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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

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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;

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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:

$$U = ku_c(y)$$

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.

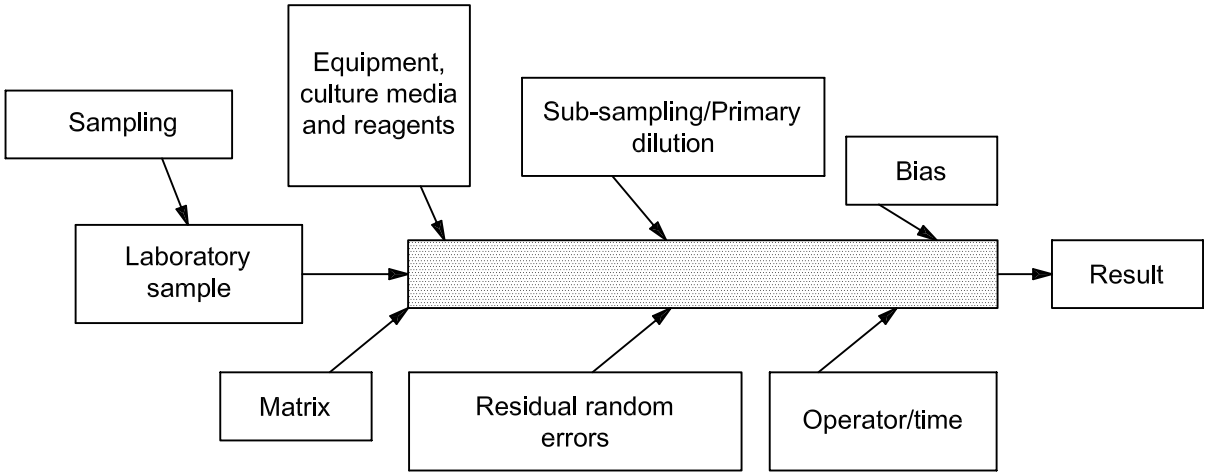


Figure 1 — Diagram of the main sources of uncertainty in food microbiology, and the “black-box” approach to measurement uncertainty

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.

2) See Note 1 in 4.4.

If spiking is required, it needs to be very tightly controlled so that it does not introduce an additional element of variability to results. Spikes should be designed to mimic real contamination as far as possible (e.g. by use of stressed organisms and inclusion of competitive/background flora).

5.2.2 Description

The protocol is described in Figure 2.

