
**Microbiology of the food chain —
Estimation of measurement
uncertainty for quantitative
determinations**

*Microbiologie de la chaîne alimentaire — 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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

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

This first edition cancels and replaces ISO/TS 19036:2006, which has been technically revised. It also incorporates the amendment ISO/TS 19036:2006/Amd.1:2009. The main changes compared with the previous edition are as follows:

- provision has been made for the estimation of technical uncertainty, and also for other relevant sources of uncertainty involved in quantitative microbiological tests, relating to:
 - the matrix uncertainty (i.e. the uncertainty due to dispersion of microbes within the actual test matrix);
 - the Poisson uncertainty that relates to colony count techniques;
 - the confirmation uncertainty associated with tests to confirm the identity of specific organisms following a count for presumptive organisms;
 - the uncertainty associated with most probable number (MPN) estimates;
- the experimental design for the estimation of intralaboratory reproducibility standard deviation described in this document in connection with the technical uncertainty is now the same as the design described in ISO 16140-3 for the verification of quantitative methods;
- worked examples have been added to illustrate ways in which uncertainty estimates should be generated and reported;
- annexes have been added to provide details of some of the important, or alternative, procedures and issues associated with uncertainty estimation.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The term “measurement uncertainty” (MU) is used to denote the lack of accuracy (trueness and precision) that can be associated with the results of an analysis. In the context of quantitative microbiology, it provides an indication of the degree of confidence that can be placed on laboratory estimates of microbial numbers in foods or other materials.

ISO/IEC Guide 98-3 (also known as the “GUM”) is a widely adopted reference document. The principal approach of ISO/IEC Guide 98-3 is to construct a mathematical or computer measurement model that quantitatively describes the relationship between the quantity being measured (the measurand) and every quantity on which it depends (input quantities). That measurement model is then used to deduce the uncertainty in the measurand from the uncertainties in the input quantities.

ISO/IEC Guide 98-3 recognizes that it might not be feasible to establish a comprehensive mathematical relationship between the measurand and individual input quantities and that in such cases the effect of several input quantities can be evaluated as a group. ISO/IEC 17025 also recognizes that the nature of the test method can preclude rigorous calculation of measurement uncertainty.

In the case of the microbiological analysis of samples from the food chain, it is not feasible to build a comprehensive quantitative measurement model, since it is not possible to quantify accurately the contribution of each input quantity, where:

- the analyte is a living organism, whose physiological state can be largely variable;
- the analytical target includes different strains, different species or different genera;
- many input quantities are difficult, if not impossible, to quantify (e.g. physiological state);
- for many input quantities (e.g. temperature, water activity), their effect on the measurand cannot be described quantitatively with adequate precision.

For the reasons given above, this document mostly uses a top-down or global approach to MU, in which the contribution of most input quantities is estimated as a standard deviation of reproducibility of the final result of the measurement process, calculated from experimental results with replication of the same analyses, as part of the measurement process. These quantities reflect operational variability and result in technical uncertainty. In food chain quantitative microbiology, assigned values or reference quantity values are usually not available so bias (which quantitatively expresses the lack of trueness) cannot be reliably estimated and is not included in the uncertainty estimated by this document.

While reproducibility provides a general estimate of uncertainty associated with the measurement method, it might not reflect characteristics associated with matrix uncertainty, resulting from the distribution of microorganisms in the food matrix.

Also, microbiological measurements often depend on counting or detecting quite small numbers of organisms that are more or less randomly distributed leading to intrinsic variability between replicates and a corresponding distributional uncertainty. For colony-count techniques, the Poisson uncertainty is determined, to which may be added, in certain cases, an uncertainty linked to confirmation tests used to identify isolated organisms. An additional uncertainty component is also required for most probable number (MPN) determinations. Relevant distributional uncertainty components, estimated from statistical theory, are calculated from individual experimental data.

These three different kinds of uncertainty (technical, matrix and distributional uncertainties) are combined using the principles of ISO/IEC Guide 98-3. This approach is similar to that followed by ISO 29201 in the field of water microbiology.

Technical uncertainty is often the largest of these three kinds and is estimated from a reproducibility standard deviation, which inevitably includes some contributions from the other two kinds. The preferred estimate of technical uncertainty is based on intralaboratory reproducibility, in the same way as ISO 16140-3. If consistent with laboratory protocols and client requirements, a general value of uncertainty may be reported as based only on a reproducibility standard deviation.

Microbiology of the food chain — Estimation of measurement uncertainty for quantitative determinations

1 Scope

This document specifies requirements and gives guidance for the estimation and expression of measurement uncertainty (MU) associated with quantitative results in microbiology of the food chain.

It is applicable to the quantitative analysis of:

- products intended for human consumption or the feeding of animals;
- environmental samples in the area of food production and food handling;
- samples at the stage of primary production.

The quantitative analysis is typically carried out by enumeration of microorganisms using a colony-count technique. This document is also generally applicable to other quantitative analyses, including:

- most probable number (MPN) techniques;
- instrumental methods, such as impedimetry, adenosine triphosphate (ATP) and flow cytometry;
- molecular methods, such as methods based on quantitative polymerase chain reaction (qPCR).

The uncertainty estimated by this document does not include systematic effects (bias).

2 Normative references

There are no normative references in this document.

3 Terms, definitions and symbols

3.1 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

3.1.1 sample

<general> one or more items (or a proportion of material) selected in some manner from a population (or from a large quantity of material) intended to provide information representative of the population, and, possibly, to serve as a basis for a decision on the population or on the process which had produced it

[SOURCE: ISO/TS 17728:2015, 3.2.2, modified — Note 1 to entry has been deleted.]

