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Uncertainty of analysis, test and measurement results. Where are we now?

Summary of the talk presented by M. PRIEL (LNE) at CECALAIT's AGM 2004

The standard ISO / CEI 17025 implicates that laboratories must have a procedure for the estimation of uncertainty of test and measurement results.
The degree of precision of the chosen procedure will depend on the requirements of the method and the client, and the spread of the limits with a declaration of conformity in mind.
The standard also indicates that the uncertainty must be declared in the following cases: if the client asks, if it affects the conformity due to the specification limits (interpretation of results), or when it is important for the validity or the application of the test results.

For laboratories that follow a method indicating the values of the main sources of uncertainty, the demands can be considered as being satisfied. Otherwise, the laboratory must identify all the components of uncertainty in order to give an estimation as exact as possible. A "reasonable" approach can be carried out using the performance values of the method (from work on standardisation or validation).

In order to indicate its position, the COFRAC established a note on the subject in October 2004, of which the main points are as follows:

The demands described in the document EA (4/02) remain unchanged for calibration laboratories. However, for testing laboratories, a new guide, EA (4/16), developing the specificity of the evaluation of uncertainty in such laboratories, has been published.
This publication foresees, for each analysis or test method, an identification of factors susceptible to influence the results (and the justification of not taking them into account, if need be), as well as a demonstration of their control. This information, associated with all other details (accuracy, inter-laboratory studies, control cards), will serve as a basis for the estimation of uncertainty.

For laboratories, this procedure may seem complicated at a first glance. However, evaluating uncertainty is firstly, the good understanding of the test process, and then the usage of all the information available to the laboratory. Finally, it is a way of controlling the test process and dialoguing with clients.

Two approaches are possible:

- An intra-laboratory approach

In this case, the test process can be modelized. The procedure described in the "GUM" (Guide to the expression of uncertainty of measurements) can be applied in 4 stages:
① Definition of the measurand, analysis of the mathematical process and determination of the mathematical model.
② Estimation of standard uncertainties of the initial magnitudes of the model
③ Estimation of the composite uncertainty (application of a principle of propagation)
④ Expression of the final result as a broadened uncertainty: $U(y) = k \times u_c(y)$
In cases where it is impossible to modelize the procedure, the laboratory should, in order to be able to estimate uncertainty, use all available information: intra-laboratory repeatability and reproducibility, effect of influencing factors and details on precision.

- An inter-laboratory approach

In this case, the following can be used:

- The results of accuracy (repeatability and reproducibility), obtained within the context of the determination of the performance of a method (according to ISO 5725).
- The laboratory's performance characteristics, obtained during participation in proficiency testing (organisation according to ISO 43-1 and statistical treatment according to ISO / FDIS 13528)

Some available references …

- **EA-4/16 :**
Guidelines for the expression of uncertainty of quantitative test results in proficiency testing (in French on-line at www.lne.fr)

- **ISO / TS 21748 :**
Guide to the use of repeatability and trueness estimates in measurement uncertainty estimation.

-Guide EURACHEM / CITAC :
Quantifying uncertainty of analytical measurement
(available on-line in French at www.lne.fr)

Uncertainty of microbiological measurements: Standardisation work in process

Summary of the talk presented by B. LOMBARD (AFSSA – LERQAP, Maisons-Alfort) at CECALAIT's AGM 2004

The standard ISO 17025 foresees general requirements in testing laboratories in terms of estimation of the uncertainty of measurements. As far as food microbiology is concerned, the ISO / TC 34 / SC9 and AFNOR V 08 B committees are actually working on the subject.

The origin of this work remounts to an ISO committee meeting in Bangkok in December 2002. A global approach for the uncertainty of measurements was adopted, firstly in quantitative microbiology with the publication of an ISO technical specification. Subsequently, work will be carried out on qualitative determinations.