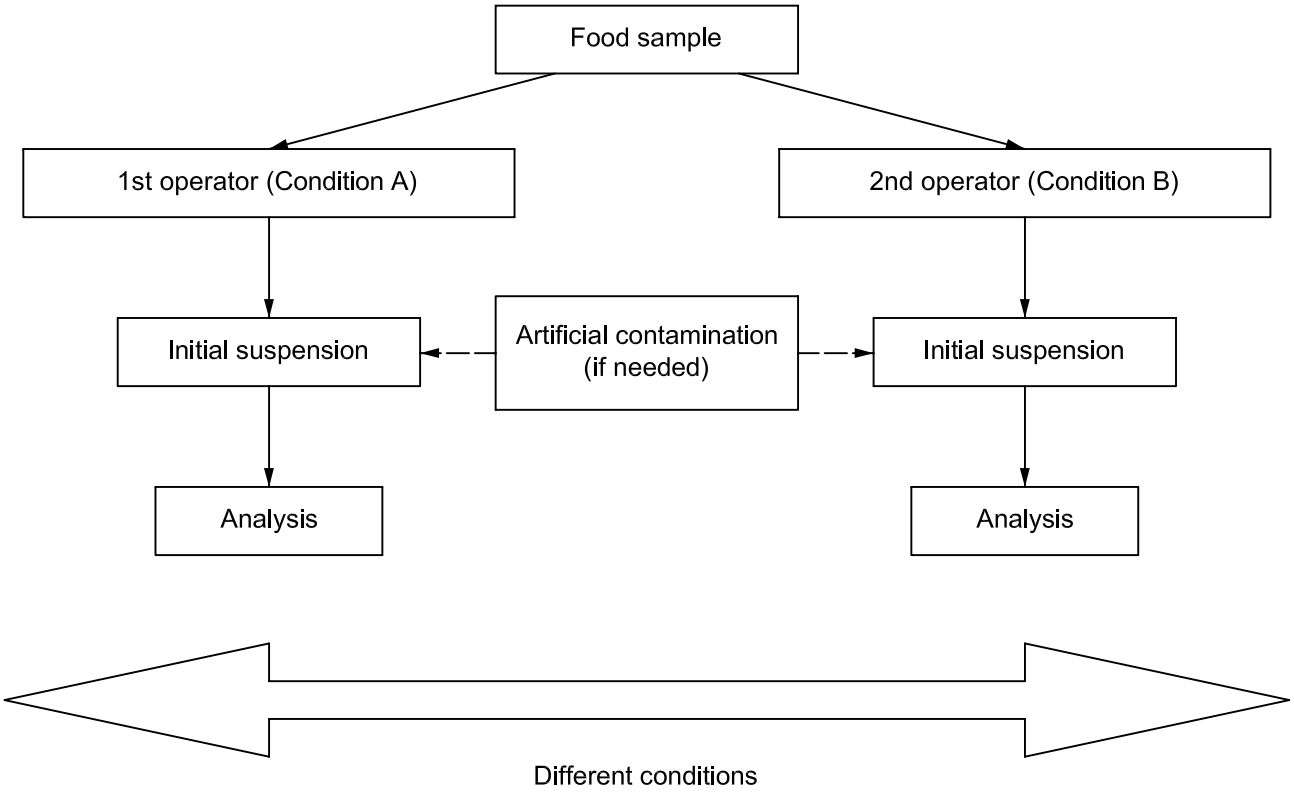


Figure 2 — Experimental protocol for the intralaboratory standard deviation of reproducibility

For each sample, each operator takes one test portion, and prepares from it one initial suspension, analysed once. Perform the analysis *as in routine testing* (e.g. preparation of one series of decimal dilutions, inoculation of 1 or 2 plates per dilution).

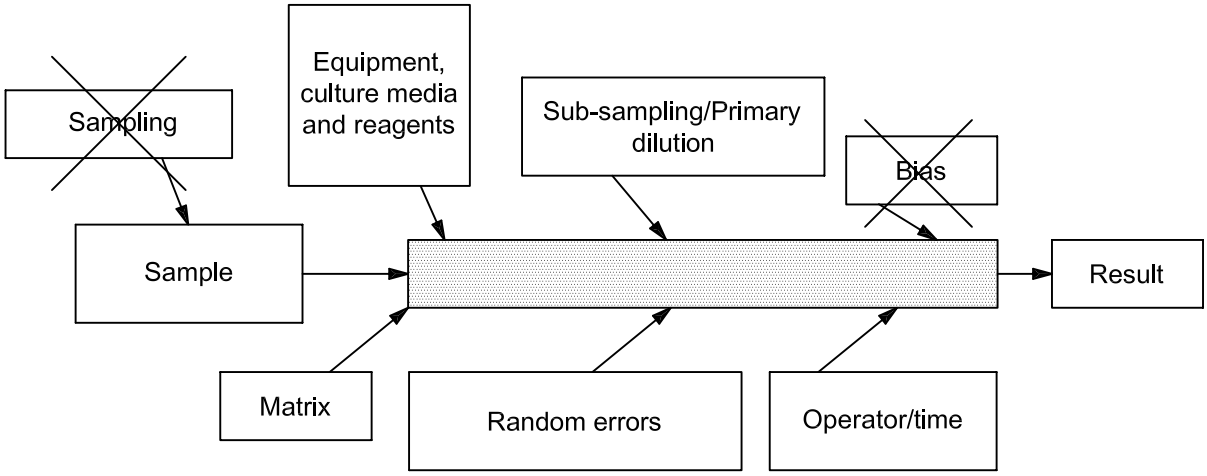
NOTE 1 In practice, the “operator” can be a team of operators (technicians), each of whom performs a given part of the procedure. In such a case, the team is considered as one operator, and any change in membership allocation of tasks is regarded as a different operator.

NOTE 2 This protocol is derived from the black box approach described in 3.1. Various sources of uncertainty, such as sub-sampling, nature of the matrix, residual random errors, operator/time, etc., are considered simultaneously but are not assessed separately.