3.1.2 laboratory sample

sample (3.1.1) prepared for sending to the laboratory and intended for inspection or testing

[SOURCE: ISO 6887-1:2017, 3.1]

3.1.3

test sample

sample (3.1.1) prepared from the *laboratory sample* (3.1.2) according to the procedure specified in the method of test and from which *test portions* (3.1.4) are taken

Note 1 to entry: Preparation of the laboratory sample before the test portion is taken is infrequently used in microbiological examinations.

[SOURCE: ISO 6887-1:2017, 3.4]

3.1.4

test portion

measured (volume or mass) representative *sample* (3.1.1) taken from the *laboratory sample* (3.1.2) for use in the preparation of the initial suspension

Note 1 to entry: Sometimes preparation of the laboratory sample is required before the test portion is taken, but this is infrequently the case for microbiological examinations.

[SOURCE: ISO 6887-1:2017, 3.5]

3.1.5

measurand

particular quantity subject to measurement

[SOURCE: ISO/IEC Guide 98-3:2008, B.2.9 modified — The example and the Note 1 to entry have been deleted.]

3.1.6

trueness

measurement trueness

closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value

Note 1 to entry: Trueness is not a quantity and thus cannot be expressed numerically, but measures for closeness of agreement are given in ISO 5725 (all parts).

Note 2 to entry: Trueness is inversely related to systematic measurement error, but is not related to random measurement error.

Note 3 to entry: “Measurement accuracy” should not be used for “trueness” and vice versa.

[SOURCE: ISO/IEC Guide 99:2007, 2.14, modified — The preferred term has been changed from “measurement trueness” to “trueness”.]

3.1.7

bias

measurement bias

estimate of a systematic measurement error

[SOURCE: ISO/IEC Guide 99:2007, 2.18, modified — The preferred term has been changed from “measurement bias” to “bias”.]

3.1.8

intralaboratory reproducibility

intermediate precision

closeness of agreement between test results obtained with the same method on the same or similar test materials in the same laboratory with different operators using different equipment

[SOURCE: ISO 8199:2018, 3.6]

3.1.9 measurement uncertainty MU

parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the *measurand* (3.1.5)

Note 1 to entry: 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 to entry: Measurement uncertainty 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.

Note 3 to entry: 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.

[SOURCE: ISO/IEC Guide 98-3:2008, 2.2.3, modified — The preferred term has been changed from “uncertainty of measurement” to “measurement uncertainty”.]

3.1.10 standard uncertainty

u

uncertainty of the result of a measurement expressed as a standard deviation

[SOURCE: ISO/IEC Guide 98-3:2008, 2.3.1, modified — The symbol has been added.]

3.1.11 combined standard uncertainty

$u_c(y)$

standard uncertainty (3.1.10) 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

[SOURCE: ISO/IEC Guide 98-3:2008, 2.3.4, modified — The symbol has been added.]

3.1.12 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* (3.1.5)

Note 1 to entry: The fraction may be regarded as the coverage probability or level of confidence of the interval.

Note 2 to entry: 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* (3.1.11). 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.

Note 3 to entry: An expanded uncertainty *U* is calculated from a combined standard uncertainty $u_c(y)$ and a *coverage factor* *k* (3.1.13) using:

$$U = k \times u_c(y)$$

[SOURCE: ISO/IEC Guide 98-3:2008, 2.3.5, modified— The symbol has been added and Note 3 to entry has been replaced.]

3.1.13
coverage factor

k
number larger than one by which a *combined standard uncertainty* (3.1.11) is multiplied to obtain an *expanded uncertainty* (3.1.12)

[SOURCE: ISO/IEC Guide 98-3:2008, 2.3.6, modified— The symbol has been added and the definition has been reworded.]

3.1.14
technical uncertainty

uncertainty resulting from operational variability associated with the technical steps of the analytical procedure

Note 1 to entry: Technical uncertainty includes the variability of the taking, mixing, and dilution of the *test portion* (3.1.4) taken from the *laboratory sample* (3.1.2) to prepare the initial suspension and subsequent dilutions. It also includes the effects of variability in incubation and media.

Note 2 to entry: Adapted from ISO 29201:2012, 3.4.2.

3.1.15
matrix uncertainty

uncertainty resulting from the extent to which the *test portion* (3.1.4) is not truly representative of the *laboratory sample* (3.1.2)

3.1.16
distributional uncertainty

uncertainty resulting from intrinsic variability associated with the distribution of microorganisms in the *sample* (3.1.1), the initial suspension and subsequent dilutions

Note 1 to entry: In microbiological suspensions, intrinsic variability is usually modelled by the Poisson distribution. When partial confirmation is practised or the MPN principle is used, the resulting distribution may differ from the Poisson distribution.

Note 2 to entry: Adapted from ISO 29201:2012, 3.4.3.

3.2 Symbols

For the purposes of this document, the following symbols apply.

ΣC	for colony-count methods, total number of counted colonies used to calculate the measurement results
n_p n_c	for colony-count methods with partial confirmation, number of presumptive colonies tested, and number of confirmed colonies, respectively
s_R	reproducibility standard deviation
s_{IR}	intralaboratory reproducibility standard deviation
$s_{IR:corr}$	intralaboratory reproducibility standard deviation, corrected by subtraction of matrix and distributional components
s_r	repeatability standard deviation
$s_{r:corr}$	repeatability standard deviation, corrected by subtraction of distributional components
$S_{unwanted}$	sum of squares of unwanted components
u	standard uncertainty

u_{distrib}	distributional standard uncertainty
u_{tech}	technical standard uncertainty
u_{conf}	confirmation standard uncertainty
u_{matrix}	matrix standard uncertainty
u_{MPN}	most probable number standard uncertainty
u_{unwanted}	standard uncertainty of the unwanted component
u_{Poisson}	Poisson standard uncertainty
$u_c(y)$	combined standard uncertainty (of output estimate)
k	coverage factor
U	expanded uncertainty (of output estimate) = $k \times u_c(y)$

4 General considerations

MU associated with any measurement value includes multiple components.

