

Using method validation and performance data for estimating measurement uncertainty

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Overview

- The main question of uncertainty evaluation
- The different approaches
 - (Modelling approach)
 - Approach based on validation and QC data
- The role of performance data
 - Precision
 - (Trueness, bias)

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The main question of uncertainty evaluation in an analytical lab:

The uncertainty sources are more or less known

There are different data available (control charts, PT results, parallel measurements ...)

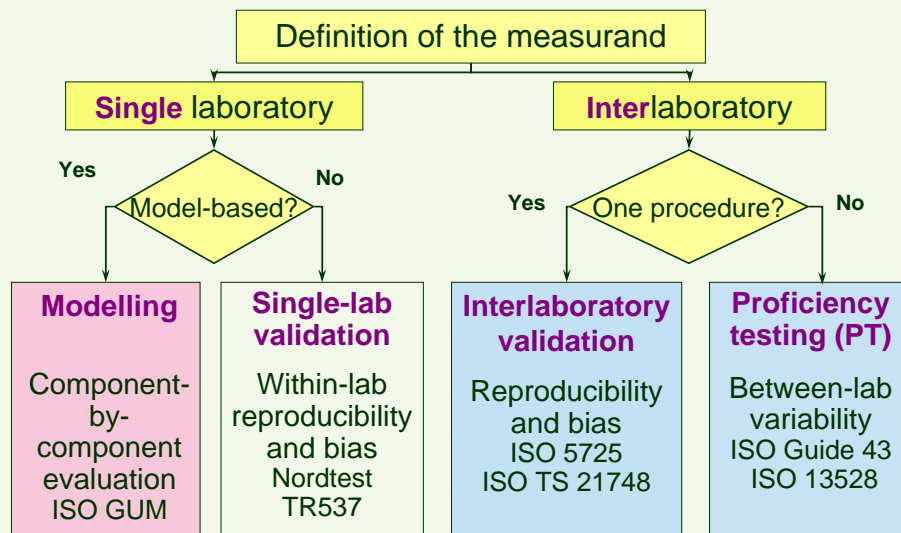
How to use these data to take these uncertainty sources into account?

Different approaches offer different solutions to this question

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Uncertainty estimation approaches



Eurolab Technical Report No 1/2007 Available from: <http://www.eurolab.org/>

Uncertainty estimates by different approaches

- Modelling (classical ISO GUM)
 - Uncertainty of an **individual result** of a measurement can be obtained
- Single-lab validation
 - Typical uncertainty of results obtained using a **procedure in the laboratory**
- Interlaboratory validation
 - Uncertainty of results obtained using the same **procedure in different laboratories**

These uncertainties refer to different situations!

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The Modelling Approach

Component by component evaluation

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Validation parameters (procedure characteristics)

- identity, selectivity, specificity
- limit of detection
- limit of quantitation
- linear range
- accuracy, trueness (recovery), precision
- sensitivity
- ruggedness/robustness

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Examples:

http://www.ut.ee/katsekoda/GUM_examples/

Examples of Measurement Uncertainty Budgets for Chemical Analysis (ppt, dissolved oxygen, sensor - Windows Internet Explorer)

Examples of Measurement Uncertainty Budgets in Analytical Chemistry

If you do not have online access to the cited publications, please contact no.letso@ut.ee