Conditions A and B should be as different as possible and should ideally include as many variations as may be encountered from one day to another within the laboratory, in terms of technicians, batches of culture media and reagents, vortex mixer, pH meter, incubators, time of analysis, etc. If the contamination of the food sample is demonstrated to be sufficiently stable (which is rarely the case in food microbiology), the conditions A and B should relate also to different days of analysis.

5.2.3 Use

Figure 3 indicates the main sources of uncertainty covered by this protocol, as well as the ones excluded (sampling and bias).



NOTE The excluded sources are marked with a cross.

Figure 3 — Main sources of uncertainty covered or excluded in an experiment on intralaboratory reproducibility

As explained in 4.2.1, this protocol incorporates the effect of the sampling of the test portion in the evaluation of the total uncertainty. Furthermore, it is well known in food microbiology that the natural contamination of food products (especially solid products, after processing, maturation, etc.) is often highly heterogeneous. The protocol takes account of the variability of the results due to this heterogeneity, which may be important in certain situations when a judgement on conformity of the sample analysed against limits (such as microbiological criteria) is to be made.

NOTE If an artificial contamination of the initial suspension is performed (a possibility in the protocol of Figure 2), the uncertainty component due to the contamination heterogeneity of the matrix is not taken into account.

However, this protocol can have a low practicability in certain cases. The distribution of natural contamination is closely linked to the type of matrix, which is why the experimental protocol should be repeated with each type of matrix (or consistent group of matrices) analysed routinely by the laboratory. This may lead to very extensive trials when the laboratory analyses a large variety of matrices.

Finally, as mentioned in 3.2, the possible contribution of bias to MU cannot be covered by this protocol.

5.3 Calculations

In accordance with the normal practice, as a preliminary step before calculations, the data (microbiological enumeration results) in cfu/g or cfu/ml shall be transformed into $\log_{10}$ (cfu/g) or $\log_{10}$ (cfu/ml).

NOTE According to ISO 31-11, the symbol for decimal logarithms is “lg”. However, in the frame of this Technical Specification, the symbol “$\log_{10}$”, largely used in the community of food microbiology laboratories, is preferred.

Calculate the reproducibility standard deviation s_R for the n samples of a given matrix as follows:

$$s_R = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\frac{\left(y_{i\mathrm{A}} - y_{i\mathrm{B}}\right)^2}{2}}$$

where

y_{ij} are the log-transformed data, in $\log_{10}$ (cfu/g) or $\log_{10}$ (cfu/ml);

i is the index of the sample, $i = 1$ to n ($n \geqslant 10$);

j is the index of the reproducibility condition, $j =$ A or B.

An example of the enumeration of aerobic mesophilic flora in mixed poultry meat is given in Table 1.

Table 1 — Calculations of standard deviations of reproducibility — Example of enumeration of aerobic mesophilic flora in mixed poultry meat

i	$x_{i\mathrm{A}}$	$x_{i\mathrm{B}}$	$y_{i\mathrm{A}} = \log_{10}(x_{i\mathrm{A}})$	$y_{i\mathrm{B}} = \log_{10}(x_{i\mathrm{B}})$	$\frac{(y_{i\mathrm{A}} - y_{i\mathrm{B}})^2}{2}$
1	$6{,}7 \times 10^4$	$8{,}7 \times 10^4$	4,83	4,94	0,006 4
2	$7{,}1 \times 10^6$	$6{,}2 \times 10^6$	6,85	6,79	0,001 7
3	$3{,}5 \times 10^5$	$4{,}4 \times 10^5$	5,54	5,64	0,004 9
4	$1{,}0 \times 10^7$	$4{,}3 \times 10^6$	7,00	6,63	0,067 2
5	$1{,}9 \times 10^7$	$1{,}7 \times 10^7$	7,28	7,23	0,001 2
6	$2{,}3 \times 10^5$	$1{,}5 \times 10^5$	5,36	5,18	0,017 2
7	$5{,}3 \times 10^8$	$4{,}1 \times 10^8$	8,72	8,61	0,006 2
8	$1{,}0 \times 10^4$	$1{,}2 \times 10^4$	4,00	4,08	0,003 1
9	$3{,}0 \times 10^4$	$1{,}3 \times 10^4$	4,48	4,11	0,065 9
10	$1{,}1 \times 10^8$	$2{,}2 \times 10^8$	8,04	8,34	0,045 3