As indicated in the Scope (see [Clause 1](#)), the uncertainty estimated by this document does not include contributions from systematic effects (bias). In food chain quantitative microbiology, assigned values or reference quantity values are usually not available so bias cannot be reliably estimated.

This document considers three distinct types of uncertainty component:

- technical uncertainty;
- matrix uncertainty;
- distributional uncertainty.

Technical uncertainty arises from operational variability and is estimated, using a global approach, from a reproducibility standard deviation of the final result of the measurement process (see [Clause 5](#)). This global approach means that the technical uncertainty estimate comes from final test results rather than by calculation using estimates of uncertainty at every individual stage of the test.

Matrix uncertainty arises from imperfect mixing of the laboratory sample, resulting in poor reproducibility of microbial levels between test portions, which can be large for solid matrices, and especially for composite food products. Matrix uncertainty is estimated for each kind of matrix (see [Clause 6](#)).

Even for homogeneous materials, the random distribution of microorganisms leads to distributional uncertainty (see [Clause 7](#)), of which three potential kinds are considered in this document. The relevance of each depends on the method used:

- for colony-count techniques:
 - Poisson uncertainty (see [7.2](#));
 - confirmation uncertainty (see [7.3](#));
- for MPN techniques: MPN uncertainty (see [7.4](#)).

The uncertainty for each distributional uncertainty source is estimated mathematically.

This document presents two options for estimating the combined uncertainty for a reported measurement.

- a) Technical, matrix and distributional uncertainty components for a reported value may be estimated separately from each other (see [Clauses 5, 6 and 7](#)), after which the three components are combined (see [8.1.2](#)).
- b) A general value of uncertainty may be reported as based only on a reproducibility standard deviation, if consistent with laboratory protocols and client requirements (see [8.1.3](#)). Technical uncertainty is indeed often the largest of the three uncertainty components.

5 Technical uncertainty

5.1 Identification of main sources of uncertainty

5.1.1 General aspects

It can be helpful to consider the sources of technical uncertainty usually associated with the main stages in a microbiological method. Typical sources for colony-count or MPN techniques are:

- taking a test portion from the laboratory (or test) sample;
- preparation of the initial suspension;
- serial dilution;
- inoculation;
- incubation;
- counting of colonies in a colony count technique, and/or detection of growth (as in a MPN technique);
- confirmation (if appropriate).

[Figure 1](#) shows the main sources of uncertainty in food chain microbiology considered in this document.

