark with the mobile phase. He analysed 10 aliquots of this solution during the same day and calculated the repeatability of the procedure as standard deviation of the results.

Did he do it right? If not, what should he do differently?

Trueness, Bias

Trueness Bias



Trueness Bias

Review
Special Focus Issue: Clinical Chemistry
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Bioanalysis

Bias in clinical chemistry

Bias in Clinical Chemistry. E. Theodorsson, B. Magnusson, I. Leito. Bioanalysis, **2014**, 6, 2855-2875.
(http://dx.doi.org/10.4155/bio.14.249)

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Clinical chemistry uses automated measurement techniques and medical knowledge in the interest of patients and healthy subjects. Automation has reduced repeatability and day-to-day variation considerably. Bias has been reduced to a lesser extent by reference measurement systems. It is vital to minimize clinically important bias, in particular bias within conglomerates of laboratories that measure samples from the same patients. Small and variable bias components will over time show random error properties and conventional random-error based methods for calculating measurement uncertainty can then be applied. The present overview of bias presents the general principles of error and uncertainty concepts, terminology and analysis, and suggests methods to minimize bias and measurement uncertainty in the interest of healthcare.

Background

Every year clinical chemistry laboratories commonly measure in the order of 20 mea- to minimize the overall measurement uncertainty for all available methods that samples from individual patients are likely to encoun-

Importance: systematic vs random effects

Trueness Bias

- Random effects have been efficiently decreased by instrument development
- Systematic effects are less affected

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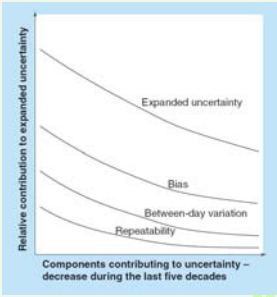

Random effects and replicates

Trueness Bias

- Random effects can be efficiently decreased by making replicate measurements
- There is no equally simple method for decreasing systematic effects

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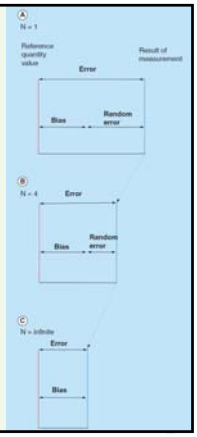


What is bias?

Trueness Bias

- Bias is …
  1. difference between the **measured value** and the **true value**
  2. Difference between the **measured value** and a **reference value**
  3. Difference between the **mean of a large number of replicate measured values** and the **true value**
  4. Difference between the **mean of a large number of replicate measured values** and a **reference value**

Which of these situations describe occurrence of bias?

Trueness Bias

1. All the results of a specific day are systematically influenced by the calibration graph of that day
2. Delicate analyte partially decomposes during sample preparation leading to lowered results
3. Samples and standards were both measured with a spectrophotometer which gives all absorbance values systematically lower by 5%
4. The titrant concentration determined on a particular day is slightly lower or higher than the true concentration
5. Because of the specifics of the used sample preparation procedure the sample is digested incompletely, leading to lowered values

Does bias depend on the time frame?

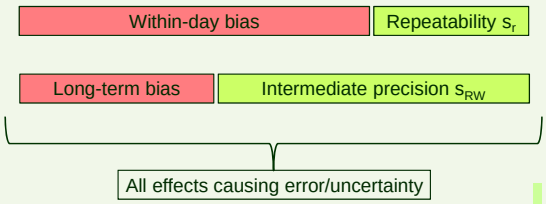
Trueness
Bias

1. Yes, bias determined within a single day is different from one determined on different days (and averaged)
2. No

Systematic and random effects

Trueness
Precision

- Random and systematic effects can be grouped differently:



The longer is the time frame the more effects change their „status“: systematic → random

Example: LC-MS determination of a delicate bioactive compound in blood plasma

Trueness
Bias

Effect	Systematic within day	Systematic in long term
Calibration graph of a specific day		
Injection volume of autosampler is 5% higher than nominal		
Delicate analyte partially decomposes at room temperature before samples are loaded into cooled autosampler		
Repeatability of peak integration		
Ionization suppression in the ESI source by a co-eluting compound		
Baseline noise		

Why is lab/method bias more useful than within-day bias?

Trueness
Bias

- Within-day bias should be redetermined every day
  - Long-term bias can be determined less frequently
- It is useful to work with the lowest possible bias
  - **s_{RW} can be determined more reliably than bias**
  - It is good if most of the uncertainty sources are included into the random component s_{RW}

From now on in this session we only address the long-term bias (lab/method bias)

Trueness / Bias

Trueness
Bias

What do we need in order to assess trueness of a procedure?

How do we express trueness numerically?

How do we choose how to express trueness?

Which are important issues in determining bias?

Trueness
Bias

Issue	*Bias*	s_{RW}
Sufficient number of replicates		
Sufficiently long timeframe		
Homogeneous sample		
Matrix match		
Concentration range match		
Reliable reference value		
Determination of one can be hindered by the other		

Which are the most reliable approaches for determining bias?

Trueness
Bias

Approach	How good?
Analysing spiked blank matrix	
Replicate measurements of a routine sample	
Using a PT sample and consensus value as reference value	
Analysing a CRM	
Analysing a routine sample with your own procedure and with a reference procedure	

How to calculate/express bias?