Using the log-transformed data y_{ij}, the reproducibility standard deviation is then:

$$s_R = \sqrt{\sum_{i=1}^{n} \frac{(y_{i\mathrm{A}} - y_{i\mathrm{B}})^2 / 2}{n}} = \sqrt{\frac{0{,}006\,4 + 0{,}001\,7 + \ldots + 0{,}045\,3}{10}} = \sqrt{0{,}023\,4} = 0{,}15\ (\log_{10})\ \text{cfu/g}$$

6 Reproducibility standard deviation of the method derived from an interlaboratory study

6.1 General

If the method used routinely by the laboratory has been submitted to an interlaboratory study for validation of the method, the laboratory may use the reproducibility standard deviation of the method for deriving the estimation of its measurement uncertainty *under certain conditions* (see below). These prerequisites are justified by the fact that the standard deviation derived from an interlaboratory study is linked to the method, and not to a given laboratory that is to report a measurement uncertainty attached to its results.

These conditions are as follows:

— *the laboratory bias* shall be compatible with that expected on the basis of the repeatability and reproducibility estimates derived from the interlaboratory study;

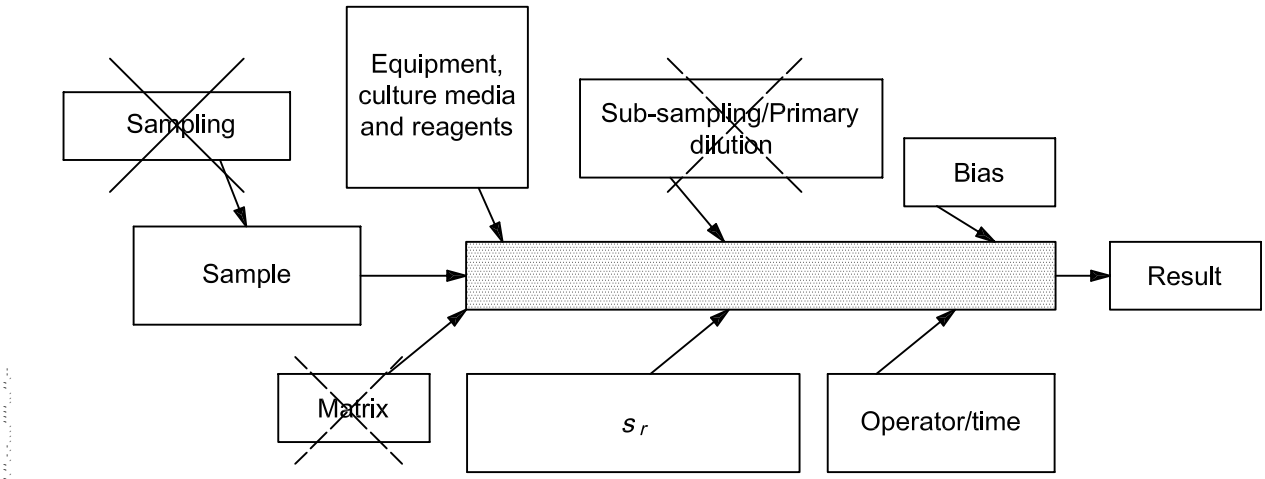
— the *precision attained by the measurements within the laboratory* shall be compatible with that expected from repeatability and reproducibility estimates derived from the interlaboratory study;

— the interlaboratory study has correctly covered all the sources of uncertainty (especially sample preparation and homogenization).

The procedure to check that these conditions are met, and how to form a combined uncertainty estimate with the possible additional factors not covered by the interlaboratory study, are described in detail in ISO/TS 21748.

6.2 Use in food microbiology

Figure 4 indicates the main sources of uncertainty covered by this protocol, as well as the one excluded (sampling).



NOTE The excluded sources are marked with a cross.

Figure 4 — Main sources of uncertainty covered or excluded by interlaboratory reproducibility

The extent to which sub-sampling and preparation of the primary dilution, as well as of the matrix effect, are covered depends on the experimental design of the trial.

This approach enables a laboratory that has taken part in an interlaboratory study to assess its laboratory bias, part of the bias component of MU. This aspect is not detailed in this Technical Specification.

However, in food microbiology, there are several limitations to this approach, which are given below. This justifies its consideration only as a 2nd option.