Measurement	Complexity of measurement	Elaboration level	Extent of comments	Description	Available files
Measurement uncertainty due to the matrix effect in LC-ESI-MS	High	High	High	This work (A. Kõiva, K. Heodes, I. Leito, <i>J. AOAC International</i> 2010, 93, 305-313) presents an empirical approach—the matrix effect graph approach—for estimating the uncertainty due to the matrix effect in LC-MS (with the electrospray (ESI) ion source) analysis of pesticide residues in fruits and vegetables. At certain time intervals (1 month), a calibration graph using extracts of different fruits/vegetables as calibration solutions is prepared, and a regression line is fitted through these data. These fruits/vegetables may be either from the commodity group of the samples or from different commodity groups. The relative residuals of the calibration point peak areas are calculated and plotted against the measurement time—the matrix effect graph is then obtained. The root mean square of the relative residuals is calculated and used as the estimate of relative uncertainty of the sample peak areas caused by the matrix effect. The matrix effect graph obtained over fruits/vegetables from different commodity groups can also be used to identify fruits/vegetables with extreme matrix effects.	Full text of the article (please contact us if you do not have online access to this article)
Measurement uncertainty of measurement with amperometric sensors	Medium	High	High	This tutorial review (E. Sõm, L. Jalakas, J. Leito, <i>Sensors</i> 2010, 10, 4430-4455) focuses on measurement uncertainty estimation in amperometric sensors (both for liquid and gas-phase measurements). The main uncertainty sources are reviewed and their contributions are discussed with relation to the principles of operation of the sensors, measurement conditions and properties of the measured samples. The discussion is illustrated by case studies based on the two major approaches for uncertainty evaluation—the ISO GUM modeling approach and the Nordtest approach. This tutorial is expected to be of interest to workers in different fields of science who use measurements with amperometric sensors and need to evaluate the uncertainty of the obtained results but are new to the concept of measurement uncertainty. The tutorial is also expected to be indicative in order to make measurement results more accurate.	Full text of the article (it is an open-access article, so the full text is freely available)
Electron probe microanalysis (SEM-EDS)	High	High	High	Determination of iron in ink writing on paper manuscripts using electron probe microanalysis (SEM-EDS). Full information, with detailed explanations on uncertainty sources and their quantification is available in publication K. Vana, E. Kõivumäe, O. Vainavaara, V. Sõnnemäe, J. Aasa, I. Paama, J. Jürgens, I. Leito, <i>Microchimica Acta</i> , published online 05.09.2007.	URL
Analysis of gold alloys by flame-AAS	High	High	High	Detailed example, covering not only uncertainty estimation but also validation and establishing traceability.	Chapter 2 in Practical Examples on Traceability, Measurement Uncertainty and Validation in Chemistry Volume 4

Examples:

http://www.ut.ee/katsekoda/GUM_examples/

Examples of Measurement Uncertainty Budgets for Chemical Analysis (pH, dissolved oxygen, sensor) - Windows Internet Explorer

Analysis of gold alloys by flame-AAS	High	High	High	Detailed example, covering not only uncertainty estimation but also validation and establishing traceability	Chapter 2 in Practical Examples on Traceability, Measurement Uncertainty and Validation in Chemistry Volume 1
Determination of calcium in serum by spectrophotometry	High	High	High	Detailed example, covering not only uncertainty estimation but also validation and establishing traceability	Chapter 3 in Practical Examples on Traceability, Measurement Uncertainty and Validation in Chemistry Volume 1
Determination of radium in water by α -spectrometry	High	High	High	Detailed example, covering not only uncertainty estimation but also validation and establishing traceability	Chapter 4 in Practical Examples on Traceability, Measurement Uncertainty and Validation in Chemistry Volume 1
Determination of polar pesticides by liquid chromatography mass spectrometry	High	High	High	Detailed example, covering not only uncertainty estimation but also validation and establishing traceability	Chapter 5 in Practical Examples on Traceability, Measurement Uncertainty and Validation in Chemistry Volume 1
Determination of ammonium in water by flow analysis (CFA) and spectrometric detection	High	High	High	Detailed example, covering not only uncertainty estimation but also validation and establishing traceability	Chapter 6 in Practical Examples on Traceability, Measurement Uncertainty and Validation in Chemistry Volume 1
Simple weighing	Simple	Medium	Medium	Uncertainty of simple weighing	GUM Workbench PDF printout XLS
Volume of 50 ml volumetric flask	Simple	Medium	Medium	Uncertainty of volume of solution contained in 50 ml volumetric flask	GUM Workbench PDF printout XLS
Volume of 10 ml pipette	Simple	Medium	Medium	Uncertainty of volume of solution delivered by 10 ml bulb pipette	GUM Workbench PDF printout XLS
Nonvolatile matter by gravimetry	Medium	Medium	Medium	Routine determination of nonvolatile matter by gravimetry. The sample was weighed before and after drying in oven at a specified temperature (please see the presentation ISO GUM Uncertainty in Chemistry)	In English: GUM Workbench PDF printout In Estonian: GUM Workbench PDF printout
pH measurement	Medium	High	Low	The uncertainty calculation for pH is available as a web application (server-based, written in PHP). This means that calculation can be carried out immediately in the browser and there is no need to install any software. The result can be displayed either as a simple or as a detailed result. In the latter case the measurement equation and detailed uncertainty budget are also displayed. Additional information is available in the help file of the web application and in the articles L. Leito, L. Struuts, E. Koort, V. Pihl, Accred. Qual. Assur. 2002, 7, 242-249 and E. Koort, K. Herodes, V. Pihl, J. Leto, Anal. Bioanal. Chem. 2004, 379, 720-729. For more information see also the PhD thesis of Eva Koort (defended on June 20, 2005).	In English: Web application
Dissolved oxygen concentration	Medium	High	High	This uncertainty estimation procedure is intended for the mainstream	In English:

Examples:

http://www.ut.ee/katsekoda/GUM_examples/

Examples of Measurement Uncertainty Budgets for Chemical Analysis (pH, dissolved oxygen, sensor) - Windows Internet Explorer

Dissolved oxygen concentration measurement	Medium	High	High	This uncertainty estimation procedure is intended for the mainstream dissolved oxygen concentration measurement with the galvanic type of equipment. Details can be found in the article L. Leito, L. Struuts, E. Koort, V. Pihl, Accred. Qual. Assur. 2002, 7, 242-249 and E. Koort, K. Herodes, V. Pihl, J. Leto, Anal. Bioanal. Chem. 2004, 379, 720-729. For more information see also the PhD thesis of Eva Koort (defended on June 20, 2005).	In English: XLS calculation file
Complexometric titration	Medium	Medium	Medium	Complexometric determination (EDTA) of total hardness of water	GUM Workbench PDF printout
Ammonium by Photometry	High	High	High	A mainstream measurement of NH_4^+ by photometry. Contains XLS import. The corresponding SMU and XLS file must be placed in the same folder. The method is based on ISO 7150-1:1984. This is a tricky example. After several years of discussion and careful study we now believe that the uncertainty estimate in this example is not underestimated if the determination is carried out carefully and if there is no strong strong chemical interference. (please see the presentation ISO GUM Uncertainty in Chemistry). In any case, use with care! For deeper coverage of uncertainty sources in photometric analysis see the paper L. Soosalu, E. J. Rõõm, A. Kutt, I. Kaljarand, J. Leto, Accred. Qual. Assur. 2006, 11, 246-255 and the PhD thesis of Liili Soosalu (defended on June 20, 2005).	In English: GUM Workbench PDF printout Auxiliary XLS
Nitrite by Photometry	High	High	High	Photometric determination of nitrite using the NEDA-sulfanilamide method. For deeper coverage of uncertainty sources in photometric analysis see the paper L. Soosalu, E. J. Rõõm, A. Kutt, I. Kaljarand, J. Leto, Accred. Qual. Assur. 2006, 11, 246-255 (published online on 25.04.06) and the PhD thesis of Liili Soosalu (defended on June 20, 2005).	GUM Workbench PDF printout
Butanol in acetone by GC	High	High	Medium	Measurement of butanol content in acetone by GC. Very small solution volumes are used in this method and all solutions are prepared by weighing. The largest uncertainty contributions are due to the imperfections of integrating peaks on the chromatogram and drift of the balance, which is mainly due to the volatility of acetone.	In English: GUM Workbench PDF printout
Sorbic acid by HPLC	High	Low	Medium	Mainstream liquid chromatography (HPLC) method for determination of preservatives (Sorbic acid in this example). Main parameters of the method: Isocratic elution (Acetate buffer - MeOH, 70:30), RP-C18 column, UV-Vis photometric detection at 235 nm.	In English: GUM Workbench PDF printout In Estonian: GUM Workbench PDF printout
Quality control of a drug product by HPLC	High	High	High	Liquid chromatography (HPLC) determination of Simvastatin in tablets. The method is a mainstream HPLC method with UV-Vis photometric detection at 238 nm. Two varieties are provided: 5-point calibration and single point calibration. This uncertainty estimation has been published in the following paper: S. Leito, K. Mälder, A. Kinnajõe, K. Herodes, L. Leto, J. Chrom. A 2006, 1121, 55-63.	In English: 5-point calibration: GUM Workbench PDF printout Single point calibration: GUM Workbench PDF printout

