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Microbiology of food and animal feeding stuffs — Guidelines for the estimation of measurement uncertainty for quantitative determinations

Microbiologie des aliments — Lignes directrices pour l'estimation de l'incertitude de mesure pour les déterminations quantitatives

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 19036 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

Laboratories operating under ISO/IEC 17025 accreditation and related systems are required to evaluate measurement uncertainty (MU) for the analyses they conduct, and to report it when relevant. The MU estimation gives a measure of the confidence that can be put on the analytical results, not on the laboratory competency.

Given this need, ISO/TC 34/SC 9 considered that it was necessary to define a general approach to the estimation of measurement uncertainty in food microbiology, based on the general guidelines for expressing MU. It reached a consensus for quantitative determinations, and was aware that there was also a need to estimate MU for qualitative determinations, but this would need more time, and would be covered by a separate later publication.

In order to expedite publication of a document to provide a harmonized approach that could be applied for accreditation purposes, ISO/TC 34/SC 9 decided to prepare a Technical Specification rather than an International Standard. It was believed that this would encourage users of this publication to report their experience on the implementation of the approach described. ISO/TC 34/SC 9 could then review the document in the light of the experience gained.

Introduction

The *Guide to the expression of uncertainty in measurement* (GUM) [15] is a widely adopted standard approach that recommends, as illustrated in the examples provided, the estimation of the individual sources of variability that contribute to uncertainty in the measurement process. The global uncertainty is then derived using formal principles of uncertainty propagation. This approach has been described in a more practical way for analytical measurements, mainly of chemical nature, by the EURACHEM/CITAC Guide [16] and also for microbiology in Reference [17].

ISO/TC 34/SC 9 considers that this "step-by-step" approach does not apply satisfactorily in the case of the microbiological analysis of food, where it is difficult to build a really comprehensive model of the measurement process. Because of the possibility of overlooking a significant source of uncertainty, there is a high risk of underestimating the true measurement uncertainty (MU) value. Furthermore, it appears difficult to quantify accurately the contribution of each individual step of the analytical process in food microbiology, where

— the analyte is a living organism, whose physiological state can be largely variable, and

— the analytical target includes different strains, different species or different genera.

In other words, the microbiological analyses do not enable a metrologically rigorous and statistically valid estimation of MU.

ISO/TC 34/SC 9 has therefore chosen a "top-down" or "global" approach to MU, which is based on a standard deviation of reproducibility of the final result of the measurement process. This is an approach based on experimental results (with replication of the same analysis) which, in the case of microbiology, seems more meaningful than the step-by-step approach.

The global approach has been endorsed for a more general use by ISO/TS 21748 elaborated by ISO/TC 69, *Application of statistical methods*, SC 6, *Measurement methods and results*. This document clarifies that the step-by-step approach and the global approach are not mutually exclusive, since all the MU components can be considered to be included in the overall performance of the analytical process, which can be characterized by the observable precision and bias.

Microbiology of food and animal feeding stuffs — Guidelines for the estimation of measurement uncertainty for quantitative determinations

1 Scope

This Technical Specification gives guidance for the estimation and expression of measurement uncertainty (MU) associated with quantitative results in food microbiology.

It is applicable to the quantitative analysis

— of products intended for human consumption and the feeding of animals, and

— of environmental samples in the area of food production and food handling,

typically carried out by enumeration of microorganisms using a colony-count technique, but applicable also to quantitative analysis by alternative instrumental methods.

This Technical Specification is not applicable to

— enumeration using a most probable number technique, or

— the analysis of low levels of microorganisms.

In this Technical Specification, MU associated with “low” numbers of organisms [1)], as described by ISO 7218, is not estimated due to a lack of a simple agreed approach to cover this case.

The approach of this Technical Specification is a global approach, based on the standard deviation of reproducibility of the final result of the measurement.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
uncertainty (of measurement)
parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand

NOTE 1 The parameter may be, for example, a standard deviation (or a given multiple of it), or the half-width of an interval having a stated level of confidence.