In addition to the need to check that the precision and bias of the laboratory conform to the corresponding values of the interlaboratory study on the method, it is essential to recognize that only a limited number of reproducibility parameters have been derived from interlaboratory studies for standardized reference methods (i.e. enumeration of *B. cereus* in ISO 7932, of *C. perfringens* in ISO 7937, of coagulase-positive staphylococci in ISO 6888-1 and ISO 6888-2, and of *L. monocytogenes* in ISO 11290-2).

Moreover, it may be difficult to generalize from the specific to the routine sample analyses performed by the laboratory. Precision values from an interlaboratory study will have been obtained under limited precisely defined combinations of matrix, strain of microorganism, contamination level, etc., and for a given background microflora (if present).

Finally, given the homogeneity requirements for the samples used for collaborative studies, the need to send to laboratories homogenized and stabilized samples induces a reduction of the "natural" variation in sample contamination that may be found in practice, which causes an under-estimation of the uncertainty.

7 Reproducibility standard deviation derived from an interlaboratory proficiency trial

If the laboratory has taken part in an interlaboratory proficiency trial, it may use the standard deviation of reproducibility from this trial to deduce its measurement uncertainty, under the following conditions:

— during the interlaboratory trial, the laboratory used the method that it uses in routine analyses;

— the samples which were used in the trial are comparable (in terms of matrix and contamination level) to the ones analysed routinely;

— the laboratories that participated in the trial did not use different empirical methods, or a sufficient number of participants used the same method, so as to allow a correct estimation of the reproducibility standard deviation.

Figure 4 indicates the main sources of uncertainty covered by this protocol, as well as the one excluded: sampling.

An objective of this approach is to enable a laboratory that has taken part to the interlaboratory trial to partly assess its bias component of MU. This aspect is not detailed in this Technical Specification.

8 Expression of measurement uncertainty in the test reports

Once the measurement uncertainty has been derived as explained above, if required it may be expressed in the report, together with the test result, as an interval in $\log_{10}$ (see Note in 5.3) or in natural values (number of cfu per gram or per millilitre), or as a percentage, as illustrated in the following example. Not more than two significant figures shall be used for reporting the values of the result or the uncertainty interval.

Denoting the test result $y = \log_{10} x$, and the reproducibility standard deviation s_R, then the expanded uncertainty U, with a coverage factor of 2 (giving a confidence level of 95 %) is given by $2s_R$.

The test result can be reported according to one of the following possibilities:

— $y \pm 2s_R$ (log);

— y log $[y - 2s_R, y + 2s_R]$;

— x cfu/g or x cfu/ml $[10^{y-2s_R}, 10^{y+2s_R}]$;

— x cfu/g or x cfu/ml $\left[10^{y} - \frac{10^{y-2s_R}}{10^{y}}\%, 10^{y} + \frac{10^{y+2s_R}}{10^{y}}\%\right]$.

EXAMPLE A standard deviation of reproducibility s_R of $\pm$ 0,15 $\log_{10}$ has been found. Thus the expanded uncertainty U, with a coverage factor of 2 (confidence level of 95 %) is $0{,}15 \times 2 = 0{,}3$ log. The test result is 5,0 log cfu/g.

Thus the test result may be reported as one of the following cases:

— 5,0 log $\pm$ 0,3 log;

— 5,0 log [4,7, 5,3];

— 10^5 cfu/g [5×10^4, 2×10^5];

— 10^5 cfu/g [10^5 – 50 %, 10^5 + 100 %].

Annex A
(informative)

Results of trials on the uncertainty component linked to the sub-sampling of test portion and to the preparation of the initial suspension

A.1 Presentation and experimental protocol

Trials were organized by AFSSA (France), on behalf of ISO/TC 34/SC 9, in 2003 and 2004. The objective was to estimate the effects of different product matrices on the components of the measurement uncertainty (MU), linked to sub-sampling of the test portion (in the analytical sample) and to the preparation of the initial suspension (see Reference [18]).

The protocol included eight enumerations per sample, as shown in Figure A.1.