Examples:

http://www.ut.ee/katsekoda/GUM_examples/

Examples of Measurement Uncertainty Budgets for Chemical Analysis [pdf, downloaded crystals, sensor - Windows Internet Explorer]

Measurement	High	High	High	Description	Files
by HPLC				The method is a mainstream HPLC method with UV-Vis photometric detection at 238 nm. Two variables are provided: 5-point calibration and single point calibration. This uncertainty estimation has been published in the following paper: S. Leito, K. Mõlder, A. Kinnasas, K. Heodes, I. Leito, <i>J. Chrom. A</i> 2006, 1127, 52-53	GUM Workbench PDF printout Single-point calibration: GUM Workbench PDF printout
Phosphorus Content in Feed by Photometry	High	High	High	Measurement uncertainty estimation example on photometric determination of phosphorus in feed using the molybdovanadate reagent. The largest uncertainty contribution is due to the sample preparation. For deeper coverage of measurement uncertainty sources in photometric analysis see the following paper: L. Soovik, E.-L. Rõm, A. Kum, I. Kaljurand, I. Leito, <i>Accred. Qual. Assur.</i> 2006, 11, 245-255	GUM Workbench PDF printout
Lead in Soil by AAS	High	High	High	Measurement of Lead content of soil by graphite furnace atomic absorption spectrometry.	GUM Workbench PDF printout XLS

Comments

Files
The uncertainty budgets are available in files of following types:

- GUM Workbench files (extension SMU) have been created by GUM Workbench TrainMC 1.3 (Metrodata GmbH)
- PDF printouts of the SMU files (not all people have the GUM Workbench software. The printout contains all the essential information about the uncertainty example)
- Excel files (extension XLS) have been created by MS Excel 97 (Microsoft Inc.). Some of them are standalone uncertainty budgets, some are just auxiliary files (containing input data) for SMU files.

 Most of the files are in English. In some cases files in other languages are also available.

Complexity, elaboration level and extent of comments
The "complexity of measurement" refers to the intrinsic complexity of the measurement itself (the more there are operations and measurements, the higher the complexity).
The "elaboration level" refers to the extent to which various uncertainty sources have been identified and taken into account. Low elaboration level does not necessarily mean that there are important uncertainty sources that have not been taken into account, instead it usually means that here and there several different uncertainty sources have been grouped. For example, instead of identifying all the repeatability contributions, they may have been grouped to give the general repeatability of the procedure that can be estimated from overall repeatability studies.
The "extent of comments" indicates how much comment is added to the file to increase the readability by users.

