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