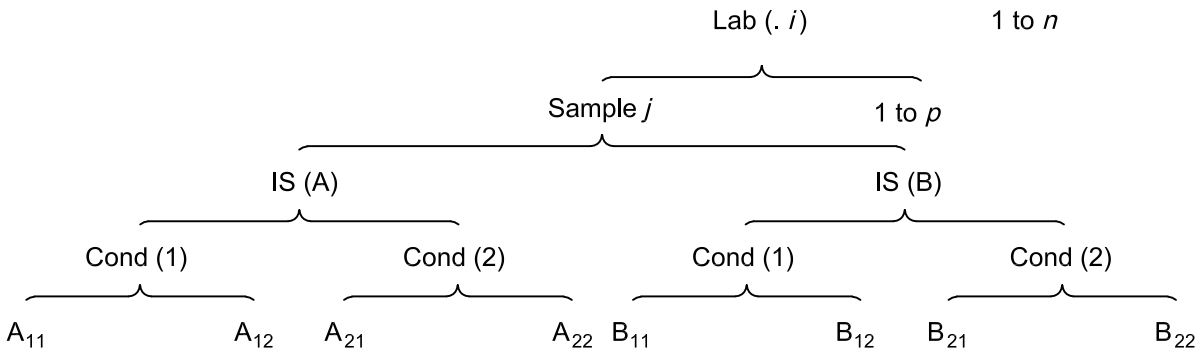


Figure A.1 — Experimental protocol of the trials

In Figure A.1:

— IS (A) and IS (B) stand for two initial suspensions prepared independently and as differently as possible (e.g. different operators, different balance, different diluent batch, etc.);

— Cond (1) and Cond (2) stand for two groups of conditions differing as much as possible (different operators, different media batches, different incubators, etc.);

— the indexes A_{11} A_{12}, etc. indicate two repetitions under repeatability conditions (i.e. two series of dilutions per initial suspension, for each set of conditions).

Note that the protocol of Figure 2 (see 5.2.2) is a simplification of Figure A.1: in Figure 2, only the enumerations noted A_{11} and B_{21} in Figure A.1 are shown.

A.2 Results

A.2.1 General

A total of 79 laboratories participated in the trials and each of them tested one or more methods in one or more matrices, so that 124 data files for {1 laboratory × 1 method × 1 matrix} were gathered. Of these, 28 data files had to be excluded because the analyses did not conform to the criteria set down for the trials.

Thus, 96 reproducibility standard deviations (s_R), each defining one laboratory, one method, and one matrix, were obtained. In addition, the theory of the variance components enabled identification for each reproducibility standard deviation the role of three uncertainty sources:

a) those linked to the matrix, the sub-sampling of the test portion, and the initial dilution;

b) those linked to the reproducibility conditions (operator/ time); and

c) the random errors under repeatability conditions.

A.2.2 Classification of matrices

Based on the results of these trials, a classification of the matrices was developed.

Four categories were distinguished on the basis of physical criteria.

— Category i): liquids and powders (e.g. milk, coconut milk, dried milk, etc.);

— Category ii): well-mixed solids (e.g. minced meat, mechanically separated meat, sausage meat, crushed meat, whipped cream, dairy ice cream, soya cream, etc.);

— Category iii): small (or very small) solids (e.g. dehydrated parsley/mushrooms, grated carrots/celeriac, salad, shrimps, cereals, feeding stuffs, chopped hazel nuts, etc.);

— Category iv): other solids (e.g. non-minced meat, cheeses, pastry, etc.).

This physical criterion significantly affected the s_R and the standard deviation for two of the sources of uncertainty: the one linked to the matrix (including sub-sampling of the test portion) and the one linked to the preparation of the initial dilution. Paired comparisons showed a significant difference between the two first matrix categories (liquids, powders and well-mixed solids), and between the two last categories (small and other solids).

For the two first categories, the matrix was responsible for about 0,1 $\log_{10}$ units (see Note in 5.3) of the overall standard deviation, regardless of the laboratory and the microflora. For the two last categories, it was not possible to assess the order of magnitude of the effect independent of the microflora and laboratory. Note that most products in the two last categories can be mixed using a blender, and then could be considered to belong to the second matrix category.

A.2.3 Detailed results

Detailed results derived from the 96 usable data files are presented in Tables A.1 to A.5, for each target microflora.

In each table:

— the category is defined as in A.2.2;

— s_{IS} represents the “component” of the standard deviation due to the initial suspension (including effects of matrix and sub-sampling, and of preparation of the initial suspension);

— s_R stands for the reproducibility standard deviation;

— s_{cond} represents the “component” due to the conditions (including effects of time/operator);

— s_{res} represents the residual standard deviation (including effects of random errors).