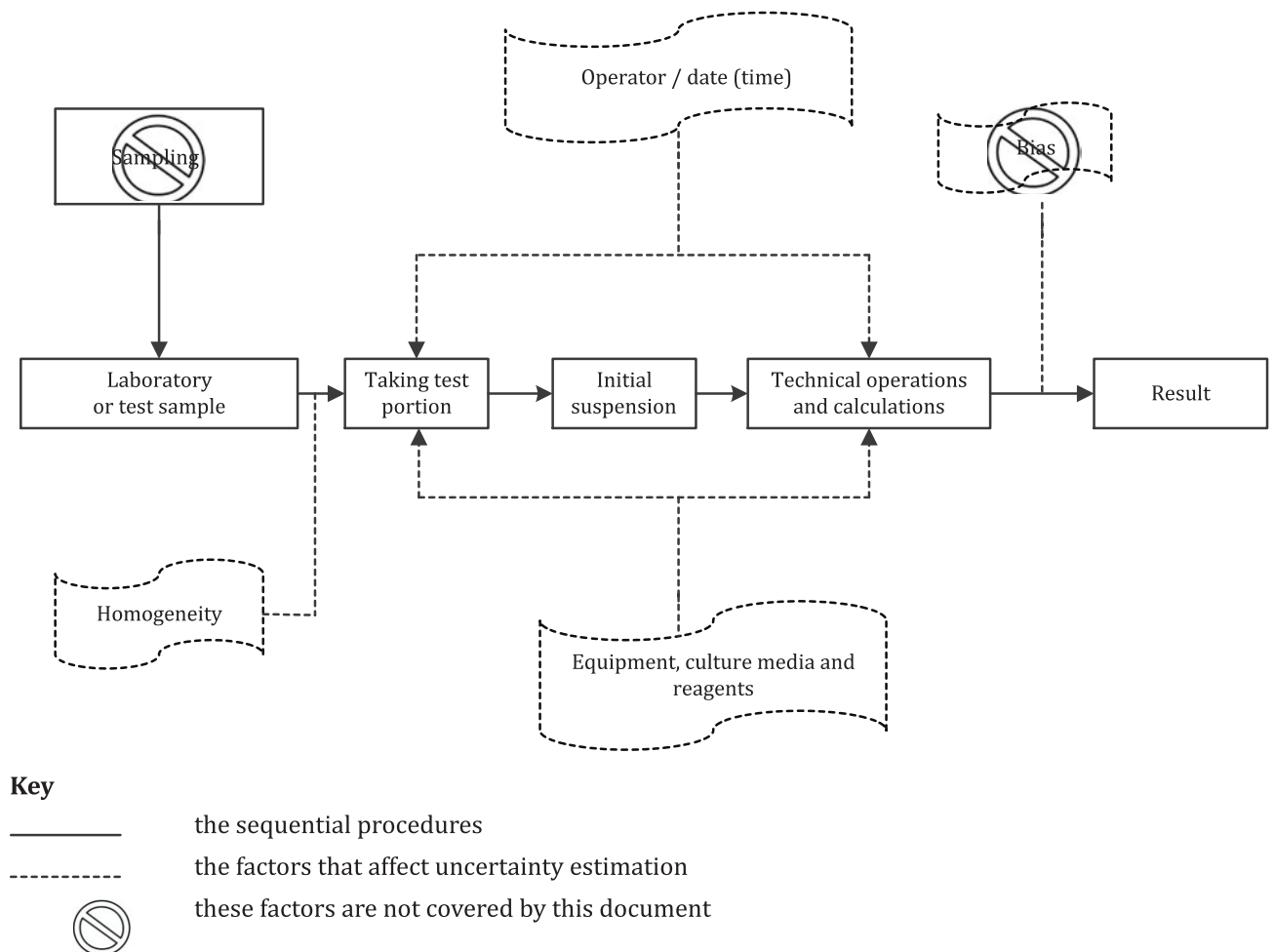


Figure 1 — Main sources of uncertainty in food chain microbiology covered in this document

5.1.2 Sampling uncertainty

Sampling uncertainty, i.e. error associated with the drawing of the laboratory sample from a lot under investigation, can contribute significantly to the overall error^[18], but it is not part of the uncertainty linked to the measurement itself and is not covered by this document.

Matrix uncertainty that arises from the inability of the test portion to perfectly represent a heterogeneous laboratory sample or test sample is covered in [Clause 6](#). The extent of such inability can depend on the size of the test portion taken for examination (see ISO 6887-1).

5.1.3 Bias

As indicated in [Clauses 1](#) and [4](#), MU estimated by this document does not include contributions from systematic effects that is bias.

5.1.4 Critical factors

Examples of critical technical factors that can influence uncertainty and need to be controlled include: the source and type of culture media and/or other reagents (such as the ones used for confirmation), the dilution, inoculation and incubation procedures, the counting techniques (manual or automated), and changes to the operator or group of operators, etc.

5.2 Estimation of technical uncertainty

5.2.1 General aspects

Technical uncertainty is estimated from the standard deviation of reproducibility, s_R , on the final result of the measurement process. As such, technical uncertainty is a characteristic of the method and technical uncertainty estimated for one method cannot be applied to other methods.

Ongoing estimation of MU should be made to show that the estimate of uncertainty remains relevant and that the test results are under control. Reassessment of MU estimate shall be made following changes to any (critical) factor (see [5.1.1](#) and [5.1.4](#)) that is likely to affect the results obtained with that method in any significant way.

Three different possibilities are presented in this document for estimation of the standard deviation of reproducibility. They are based upon repeated measurements of nominally identical material. The preference order is as follows:

- option 1: intralaboratory reproducibility, i.e. reproducibility estimated within a laboratory (see [5.2.2](#));
- option 2: reproducibility derived from results of a method validation interlaboratory study (see [5.2.3.1](#));
- option 3: reproducibility derived from results of an interlaboratory proficiency test (PT) (see [5.2.3.2](#)).

5.2.2 Reproducibility standard deviation derived from intralaboratory experiments, s_{IR}

5.2.2.1 General aspects

Option 1, intralaboratory reproducibility, is the preferred option for deriving technical MU since it enables a laboratory to attach the MU value to the results that it reports, in line with the definition of MU.

The experimental protocol described in this clause should take into account as many as possible of the uncertainty sources identified in (see [5.1](#)).

5.2.2.2 Experimental protocol

5.2.2.2.1 General aspects

The protocol for analysis of exactly two test portions for each laboratory sample is shown in [Figure 2](#), for which the corresponding calculations are provided in [5.2.2.3](#). For other cases (i.e. more than two test portions for some or all laboratory samples), the protocol and calculations are given in [Annex A](#).

For each test method, perform the experimental protocol of [Figure 2](#) for at least ten laboratory samples and repeat it to give at least two acceptable results for each laboratory sample. [5.2.2.3.1](#) provides indications of acceptable values. Depending on the circumstances, this can require more than ten laboratory samples and/or more than two test portions for each laboratory sample.

The data from different laboratory samples are collected over a period of time as part of a special exercise or as part of a laboratory's routine quality management procedure. In that case, it should be ensured that the experimental design principles in this clause are followed.