Estimates of uncertainty components
Generally the uncertainty components have been estimated according to the particular equipment and working practices used in our lab. In some cases reasonable estimates (based on experience or literature data) are used. The obtained uncertainty values have proved to be adequate for those conditions. However, these uncertainty values are not directly applicable to results obtained in other laboratories using different instrumentation and working practices (even if exactly the same measurement procedures are used) because they are dependent on the conditions. These values should thus be used for guidance only. The users of the examples are strongly recommended to do their own estimation of uncertainty components based on their own equipment and working practices and then insert the uncertainty data into the files from this page. Also, it is strongly recommended to see the presentations [ISO/GUM Uncertainty in Chemistry](#) (presented in 2004 in Stockholm on MC teaching workshop) and [Different Approaches to Estimation of Measurement Uncertainty in Analytical Chemistry](#) (presented in 2010 in Tallinn at InAnalytica XII).
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Feedback
Any feedback (comments, minor corrections, criticism) is most welcome! The feedback should be sent to Ivo Leito (ivo.leito@ut.ee, +372 5 194 177, I. University of Tartu)

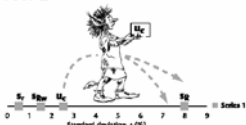
Approach Based on Validation and Quality Control Data

on the example of the Nordtest approach

NORDTEST Report TR 537
Approved 2004-02

HANDBOOK FOR CALCULATION OF MEASUREMENT UNCERTAINTY IN ENVIRONMENTAL LABORATORIES

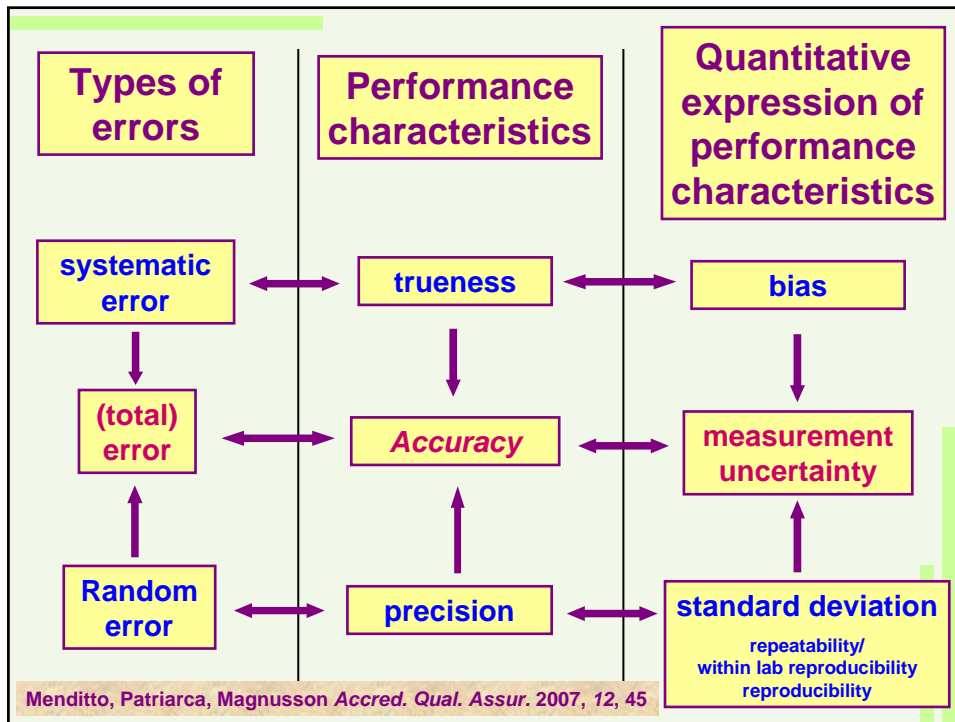
EDITION 2



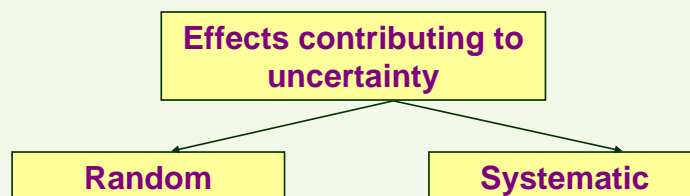
Bertil Magnusson
Teemu Näykki
Håvard Hovind
Mikael Krysell

Nordtest Technical Report 537, 2nd ed (2004)

<http://www.nordtest.info/index.php/technical-reports/category/chemistry.html>



Single-laboratory validation approach



- The two groups of uncertainty contributions are quantified separately and then combined