All standard deviations are expressed in $\log_{10}$ (cfu/g) or $\log_{10}$ (cfu/ml).

Table A.1 — Standard deviations for aerobic mesophilic flora

Laboratory code	Food	Category	s_{IS}	s_R	s_{res}	s_{cond}
2	fish	iv	0,36	0,43	0,23	0,06
2	frozen minced veal meat	ii	0,07	0,25	0,24	0,06
3	pastries	iv	0,12	0,18	0,11	0,07
4	fish	iv	0,37	0,51	0,29	0,20
7	ready-to-eat cooked meals	iv	0,24	0,33	0,17	0,13
8	vacuum-packed minced beef meat	ii	0,09	0,15	0,10	0,06
10	packed green salad	iii	0,10	0,45	0,17	0,41
10	dehydrated onion powder	ii	0,17	0,24	0,13	0,11
11	pâté	iv	0,72	0,78	0,10	0,29
11	pastries	iv	0,05	0,19	0,12	0,13
11	dehydrated mushrooms	iii	0,14	0,26	0,15	0,16
12	chicken neck skin	iv	0,19	0,20	0,06	0,02
13	cooked snails	iv	0,06	0,13	0,10	0,05
14	pastries	iv	0,32	0,35	0,11	0,08
20	chicken neck skin	iv	0,14	0,16	0,05	0,06
20	mechanically separated turkey meat	ii	0,10	0,13	0,06	0,05
20	mechanically separated chicken meat	ii	0,10	0,14	0,09	0,05
25	pâté	iv	0,46	0,47	0,07	0,03
26	raw milk cheese	iv	0,16	0,26	0,09	0,19
27	sliced ham	iv	0,30	0,31	0,06	0,05
30	pastries	iv	0,09	0,12	0,06	0,05
31	grated carrots	iii	0,09	0,14	0,08	0,08
32	fresh pork sausages	iv	0,20	0,24	0,12	0,05
34	fresh pork meat	iv	0,70	0,70	0,06	0,05
38	vanilla ice cream	ii	0,03	0,10	0,09	0,02
41	milk powder (environment)	i	0,05	0,14	0,10	0,08
42	milk powder	i	0,02	0,05	0,04	0,02
43	frozen shrimps	iii	0,19	0,20	0,05	0,05
44	frozen shrimps	iii	0,09	0,18	0,14	0,08
48	milk	i	0,04	0,12	0,06	0,09
49	corn starch	i	0,09	0,14	0,06	0,08
55	packed green salad	iii	0,15	0,20	0,06	0,11
72	caseinate	i	0,03	0,09	0,08	0,04
76	water	i	0,04	0,10	0,09	0,03
77	minced beef meat	ii	0,07	0,09	0,05	0,03
79	mixed poultry meat	ii	0,03	0,13	0,09	0,07
With spiral system:						
1	raw milk cheese	iv	0,29	0,38	0,12	0,21
24	dry feed for dogs	iii	0,18	0,24	0,13	0,09
59	minced meat	ii	0,17	0,23	0,15	0,05

Table A.2 — Standard deviations for coliforms

Laboratory code	Food	Category	s_{IS}	s_R	s_{res}	s_{cond}
1	vacuum-packed minced beef meat	iv	0,32	0,35	0,11	0,07
3	pastries	iv	0,16	0,23	0,15	0,07
6	fresh beef meat	iv	0,33	0,35	0,05	0,09
10	packed green salad	iii	0,41	0,78	0,33	0,58
12	chicken neck skin	iv	0,15	0,20	0,12	0,06
20	chicken neck skin	iv	0,07	0,12	0,09	0,05
26	raw milk cheese	iv	0,30	0,33	0,09	0,10
29	mechanically separated chicken meat	ii	0,10	0,15	0,07	0,08
30	pastries	iv	0,15	0,19	0,09	0,07
32	fresh pork sausages	iv	0,15	0,31	0,21	0,13
44	raw milk cheese	iv	0,11	0,21	0,10	0,14
45	fresh meat	iv	0,17	0,22	0,10	0,09
58	frozen coconut milk	i	0,12	0,18	0,11	0,08
74	whipped cream	ii	0,07	0,20	0,13	0,13