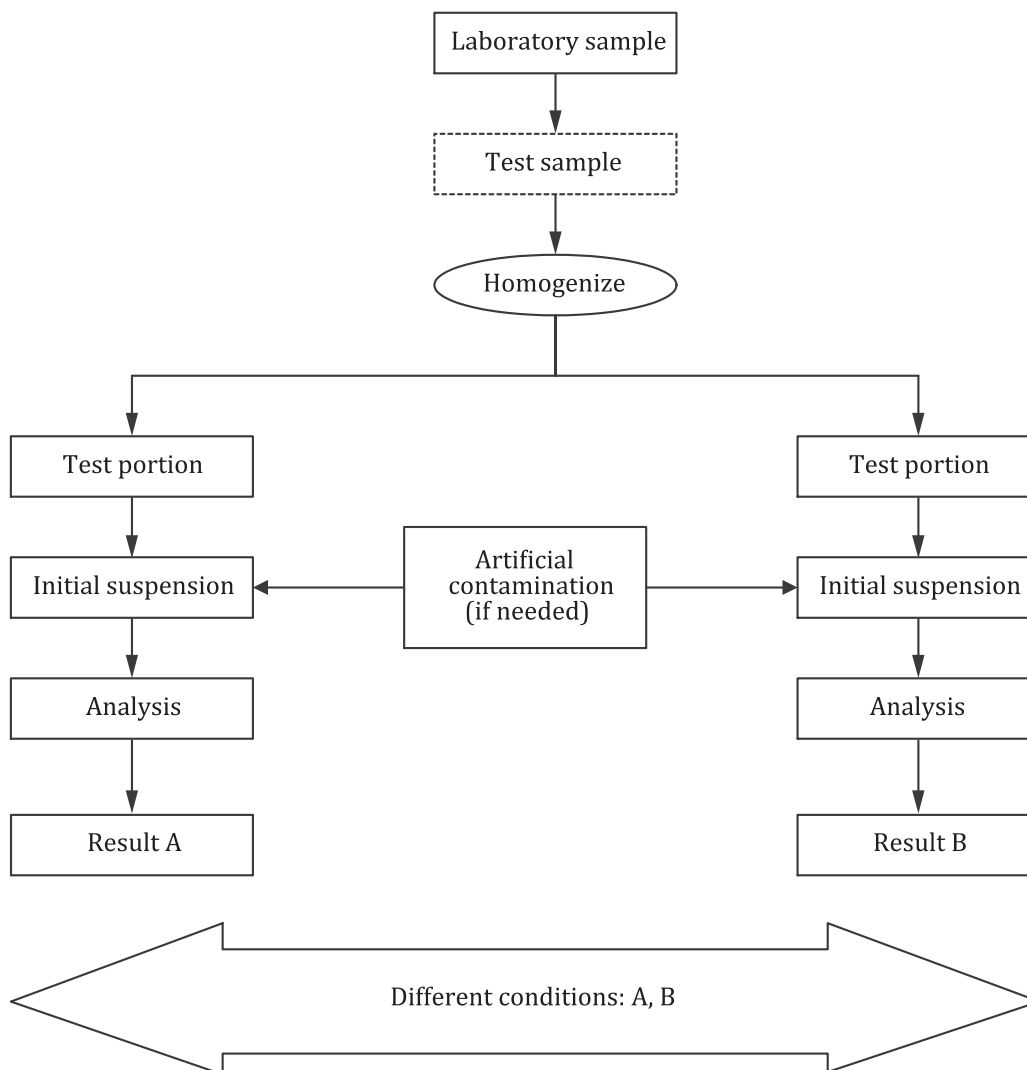


Figure 2 — Experimental protocol for estimation of intralaboratory reproducibility — Two determinations on each laboratory sample

5.2.2.2.2 Choice of laboratory samples

The estimation of intralaboratory reproducibility is designed to exclude contributions from heterogeneity within the laboratory sample, so it is not necessary to repeat this estimation for different matrices, and this estimate may be based on a single matrix.

The calculation (see 5.2.2.3) uses log-transformed data to normalize the intralaboratory reproducibility variance, so it is not necessary to repeat the experimental protocol for different contamination levels. However, where possible, the laboratory samples should be chosen to cover the expected natural variation in contamination levels.

5.2.2.2.3 Samples from interlaboratory proficiency tests

If a laboratory participates in interlaboratory PTs, the results of that laboratory's analyses may be used to contribute to the intralaboratory reproducibility estimate of uncertainty, provided that:

- the PT samples are representative of routine samples analysed by the laboratory (matrix type, test portion size);

and

- b) the laboratory carries out estimates on two, or more, test portions under different measurement conditions, as indicated in [5.2.2.2.6](#).

However, if the intralaboratory reproducibility estimates from PT samples differ widely from in-house estimates on real samples of a similar type, the differences shall be recognized and recorded since they can reflect differences in the matrix and microbial inoculum used in the PT samples.

5.2.2.2.4 Preparation of laboratory sample

In order to minimize matrix uncertainty contributions, the laboratory sample or the test sample, in cases where the laboratory sample is too big to homogenize, should be made as homogeneous as possible. Laboratory samples that comprise the following should be mixed well prior to drawing test portions:

- non-viscous liquids and powders (e.g. milk, coconut milk, dried milk);
- minced/finely chopped solids or suspensions/emulsions (e.g. minced meat, mechanically separated meat, sausage meat, crushed meat, whipped cream, dairy ice cream, soya cream).

Prior to drawing test portions, other laboratory samples or test samples should be mixed using an appropriate homogenization procedure. For possibilities suited to each type of sample material, see ISO 6887 (all parts).

5.2.2.2.5 Test portions

Take at least two test portions from each laboratory (or test) sample to allow repeated measurements.

5.2.2.2.6 Initial suspension, artificial contamination (if needed) and conditions of analysis

If artificial contamination is required, perform it in the initial suspension. Detailed procedures for the preparation of artificially inoculated food are described in ISO 16140-3.

Perform the analyses on each test portion as in routine testing (e.g. preparation of one series of decimal dilutions, inoculation of one or two plates per dilution).

The measurement conditions A and B for the two test portions (see [Figure 2](#), or [Annex A](#) if more than two test portions are examined) should differ in as many ways as possible. Ideally, include as many variations in all relevant sources of technical uncertainty (see [5.1](#)) as could be encountered from one day to another within the laboratory. These will typically include, but not be limited to, batches of culture media, reagents and membrane filters, vortex or other mixer, pH meter, incubators, time of analysis, etc. If possible, the two test portions should be tested by at least two different technicians. As the contamination of the food sample is rarely stable in food chain microbiology, the measurement repetitions should be done within a short period of time on a single day. Repetitions may be performed on more than one day only if contamination levels can be shown to be stable.