The quantitative microbiology approach

During discussions on the subject, the GUM resolution approach was put aside due the complexity of its implementation in a food microbiology laboratory and the risk of underestimating the values.
In agreement with numerous normative documents (AFNOR FD X 07 021, FD V 03-116, ISO/DTS 21-748) and with the benefit of a wide consensus of opinion, a global approach, based on the standard deviation of experimental reproducibility, was retained. This served as a basis for the first version of the document ISO / TS 19036 « Food microbiology – Guide to the expression of uncertainty of measurements of quantitative determinations ».
The general principal of this approach is an estimation of the standard uncertainty per standard deviation of reproducibility, specific to each micro-organism. This is expressed in the form of a broadened uncertainty of measurement equal to 2 S_R.

Concerning the methods of estimation of the standard deviation of reproducibility, S_R, three options were chosen:

- The use of an intra-laboratory S_R obtained for each laboratory with the help of an experimental protocol. The protocol includes a choice of repetitions with each test sample (protocol I), or with the initial dilution (protocol II).

- The use of an inter-laboratory S_R obtained during the normalisation or validation of the method.

- The use of an inter-laboratory S_R from proficiency testing, subject to the following conditions:

- The laboratory took part in the proficiency testing, used as the basis of the calculation, using the method employed in routine analysis
- S_R was calculated with "robust" methods and with samples similar to those used in routine analysis.

Tests, grouping 72, laboratories were carried out in 2003 and 2004. The objective was to quantify, for each matrix analysed, the proportion of uncertainty of measurements linked to sampling and to preparation of the initial suspensions (IS).
This, to enable each laboratory to estimate the total uncertainty of measurements (UM):

$$UM_{total} = UM_{IS} + UM_{Protocol\ II}$$

On the basis of these results, the ISO project group confirmed the global approach according to protocol I, but without repetitions under repeatability conditions (one analysis par operator). A modified protocol I was therefore established, including:
- 8 samples per matrix, representative of food types analysed in routine analyses, and tests over a prolonged period of time.
- An estimation of uncertainties excluding, for the moment, weaker contaminations (< 100 cfu / g).
- Expressed as $\log_{10}$ or as % (RSD_R).

The results obtained by the voluntary laboratories applying this protocol, will allow the revision of the first version of the ISO document. This document will be submitted to a vote before publication.

The qualitative microbiology approach

The first exchanges on this subject took place during the TC 34 / SC 9 meeting in April 2004 (Parma, Italy). Several lines of thought were envisaged around the notions of confidence intervals, detection limits (LoD_{50}) and a reproducibility equivalent for qualitative methods.

GUM : guide to the expression of uncertainty of measurements

Examples of applications for the estimation of uncertainty of chemical tests on milk and dairy products

Summary of the talk presented by Ph. TROSSAT (CECALAIT) at CECALAIT's AGM 2004

Amongst the different methods of estimation possible, 3 lines of approach are frequently encountered in testing laboratories:

- **Analysis of the measuring process and application of a principle of propagation on the sources of uncertainty**
- **Use of accuracy values of the method**
- **Use of performance criteria obtained during participation in proficiency testing**

1) Using the application of a principle of propagation:

The different stages of this approach are:

- *Characterisation of the test process, which defines the means necessary for carrying out the test considered (object, tools, environment, method and competencies).*
- *Creation of an uncertainty budget using the principle of propagation for the estimation of each uncertainty component.*
- *An inventory of causes of error and the possibility of applying a correction to cancel these errors.*

Example: determination of fat content using the Rose Gottlieb method.

CHARACTERISATION OF THE TEST PROCESS

Method : Determination of fat content using the Rose Gottlieb method

1 - OBJECT Milk samples
2 - TOOLS - Water bath at 40°C - Class I balance - 150 ml Tubes + balls - Rotary evaporator - Oven at 102°C
3 - ENVIRONMENT Temperature regulated chemistry laboratory
4 – METHODE Ammoniacal attack of a milk test sample, fat extraction using a mixture of solvents. Elimination of the ether phase by evaporation and oven drying and weighing of the residue.
5 - COMPETENCIES Qualified operator

PREVISIONNAL BUDGET OF UNCERTAINTY

Method: Determination of fat content using the Rose Gottlieb method (g/kg)