NOTE 2 Uncertainty of measurement comprises, in general, many components. Some of these components may be evaluated from the statistical distribution of the results of a series of measurements and can be characterized by experimental standard deviations. The other components, which also can be characterized by standard deviations, are evaluated from assumed probability distributions based on experience or other information.

1) That is below 10 colonies counted on at least one plate, normally corresponding to less than 100 cfu per gram or per millilitre, or 1 000 cfu per gram or per millilitre of product depending on the volume of the inoculum (1 ml or 0,1 ml).

NOTE 3 It is understood that the result of the measurement is the best estimate of the value of the measurand and that all components of uncertainty, including those arising from systematic effects, such as components associated with corrections and reference standards, contribute to the dispersion.

[GUM:1993 [15]]

2.2
standard uncertainty
$u(x_i)$
uncertainty of the result of a measurement expressed as a standard deviation

[GUM:1993 [15]]

2.3
combined standard uncertainty
$u_c(y)$
standard uncertainty of the result of a measurement when that result is obtained from the values of a number of other quantities, equal to the positive square root of a sum of terms, the terms being the variances or covariances of these other quantities weighted according to how the measurement result varies with changes in these quantities

[GUM:1993 [15]]

2.4
expanded uncertainty
U
quantity defining an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand

NOTE 1 The fraction may be regarded as the coverage probability or level of confidence of the interval.

NOTE 2 To associate a specific level of confidence with the interval defined by the expanded uncertainty requires explicit or implicit assumptions regarding the probability distribution characterized by the measurement result and its combined standard uncertainty. The level of confidence that may be attributed to this interval can be known only to the extent to which such assumptions may be justified.

[GUM:1993 [15]]

NOTE 3 An expanded uncertainty U is calculated from a combined standard uncertainty $u_c(y)$ and a coverage factor k using:

$$U = k u_c(y)$$

2.5
coverage factor
k
numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty

NOTE A coverage factor, k, is typically in the range 2 to 3.

[GUM:1993 [15]]

2.6
bias
difference between the expectation of the test results and an accepted reference value

NOTE Bias is the total systematic error as contrasted to random error. There may be one or more systematic error components contributing to bias. A larger systematic difference from the accepted reference value is reflected by a larger bias value.

[ISO 3534-1:—]

3 Principles

3.1 Global approach for the estimation of measurement uncertainty (MU)

A global approach is adopted by this Technical Specification for the estimation of MU. It is based on the overall variability of the analytical process whose outcome is the test result. This overall variability includes both observable precision (random component) and bias (systematic component). In practice in the field of food microbiology, only precision is taken into account (see 3.2).

The global approach to MU estimation in this Technical Specification is derived from an experimental estimation of the standard deviation of reproducibility of the final result of the complete measurement process. This standard deviation corresponds to the combined standard uncertainty (see 4.1).

The global approach can be considered as a "black-box" system, as illustrated in Figure 1, where the main sources of uncertainty in food microbiology are identified. Such a diagram can be helpful in identifying the uncertainty sources that are covered or not by the experimental protocol chosen.