The pattern of variation should not be the same for all laboratory (test) samples. For example, if sample 1 is tested by technician A using media batch B on day 1, then sample 2 should vary this pattern, e.g. sample 2 is tested by technician A using media batch A on day 1 or on day 2. The objective is to maximize the variation between repeated measurements while maintaining, at the same time, a realistic representation of the laboratory's operations.

5.2.2.3 Calculations

5.2.2.3.1 Acceptable results

For colony count techniques, ensure that a sufficiently large number of counted colonies can be used for the calculations. Enumeration results based on less than 30 counted colonies should be excluded as well

as counts above the maximum number per plate (in most cases 300 cfu/plate or lower as specified in the specific standard).

NOTE 1 The limit of 30 colonies relates to the sum of the total numbers of counted colonies on all retained plates, ΣC , for a single result.

NOTE 2 The limit of 30 colonies is specific to this experimental protocol for estimating the standard deviation of intralaboratory reproducibility (i.e. experiments aiming specifically to assess the uncertainty) and not the use of this standard deviation to assess MU for new samples (see [Clause 8](#)).

For methods including partial confirmation, any results for which less than half of the colonies tested were confirmed should be excluded, i.e. it is recommended to exclude results for which $n_c < n_p/2$ (see [7.3](#) for the symbols).

For MPN-based methods, where a single measurement result arises from a number of positive or negative test results, measurement results based on less than five positive test results should be excluded from the calculation of intralaboratory reproducibility.

NOTE 3 The limit of five positive test results relates to the sum of positive results across all dilutions tested for a single measurement result. This limit does not depend on the number of negative test results, or on the total number of test results.

5.2.2.3.2 Intralaboratory reproducibility standard deviation

In accordance with normal practice in food chain microbiology, transform the result from each test portion in cfu/g or ml into \log_{10} cfu/g or ml before calculations are done.

This subclause describes the calculation procedure for exactly two values from each laboratory sample. Refer to [Annex A](#) for the calculation procedure to be used when there are more than two values from each laboratory sample. [Annex A](#) also provides an alternative calculation for two values from each laboratory sample.

For the n (at least 10) laboratory samples from a given implementation of the protocol (see [5.2.2.2](#)), the results (y_{iA} and y_{iB}) for each of the test portions are used to calculate the intralaboratory reproducibility standard deviation, s_{IR} , as shown in [Formula \(1\)](#):

$$s_{IR} = \sqrt{\frac{1}{2n} \sum_{i=1}^n (y_{iA} - y_{iB})^2} \quad (1)$$

where

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

y_{iA}, y_{iB} are the log-transformed data, in \log_{10} cfu/g or ml, from conditions A and B respectively.

An example of the manual calculation is given in [Table 1](#). Calculations were performed in Excel^{®1)} from values shown for the dilution factors (d) and colonies counted (C). Derived values have been rounded for display, but accuracy was kept in the calculations without rounding.

1) Excel is the trade name of a product supplied by Microsoft. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Table 1 — Calculation of intralaboratory reproducibility standard deviation — Example of enumeration of aerobic mesophilic flora in mixed poultry meat with one replicate at each of two dilutions tested

Laboratory sample	Test portion	Dilution factors (<i>d</i>) Colonies counted (<i>C</i>)				Total colonies counted ΣC_{ij}	Colony count result in cfu/g or ml weighted mean (see ISO 7218) x_{ij}	\log_{10} cfu/g or ml $y_{ij} = \log_{10}(x_{ij})$	Difference \log_{10} cfu $y_{iA} - y_{iB}$	Squared difference $(y_{iA} - y_{iB})^2$
		d_1	C_1	d_2	C_2					
1	A	3	102	4	8	110	$1,0 \times 10^5$	5,000 0	0,242 1	0,058 6
1	B	3	59	4	4	63	$5,7 \times 10^4$	4,757 9		
2	A	5	61	6	6	67	$6,1 \times 10^6$	6,784 7	-0,043 2	0,001 9
2	B	5	66	6	8	74	$6,7 \times 10^6$	6,827 8		
3	A	4	168	5	18	186	$1,7 \times 10^6$	6,228 1	0,301 0	0,090 6
3	B	4	86	5	7	93	$8,5 \times 10^5$	5,927 1		
4	A	5	266	6	25	291	$2,6 \times 10^7$	7,422 5	0,270 8	0,073 3
4	B	5	140	6	16	156	$1,4 \times 10^7$	7,151 7		
5	A	6	45	7	5	50	$4,5 \times 10^7$	7,657 6	0,540 6	0,292 3
5	B	5	129	6	15	144	$1,3 \times 10^7$	7,117 0		
6	A	4	129	5	12	141	$1,3 \times 10^6$	6,107 8	0,045 4	0,002 1
6	B	4	117	5	10	127	$1,2 \times 10^6$	6,062 4		
7	A	2	92	3	8	100	$9,1 \times 10^3$	3,958 6	-0,158 4	0,025 1
7	B	2	131	3	13	144	$1,3 \times 10^4$	4,117 0		
8	A	3	139	4	13	152	$1,4 \times 10^5$	5,140 5	-0,016 8	0,000 3
8	B	3	143	4	15	158	$1,4 \times 10^5$	5,157 3		
9	A	1	49	2	5	54	$4,9 \times 10^2$	2,691 0	-0,419 9	0,176 3
9	B	1	129	2	13	142	$1,3 \times 10^3$	3,110 9		
10	A	4	142	5	13	155	$1,41 \times 10^6$	6,148 9	0,787 2	0,619 7
10	B	3	227	4	26	253	$2,30 \times 10^5$	5,361 7		
								sum =	1,340 1	
								$1,340 1 / (2 \times 10) =$	0,067 0	
								$s_{IR} = \sqrt{0,067} =$	0,258 9	
x_{ij} are the calculated colony counts on test portions, e.g. $x_{1B} = 10^{d_1} \frac{\Sigma C_{1B}}{1,1} = 10^3 \frac{63}{1,1} = 10^3 \times 57,3 = 5,73 \times 10^4$.										