Trueness
Bias

Way of expressing	Formula	When to use?
Absolute bias	$bias = C_{lab\_mean} - C_{ref}$	
Relative bias	$bias = \frac{C_{lab\_mean} - C_{ref}}{C_{ref}}$	
Recovery	$R = \frac{C_{\text{uncorrected}}}{C_{\text{Ref}}}$	
Recovery	$R = \frac{C_1 - C_0}{\Delta C}$	

Recovery, R

Trueness
Recovery

When is recovery an important parameter?

Relation between recovery and bias?

Can recovery be above 1 (above 100%)? If yes, then what could this mean?

How can recovery be determined?

Recovery from spiking

Trueness
Recovery

$$R = \frac{C_1 - C_0}{\Delta C}$$

What are the meanings of the terms in the equation?

How will the equation change if it is possible to obtain a blank sample?

How to conduct a spiking experiment?

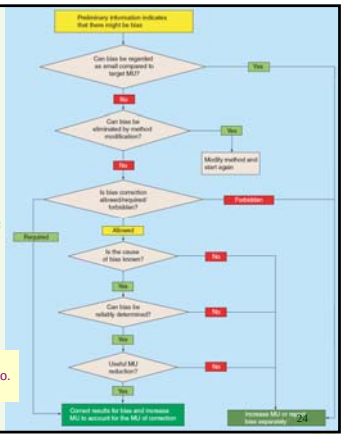
Trueness
Recovery

- *Two analysts determined meropenem (an antibiotic) in blood plasma. Both needed to determine the recovery of the procedure. They obtained blank plasma samples and did the following:*
- ***Analyst 1** took 500 µl of the blank plasma and added 400 µl of methanol for protein precipitation. He separated the precipitated proteins by centrifugation and transferred the supernatant into an HPLC vial. 100 µl of meropenem standard solution with suitable concentration was added to the supernatant and the resulting solution was injected into the HPLC system for analysis.*
- ***Analyst 2** took 500 µl of the blank plasma, added 100 µl of meropenem standard solution and mixed well. She then added 400 µl of methanol for protein precipitation. She separated the precipitated proteins by centrifugation and injected the resulting supernatant into the HPLC system for analysis.*

Which analyst did it more correctly? Why?

Bias/recovery correction?

- What should we do with our results if there is evidence of bias?



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Measurement uncertainty sources

Uncertainty sources

What is the uncertainty source?

Why do we need to know them?

How chall we use this knowledge in uncertainty estimation?

Which of the following are uncertainty sources in chemical analysis? Please explain. Part I

Uncertainty sources

- Spectrophotometric cell pathlength is not exactly 1 cm
- Analyte partially decomposes during sample preparation
- The sample contains a substance that interferes with the derivatisation reaction and leads to less than 100% derivatisation efficiency
- Part of the sample solution was spilled during quantitative transfer
- Subsample is taken for analysis from a sample that is inhomogenous
- There is scatter of data points around the calibration line

Which of the following are uncertainty sources in chemical analysis? Please explain. Part II

Uncertainty sources

- On a chromatogram a small peak is found, which partially interferes with the analyte peak
- Injection volume in GC varies between chromatograms
- In HPLC wavelength 290 nm is accidentally used instead of 280 nm
- A C18 column is used in HPLC, but not the same brand as defined in the procedure
- Unstable analyte partially decomposes in autosampler
- Partial ionization of the analyte at the mobile phase pH

What are the uncertainty sources in photometric NO_2^- determination?

Uncertainty sources

- **Sample: wastewater**
- **Sample preparation**: 25 ml of sample (not filtered) is measured with a graduated cylinder then 0.5 ml of sulphanylamide is added and the solution is allowed to stand for 3 minutes. After that 0.5 ml of diamine (NEDA) is added additionally. The absorbance of the sample solution is then measured at 540 nm.
- **Calibration graph**: A series of calibration standard solution containing 0.2, 0.7, 1.0, 1.2, 1.4, 1.6 ml of nitrite standard solution were prepared in 50 ml volumetric flasks. The flasks were then made up to the mark with distilled water. Then 25 ml of each solution is measured with graduated cylinder. 0.5 ml of sulphanylamide is added to each graduated cylinder and after the solutions have been left to stand for 3 minutes, 0.5 ml of diamine is added to each graduated cylinder. The absorbances of these solutions are then measured at 540 nm and calibration graph is built.
- Nitrite **concentration in sample** is deremined from the graph

How to take into account the uncertainty sources?

Uncertainty sources

- Uncertainty sources from the previous slide
- Peak overlap in chromatography
- Irreproducible injection volume in GC
- Partial decomposition of the analyte during sample preparation

Uncertainty estimation approaches

Uncertainty estimation approaches

What approaches exist?

How to choose, which one to use?

What data are needed?

Thank you for your participation!

- The materials are available from:
  http://tera.chem.ut.ee/~ivo/Temp/QA_Hg_Ljubljana_2015/
- More explanations and examples:
  http://sisu.ut.ee/measurement/
- You are always welcome to contact me:
  ivo.leito@ut.ee

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32