Table A.3 — Standard deviations for *E. coli*

Laboratory code	Food	Category	s_{IS}	s_R	s_{res}	s_{cond}
9	raw milk cheese	iv	0,45	0,47	0,10	0,06
16	raw milk cheese	iv	0,09	0,13	0,07	0,07
17	poultry meat (without skin)	iv	0,27	0,35	0,10	0,20
18	raw milk cheese	iv	0,25	0,27	0,07	0,06
19	poultry liver	iv	0,12	0,16	0,09	0,05
35	raw milk cheese	iv	0,13	0,18	0,12	0,03
37	frozen minced beef meat	ii	0,13	0,17	0,10	0,05
47	soya cream	ii	0,13	0,44	0,15	0,39
50	raw milk cheese	iv	0,29	0,30	0,04	0,02
50	raw milk cheese	iv	0,24	0,26	0,08	0,05
51	raw milk cheese	iv	0,13	0,15	0,07	0,02
52	sausage meat	ii	0,08	0,11	0,07	0,03
59	minced meat	ii	0,15	0,19	0,08	0,09
Most probable number:						
78	mussels	iii	0,15	0,31	0,15	0,11

Table A.4 — Standard deviations for coagulase-positive staphylococci

Laboratory code	Food	Category	s_{IS}	s_R	s_{res}	s_{cond}
1	raw milk cheese	iv	0,26	0,33	0,16	0,14
16	raw milk cheese	iv	0,08	0,16	0,11	0,09
28	raw milk cheese	iv	0,15	0,24	0,17	0,08
46	dry noodles	iii	0,09	0,13	0,08	0,05
50	raw milk cheese	iv	0,15	0,16	0,05	0,01
50	raw milk cheese	iv	0,12	0,14	0,05	0,04
9	raw milk cheese	iv	0,43	0,45	0,10	0,05
36	raw milk cheese	iv	0,21	0,22	0,06	0,04
71	raw milk cheese	iv	0,20	0,23	0,09	0,04
73	raw milk cheese	iv	0,32	0,48	0,28	0,03

Table A.5 — Standard deviations for other flora

Laboratory code	Food	Microorganisms	Category	s_{IS}	s_R	s_{res}	s_{cond}
10	dehydrated onion powder	yeasts + moulds	i	0,08	0,23	0,09	0,20
11	dehydrated mushrooms	*Bacillus cereus*	iii	0,21	0,26	0,12	0,09
15	poultry meat (without skin)	*Pseudomonas*	iv	0,20	0,34	0,14	0,24
20	mechanically separated turkey meat	sulfite-reducing bacteria	ii	0,05	0,10	0,08	0,03
20	mechanically separated chicken meat	sulfite-reducing bacteria	ii	0,09	0,14	0,09	0,06
21	raw milk cheese	*L. monocytogenes*	iv	0,59	0,60	0,10	0,05
22	cattle feeding powder	Enterobacteriaceae	iii	0,31	0,33	0,11	0,05
33	grated celeriac	lactic acid flora	iii	0,08	0,25	0,14	0,20
39	milk powder	*Bifidobacterium*	i	0,09	0,14	0,08	0,08
40	dry parsley	*Bacillus cereus*	iii	0,17	0,27	0,18	0,12
45	fresh meat	*Salmonella*	iv	0,21	0,24	0,09	0,07
53	minced turkey meat	sulfite-reducing bacteria	ii	0,07	0,25	0,12	0,21
54	dried figs	yeasts + moulds	iv	0,74	0,75	0,07	0,01
55	corn flakes	moulds	iii	0,32	0,36	0,11	0,12
56	fresh chicken meat	Enterobacteriaceae	iv	0,35	0,52	0,29	0,27
57	minced beef meat	Enterobacteriaceae	ii	0,01	0,04	0,03	0,02
70	hazel nuts	yeasts + moulds	iii	0,28	0,29	0,03	0,07
76	water	*Streptococcus*	i	0,08	0,17	0,14	0,03
76	water	*Enterococcus*	i	0,12	0,16	0,09	0,02

A.3 Use

In the frame of this Technical Specification, the outcome of these trials, i.e. the 4 main categories explained in A.2.2 and the detailed results in A.2.3, can serve as guidance to the laboratories for selecting the matrices to be tested for MU estimation (see 5.2.1).

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