[Annex A](#) describes the calculations for the general case of more than two values from each laboratory sample and also illustrates how the calculations may be performed using analysis of variance (ANOVA) in the general case of two and more values from each laboratory sample.

NOTE The reproducibility standard deviation inevitably includes any of the matrix and distributional components relevant to the reproducibility data. If uncertainty of a test result is calculated by combining this reproducibility standard deviation with matrix and distributional components relevant to the test result, there will be an overestimate of uncertainty. The laboratory can choose to avoid this overestimation, at the expense of more complicated calculations, by subtracting any of the relevant matrix and distributional uncertainty components from the reproducibility standard deviation. This optional alternative approach is described in [Annex D](#), to give a corrected standard deviation, $s_{IR:corr}$

5.2.3 Reproducibility standard deviation derived from interlaboratory studies

5.2.3.1 Interlaboratory method validation studies

5.2.3.1.1 General aspects

Where a method used by a laboratory has been submitted to an interlaboratory validation study, the laboratory may use the reproducibility standard deviation of the method as an estimate of its technical MU, subject to the following condition: the repeatability and reproducibility estimates of precision attained by measurements within the laboratory shall not be larger than the corresponding values obtained in the interlaboratory study.

The procedure used to check this condition is met, and to form a combined uncertainty estimate with the possible additional factors not covered by the interlaboratory study, is described in detail in ISO 21748.

5.2.3.1.2 Use in food microbiology

Limitations to the use of interlaboratory method validation studies to estimate technical uncertainty are summarized below.

- Reproducibility parameters derived from interlaboratory method validation studies are not available for all methods.
- The extent to which taking the test portion and preparation of the initial suspension includes matrix effects will depend on the experimental design of the interlaboratory method validation study.
- Precision values from an interlaboratory method validation study will have been obtained under limited and precisely defined conditions. Combinations of matrix, strain of test microorganism, contamination level, stress treatment, etc. are used to provide homogeneous and standardized samples for interlaboratory studies with or without a defined background microflora. Hence, the natural variation in sample contamination that may be found in practice is reduced, thereby leading to an under-estimate of the uncertainty. So, even if reproducibility data are available, it may be difficult to generalize from artificial trials to routine analyses performed by the laboratory.
- It is unlikely that adequate detail is available to correct the reproducibility standard deviation for unwanted uncertainty components, in accordance with [Annex D](#).

For these reasons, the use of the reproducibility standard deviation from an interlaboratory study of the method is only a second option, after reproducibility standard deviation from intralaboratory experiments (see [5.2.2](#)).

5.2.3.2 Interlaboratory proficiency tests

For uncertainty estimation, the estimate of reproducibility derived from participants in a PT may be used only in the following situation.

- When the same method has been used by all participants in a PT, a participant whose result was assessed as satisfactory by the PT organizer may estimate technical uncertainty as the standard deviation of all results assessed as satisfactory by the PT organizer.
- As for [5.2.3.1](#), the extent to which taking the test portion and preparation of the initial suspension includes matrix effects will depend on the PT experimental design.
- As for [5.2.3.1](#), reproducibility values from a PT will have been obtained under limited and precisely defined conditions. Combinations of matrix, strain of test microorganism, contamination level, stress treatment, etc. are used to provide homogeneous and standardized samples for PT with or without a defined background microflora. Hence, the natural variation in sample contamination that may be found in practice is reduced, thereby leading to an under estimate of the uncertainty.

- As for [5.2.3.1](#), it is unlikely that adequate detail is available to correct the reproducibility standard deviation for unwanted uncertainty components, in accordance with [Annex D](#).

Therefore, it can be difficult to generalize from artificial trials to routine analyses performed by the laboratory.

Given these limitations, the use of the reproducibility standard deviation from an interlaboratory PT is only a third option, after reproducibility standard deviation from intralaboratory experiments (see [5.2.2](#)) and reproducibility standard deviation from an interlaboratory study of the method (see [5.2.3.1](#)).

When this option is followed, care shall be taken that the standard deviation used is that of the PT participants' results, as described above. This normally differs from the standard deviation for proficiency assessment used to calculate z-scores (a performance statistic defined in ISO 13528).

However, as described in [5.2.2.2.3](#), results obtained in a laboratory by analysis of at least two test portions of an interlaboratory PT sample may be included in that laboratory's assessment of intralaboratory reproducibility.

6 Matrix uncertainty

6.1 General aspects

A test result can be affected by both matrix composition and microbial distribution. In this document, the term “matrix uncertainty” refers only to the effects of microbial distribution in a given matrix, i.e. the variation between results from different test portions taken from the same laboratory sample. It reflects the extent to which the individual test portions are not representative of the overall laboratory sample. Matrix uncertainty differs from sampling uncertainty (see [5.1.2](#)), which is not covered in this document. The matrix uncertainty is regarded as being independent of the analytical method used. This means that the matrix uncertainty estimated for a matrix can be applied as the matrix uncertainty contribution for all quantitative tests in this matrix. Consideration should also be given to the distribution of the different types of microorganisms in the sample and the sample history (e.g. whether the sample was contaminated after production).

If the material is effectively homogeneous, such as well-mixed liquids (milk, water, drinks), the matrix uncertainty is expected to be small. However, it is well known^[17] that the natural microbial contamination of certain food products (especially solid, processed, or fermented products, etc.) can be highly heterogeneous and this can contribute a large uncertainty component. This is especially true for multi-component products with several distinct parts, such as pizzas or ready-to-eat cooked meals. It can also occur with other foods including powders (e.g. dried milk powder), cheeses and fresh-cut vegetables. For such heterogeneous materials, the uncertainty can be reduced by taking a larger test portion (see ISO 6887-1).