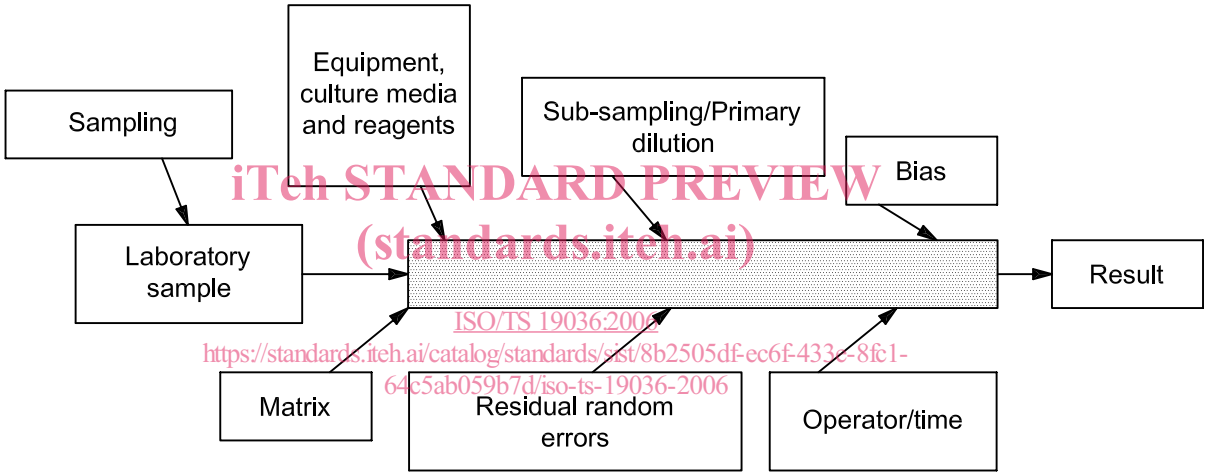

Figure 1 — Diagram of the main sources of uncertainty in food microbiology, and the "black-box" approach to measurement uncertainty

In Figure 1, sampling [drawing of the sample unit(s) to be tested from the lot to be controlled] introduces a significant (if not major) part of the total error, but it is not part of the uncertainty linked to the measurement itself. Sub-sampling means the drawing of the test portion from an analytical sample (one of the units drawn from the lot). This test portion is used in the preparation of the initial suspension in bacterial enumeration techniques, according to ISO 6887-1. The main sources of uncertainty during the analytical process are the operator/time and the equipment/culture media/reagents. Finally, the residual random errors are the ones not explained by the previous factors, and usually assessed within a laboratory under repeatability conditions.

Meanwhile, the adoption of this global approach necessitates that results come from a measurement procedure demonstrated to be under control.

3.2 Consideration of bias

It is generally considered that the bias is not taken into account in the MU estimation, given the empirical nature of microbial enumerations. In other words, the analytical procedure directly determines the result of the measurement, e.g. the number of colony-forming units per unit of sample. Thus it is not possible in practice to determine a true value, which is required to determine bias. Even when using Certified Reference Materials, or values derived from interlaboratory trials, only part of the total bias can be assessed.

Meanwhile, it is recognized that part of the bias can be estimated through interlaboratory studies that are used in two of the options retained in this Technical Specification for evaluating the standard deviation of reproducibility (see Clauses 6 and 7). The method of taking into account the bias component of uncertainty is not described in this Technical Specification. However, even if the bias component of MU is not formally assessed, the laboratory bias can be shown to be in control by participating, for example, in interlaboratory proficiency testing or by testing (Certified) Reference Materials.

4 General aspects

4.1 Combined standard uncertainty

When the main components of uncertainty are under control (see 3.1), the combined standard uncertainty $u_c(y)$ (2.3) is, in general, the combination of a standard uncertainty related to observable precision and, where appropriate, to bias.

The combined standard uncertainty is estimated in this Technical Specification by the experimental standard deviation of reproducibility on the final result of the measurement (4.2).

NOTE The method of combining a standard uncertainty related to bias is not described here.

4.2 Standard deviation of reproducibility

Three different possibilities have been selected for the estimation of the standard deviation of reproducibility (s_R), with a priority order as follows:

— 1st option: intralaboratory standard deviation of reproducibility;

— 2nd option: standard deviation of reproducibility of the method derived from an interlaboratory study;

— 3rd option: standard deviation of reproducibility derived from an interlaboratory proficiency trial.

A clear priority is given for the first option, which has been tested and an experimental protocol is described in detail.

General rules for the estimation of the reproducibility standard deviation are given in 4.4, and each option is detailed in Clauses 5 to 7.