Single lab validation approach: in practice (1)

- The main equation:

$$u_c = \sqrt{u(R_w)^2 + u(bias)^2}$$

Within-laboratory
reproducibility

This component
accounts for the
random effects

Uncertainty of the
estimate of the
laboratory and the
method bias

This component
accounts for the
systematic effects

- This and subsequent equations work with absolute and relative values

Nordtest Technical Report 537, 2nd ed (2004)

<http://www.nordtest.info/index.php/technical-reports/category/chemistry.html>

Validation parameters (procedure characteristics)

- identity, selectivity, specificity
- limit of detection
- limit of quantitation
- linear range
- **accuracy, trueness (recovery), precision**
- sensitivity
- ruggedness/robustness

Precision component $u(R_w)$

$u(R_w) = s_{RW}$ is usually found from:

- the warning limits of X chart
– using a stable control sample
- long term pooled standard deviation

Ideally: separately for different matrices and different concentration levels!

The control sample analysis has to cover the whole analytical process

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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 50 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 within-lab reproducibility as standard deviation of the results.

Did he do it right?

If not, what should he do differently?

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Determining precision when sample is stable for a long time

Precision

Determination of fat content

Date	Sample	Result (g/100g)
10.02.2008	27	22.5
16.02.2008	27	21.8
26.02.2008	27	22.4
7.03.2008	27	23.6
17.03.2008	27	23.9
27.03.2008	27	23.4
6.04.2008	27	23.7
16.04.2008	27	23.9
26.04.2008	27	22.1
6.05.2008	27	25.8
16.05.2008	27	22.1
26.05.2008	27	23.2
5.06.2008	27	22.2

Mean: 23.1 g/100g

St Dev: 1.1 g/100g

DF: 12

Within-lab reproducibility s_{RW}

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Pooled Standard Deviation

- General formula:

$$s_{\text{pooled}} = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \dots + (n_k - 1)s_k^2}{n_1 + n_2 + \dots + n_k - k}}$$

- Symbols:
 - k number of groups (in this case samples)
 - s_1, s_2, \dots are within group standard deviations
 - n_1, n_2, \dots are numbers of measurements made with different samples

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Determining precision when sample is not stable for a long time

Precision

Pooled Std Dev
Determination of protein content

Date	Sample	Result (g/100g)	Sample	St dev g/100g	Comp.
10.02.2008	1	10.2	1	0.354	0.125
10.02.2008	2	13.4	2	0.572	0.980
10.02.2008	3	17.6	3	0.473	0.447
13.02.2008	1	10.7	4	0.681	0.927
13.02.2008	2	14.2	5	0.707	0.500
13.02.2008	4	16.9	6	0.663	1.320
18.02.2008	2	12.9			
18.02.2008	3	16.7			
18.02.2008	6	12.1			
25.02.2008	6	13.5			
25.02.2008	4	17.2			
25.02.2008	5	19.2			
4.03.2008	2	13.1			
4.03.2008	6	12.9			
8.03.2008	3	17.4			
8.03.2008	4	18.2			
8.03.2008	5	13.2			
8.03.2008	6	13.5			

s_pooled = 0.598 g/100g

DF: 12

Within-lab reproducibility s_{RW}

Different sample matrixes!

But less "long-term"

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$u(bias)$

Trueness, bias

- The **bias** of lab's results from the best estimate of true value is taken into account
- $u(bias)$ can be found:
 - From analysis of the same samples with a reference procedure
 - From analysis of certified reference materials (CRMs)
 - From interlaboratory comparison measurements
 - From spiking experiments

Ideally: several reference materials, several spikings (bias will in most cases vary with matrix and concentration range)

Necessarily: several replicate measurements for the same CRM

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u(bias) Trueness, bias

$$bias_i = C_{lab_i} - C_{ref}$$

$$RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n}} \quad u(C_{ref}) = \sqrt{\frac{\sum u(C_{ref}_i)^2}{n}}$$

$$u(bias) = \sqrt{RMS_{bias}^2 + u(C_{ref})^2}$$

This component accounts for the **average bias** of the laboratory results from the C_{ref}