Origin	Uncertainty component
A : REPEATABILITY (Sr/√n)	0.067/√2 = 0.047
B : IDENTIFIED CAUSES - B1 : Balance accuracy → B11 : sample weight ± 2 mg : that is 0.018 % for a weight of 11 g. For a milk with 40 g/kg of fat : ± 0.0072 g/kg	Rectangle principle 0.0072/√3 = 0.042
→ B12 : final weight ± 2 mg: that is 0.5 % for 400 mg of residue. For a milk with 40 g/kg of fat : ± 0.20 g/kg	Rectangle principle 0.20/√3 = 0.115
- B2 : Numerical indication of final weight 0.1 mg / 400 g → ± 0.01 g/kg	0.01/2√3* = 0.0289
- B3 : Constant weight tolerance 0.5 mg → ± 0.025 g/kg	Rectangle principle 0.025/√3 = 0.0144
COMPOSED STANDARD UNCERTAINTY	0.135 g/kg

* special case of standard uncertainty for numerical indications, where standard uncertainty = a/2√3

INVENTORY OF CAUSES OF ERROR

Method: Determination of fat content using the Rose Gottlieb method

Identified cause of error	Correction yes/no
1 – MESURAND Representativity of test sample	no
2 – MEASURING INSTRUMENTS - Balance → accuracy → numerical indication - Oven at 102 °C	 no no no
3 – MEASURING METHOD - Extraction yield - Stirring method - Tolerance of constant weight	 no no no
4 – MAGNITUDE OF INFLUENCE Laboratory temperature	no

The broadened uncertainty U(y) is equal to the composite uncertainty Uc (y) x k (broadening coefficient)

U(y) = 2 x 0.135 = 0.27 g/kg

2) Using the accuracy values of the method:

The principal of this approach is to assimilate the standard deviation of reproducibility of the method used to the standard composite uncertainty [Uc(y)].
Therefore, the broadened uncertainty is U (y) = k x SR (k = 2)

Examples :

- Determination of fat content using the Rose Gottlieb method: SR = 0.144 g/kg → **U(y) = 0.29 g/kg**

- Determination of dry matter by oven drying: SR = 0.072 g/100 g → **U(y) = 0.14 g/100 g**

3) Using performance results obtained during proficiency testing:

The principal of this approach is to use the information relative to the repeatability of the method (Sr), the precision (mean bias) and the dispersion (standard deviation), observed whilst participating in proficiency testing, in order to estimate the standard composite uncertainty.

- **$U^2(x) = Sr^2/n$**

- calculation of the superior and inferior maximum limit values = $\bar{d} \pm 2$ Sd. The standard uncertainty is calculated with the help of the rectangle principle: **$u^2(y) = [(a^2/3)]$,** a being the biggest limit value (superior or inferior, absolute value).

$$U^2c(y) = U^2(x) + U^2(y)$$

To increase the pertinence of the estimation, this approach can be carried out using the "mean" performance values, taking into account the results of participation in several proficiency tests.

Example: determination of fat content using the Rose Gottlieb method

FAT EXTRACTION

Name	d	Sd	SL	Lim sup	Lim inf
1	0.02	0.07	0.05	0.16	-0.12
2	0.07	0.11	0.07	0.29	-0.15
3	0.15	0.06	0.08	0.27	0.03
4	0.04	0.10	0.05	0.24	-0.16
Mean	**0.07**	**0.09**	**0.06**	**0.24**	**-0.10**

$U^2c(y) = 0.06^2/2 + 0.24^2/3 = 0.0210$ and Uc(y) = 0.15
U(y) = 2 x 0.15 = 0.30 g/kg.

CONCLUSION

Although the approach using the propagation principle is described in the GUM, it is certainly the most difficult to set up in a testing laboratory. Furthermore, certain error components (matter reagent interactions, for example) are impossible to quantify.

Using accuracy results can be a simple method. However, laboratories should apply the method such as it is described and verify that the limits used to estimate uncertainty are prescribed to (as far as accuracy is concerned). In any case, the calculated uncertainty will not be totally specific to the laboratory.