4.3 Expanded uncertainty

The expanded uncertainty U (2.4), as defined by GUM, is derived from the combined standard uncertainty $u_c(y)$ (see 4.1), with a coverage factor k (2.5) chosen in this Technical Specification as a value of 2 (so as to correspond approximately to a confidence level of 95 %):

$$U = 2\, u_c(y) = 2\, s_R$$

4.4 General rules for the estimation of the reproducibility standard deviation

The black-box concept described in this Technical Specification should take into account as many as possible of the uncertainty sources considered in Figure 1. In particular, the laboratory should have an understanding of the distribution of microorganisms within the matrices it tests, in order to take them into account for estimating the sub-sampling component of uncertainty (see 3.1).

The standard deviation of reproducibility shall be estimated for each type of target microorganism (or consistent group of target microorganisms) and for each matrix (or consistent group of matrices), for a given method that the laboratory uses for producing its routine results.

NOTE 1 The term “consistent” means that the group of microorganisms/methods or the group of matrices gives equivalent values of MU.

NOTE 2 The MU estimate is associated with the laboratory and links a given MU to the relevant test result, determined under defined conditions, such as operators, operating procedure, equipment, reagents, etc. The MU estimate does not characterize the analytical method itself independently from the laboratory which implements it.

According to the principles of ISO/IEC 17025, the critical factors associated with the method or the laboratory that are likely to affect the measurement result should be identified and demonstrated to be under control. Examples of such critical factors are the source and type of culture media and/or other reagents (such as the ones used for confirmation), the counting techniques (manual or automated), the operator or group of operators, etc. Ongoing monitoring of the MU estimation is needed to show that this estimation remains relevant and that the test results are under control. A re-assessment of the MU estimation is required following changes to any of the critical factors.

5 Intralaboratory standard deviation of reproducibility

5.1 General

The intralaboratory standard deviation of reproducibility is the preferred option for deriving MU since it enables a laboratory to attach the MU value to the results that it reports, thus respecting the principle of the definition of MU. This corresponds to a particular case of intermediate precision, as introduced in ISO 5725-3. A theoretical drawback of this option is that it cannot take bias into account.

5.2 Experimental protocol

5.2.1 General

In food microbiology, the effect of the matrix on MU cannot be avoided; thus the experimental protocol takes into account the effect of sub-sampling to obtain the test portion from the laboratory sample (i.e. the food sample tested).

For each target microorganism [or consistent group of organisms [2)]] and for a given type of matrix, the experimental protocol (5.2.2) shall be performed for at least 10 samples of the same matrix. The repetition of the protocol should take place on different days, in order to cover variation in the operating conditions over time. This also enables accumulation of data over a period of time.

The number of types of matrices to be tested depends on the diversity of the matrices analysed routinely by the laboratory. The selected matrices should be representative, in terms of MU values, of the types of matrices analysed by the laboratory, and also relevant to the microorganisms for which the test is to be done. Annex A gives guidance on this selection, by providing the outcome of trials performed at the international level which aimed to assess the MU component linked to the sub-sampling of the test portion from the laboratory sample, and to the preparation of the initial suspension. Further guidance is also available in Annex B of ISO 16140:2003.

The calculation of the standard deviation on log-transformed data (5.3) stabilizes the reproducibility variance over the contamination levels, given that low levels are not considered here. It is therefore not necessary to estimate the reproducibility standard deviation per contamination level. However, where possible, the samples and/or the dilutions should be chosen as to cover the concentration range in routine testing.

Naturally contaminated samples should be used whenever possible, since they enable a more realistic estimation of MU, which is to be used for characterizing results obtained on naturally contaminated samples. Moreover, the physiological state of the microorganism (e.g. stressed) may also influence the variability of the results, and should therefore be similar to the conditions encountered in routine testing.

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2) See Note 1 in 4.4.