This component accounts for the **average uncertainty** of the reference values C_{ref}

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How to conduct a spiking experiment? Trueness, bias

- Two analysts determined meropenem (an antibiotic) in blood plasma. Both needed to determine the bias 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. He separated the precipitated proteins by centrifugation and transferred the supernatant into an HPLC vial. He then added 100 µl of meropenem standard solution with suitable concentration to the supernatant and injected the resulting solution into the HPLC system for analysis.

Analyte has to be added at as early stage as possible!
- Analyst 2** took 500 µl of the blank plasma and added 500 µl of methanol, which contained a suitable amount of meropenem. She separated the precipitated proteins by centrifugation and injected the resulting supernatant into the HPLC system for analysis.

Which analyst did it correctly? Why?

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Absolute vs relative uncertainties: Rules of Thumb

- **At low concentrations (near detection limit, trace level) use absolute uncertainties**
 - Uncertainty is not much dependent on analyte level
- **At medium and higher concentrations use relative uncertainties**
 - Uncertainty is roughly proportional to analyte level
- **In general: whichever is more constant**

Appendix E.5 from *Quantifying Uncertainty in Analytical Measurement*, EURACHEM/CITAC Guide, Third Edition (2012)
Available from: <http://www.eurachem.org/>

Single lab validation approach: Determination of ammonium in water

- According to EN/ISO 11732
- Concentration level 200 mg/L
- From the X chart: warning limits are set to $\pm 3.34\%$
 - Warning limits are set to $2s$
 - Thus $u(R_w) = s_{RW} = 3.34\% / 2 = 1.67\%$

This is the result of a particular analysis for which we want to calculate uncertainty

Example from: Nordtest Technical Report 537, 2nd ed (2004)
<http://www.nordtest.info/index.php/technical-reports/category/chemistry.html>

Single lab validation approach: Determination of ammonium in water

- From the interlaboratory comparison results bias over 3 years has been: +2.4%, +2.7%, +1.9%, +1.4%, +1.8% and +2.9%.
 - Thus $RMS_{\text{bias}} = 2.25\%$
 - Uncertainty of consensus values is estimated as $u(\text{Cref}) = 1.5\%$
 - Thus $u(\text{bias}) = 2.71\%$
- Standard uncertainty:
$$u_{\text{bias}} = \sqrt{2.25^2 + 1.5^2} = 2.71\%$$
$$u_c = \sqrt{1.67^2 + 2.71^2} = 3.18\%$$
- Relative expanded uncertainty: $U = 6.4\% (k = 2)$
- Absolute expanded uncertainty:
 $U = 200 \text{ mg/l} * 6.4\% / 100\% = 12.8 \text{ mg/l} (k = 2)$

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Choosing the approach

- If you have
 - Competence and time
 - Data on all important influencing quantities
 - Use the Modeling approach
- If you have
 - Quality control data and results of participation in ILC-s or CRM analysis
 - Use the Single-lab validation approach
- Interlab approaches are not generally recommended

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Credits

- Parts of this presentation have been created in collaboration with **Bertil Magnusson** (SP, Sweden)
- The thoughts expressed yesterday by **Steve Ellison, Ricardo da Silva and Wolfhard Wegscheider** were very inspiring
- A part of this presentation has been used in the



training materials

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Thank you for your attention!

- The presentation is available from:
<http://www.ut.ee/ams/>
- You are always welcome to contact me:
ivo.leito@ut.ee

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AMS blog and news

Validation, Traceability, Measurement Uncertainty: the Challenges for the 21st Century Analysts

On May 21-22, 2012 a workshop *Validation, Traceability, Measurement Uncertainty: the Challenges for the 21st Century Analysts* took place in Berlin. This workshop focused on the relationship between method validation, [...]

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