The use of proficiency testing results, although they necessitate a rather large amount of data, will enable obtainment of a realistic estimation of laboratory performance.

STANDARDS, DRAFT STANDARDS

1.2 - IDF published standards

MILK AND DAIRY PRODUCTS		
MILK / DAIRY PRODUCTS / PHOSPHATASE	IDF 82 (ISO/TS 6090) Mars 2004	Milk and dried milk, buttermilk and buttermilk powder, whey and whey powder – detection of phosphatase activity
MILK / EVAPORATED MILK	IDF 160 (ISO/TS 9941) December 2003	Milk and canned evaporated milk – Determination of tin content (spectrometric method)

NEW EU STANDARDS AND REGULATIONS

Classification is established in alphabetic order of the first keyword

HYGIENE / FOODSTUFFS
Official Journal L139, 30th April 2004 – REGULATION (EC) No 852/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29th April 2004 on the hygiene of foodstuffs http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l_139/l_13920040430en00010054.pdf
Official Journal L139, 30th April 2004 – REGULATION (EC) No 853/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29th April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l_139/l_13920040430en00550205.pdf
Official Journal L226, 25th June 2004 – Corrigendum to Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29th April 2004 on the hygiene of foodstuffs (OJ L 139, 30.4.2004) http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l_226/l_22620040625en00030021.pdf
Official Journal L226, 25th June 2004 – Corrigendum to Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29th April 2004 laying down specific hygiene rules for food of animal origin (OJ L 139, 30.4.2004) http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l_226/l_22620040625en00220082.pdf
HYGIENE / FOODSTUFFS / FOODSTUFFS OF ANIMAL ORIGIN
Official Journal L195, 2nd June 2004 – Corrigendum to Directive 2004/41/EC of the European Parliament and of the Council of 21st April 2004 repealing certain Directives concerning food hygiene and health conditions for the production and placing on the market of certain products of animal origin intended for human consumption and amending Council Directives 89/662/EEC and 92/118/EEC and Council Decision 95/408/EC (OJ L 157, 30.4.2004) http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l_195/l_19520040602en00120015.pdf
MICROBIOLOGY / SALMONELLA
Official Journal L251, 27th July 2004 – Commission Decision of 20th July 2004 concerning Community reference laboratories for the epidemiology of zoonoses and for salmonella and national reference laboratories for salmonella (notified under document number C (2004) 2781) (1) http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l_251/l_25120040727en00140017.pdf

BOOKSHOP: LATEST PUBLICATIONS

The classification in alphabetic order of the first keyword allows you to consult the references according to your interests. The web site allows you to know more, or to order the book.

MICROBIOLOGY / FOOD / PATHOGENS

Ahmed E YOUSEF et Carolyn CARLSTROM – **Food Microbiology: A laboratory manual – Wiley Europe ISBN: 0-471-39105-0**

http://www.wileyeurope.com/WileyCDA/WileyTitle/productCd-0471391050.html

Summary: This book presents an assessment of basic microbiological techniques, analytical methods and tests for food-borne pathogens such as *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella*.

Thomas A McMEEKIN – **Detecting Pathogens in Food – Woodhead Publishing ISBN: 0-8493-1756-8**

http://www.crcpress.com

Summary: This book presents a selection of the latest microbiological analysis techniques. This edition also presents general aspects of food safety management and sampling, as well as a selection of techniques for detection.

MICROBIOLOGY / LISTERIA /

Chris BELL et Alec KYRIAKIDES **– Listeria (Practical food microbiology, 2nd Ed.) - Lavoisier**

http://www.lavoisier.fr/fr/livres/index.asp?texte=List%E9ria&select=motcle&exact=on&togo=&support=NULL&from=

Summary: This book gives a round-up on the taxonomy of *Listeria*, on details of certain recent food-borne diseases, as well as on legislation, etc.

MICROBIOLOGY / MEDIA

Ronald M. ATLAS – **Handbook of Microbiological Media - Third Edition – CRC Press ISBN: 0-8493-1818-1**

http://www.crcpress.com

Summary: This book lays out a large number of culture media formulae for microbiological applications.

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