If the composition of the material is likely to affect substantially the performance of the method, then the applicability of the intralaboratory reproducibility value should be considered as restricted to similar materials; e.g. direct enumeration of sub-lethally damaged organisms in processed foods or in plant hygiene samples; see also ISO 6887-1.

See [Annex B](#) for more details.

Three approaches are described in [6.2](#) to [6.4](#) for estimating matrix uncertainty:

- use of a fixed value (see [6.2](#)): for well-mixed homogeneous laboratory (or test) samples, the matrix uncertainty is expected to be small, and a fixed (minimum) value can be used;
- examination of multiple test portions from laboratory (or test) samples from which the within-sample variance can be determined (see [6.3](#));
- relevant characteristics of the matrix and method are well known and the matrix uncertainty may be estimated from prior knowledge (see [6.4](#)).

6.2 Case of homogeneous laboratory (or test) sample

Experience indicates that liquids (thin, non-viscous fluids) are regarded as being homogeneous and thus have a relatively low matrix uncertainty, typically $u_{\text{matrix}} = 0,1 \log_{10}$ cfu/g or ml derived from experiments reported in Reference [14] (freely available for download at <http://standards.iso.org/iso/19036>). However, in some cases, matrix uncertainty of such materials can be larger.

Provided that the whole of the laboratory sample can be made homogeneous before taking the test portion, then the matrix uncertainty can be taken at a fixed value of $u_{\text{matrix}} = 0,1 \log_{10}$ [14]. Homogenization may include treatment using, for example, a rotating knife blade, a peristaltic paddle system or an ultrasonic system (e.g. a Pulsifier®²⁾). Advice on homogenization techniques is given in ISO 6887-1 and ISO 7218.

6.3 Multiple test portions from laboratory samples

Matrix uncertainty may be estimated as the within-laboratory-sample repeatability standard deviation, by analysing multiple test portions in repeatability conditions from one or more laboratory samples, using the experimental design in Figure 3.

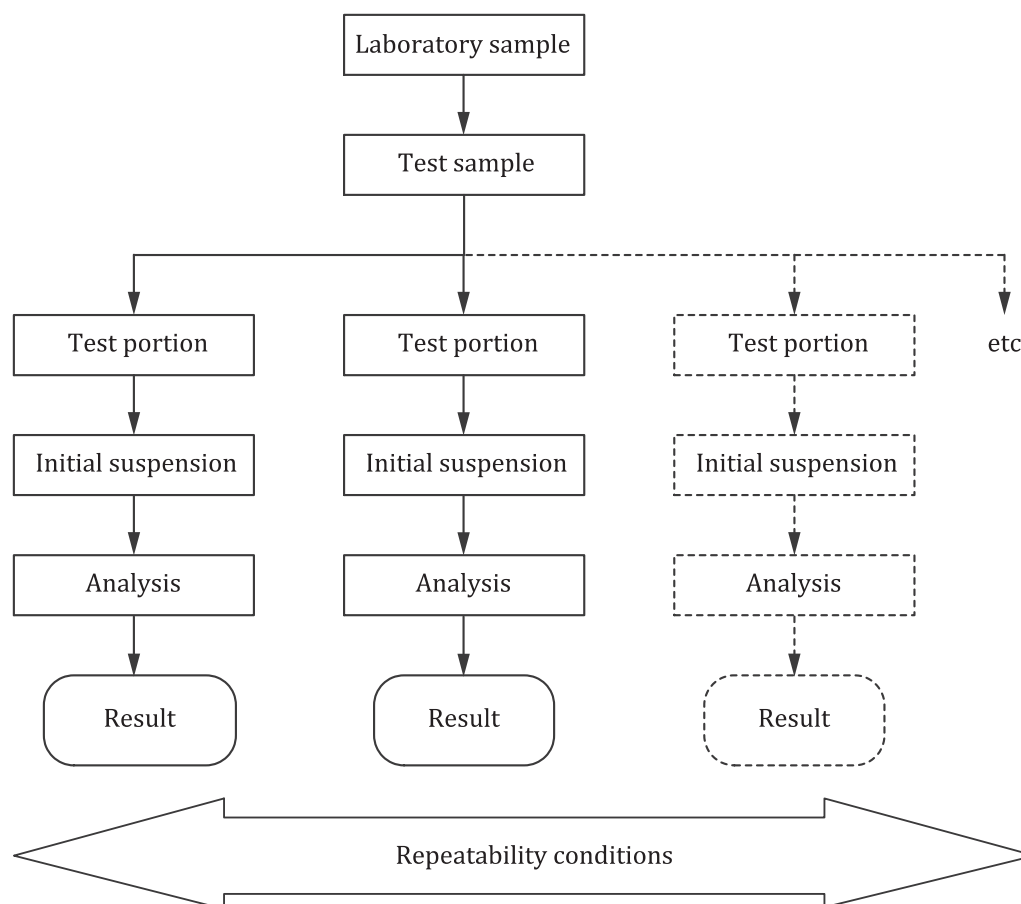


Figure 3 — Experimental design to estimate matrix uncertainty from at least two test portions from each laboratory sample — Design for each laboratory sample

This estimate unavoidably leads to an overestimate of matrix uncertainty since it includes some technical uncertainty components due to operational variation between the repeated analyses. To minimize this overestimation, the repeated analyses from a single laboratory sample are performed

2) Pulsifier is the trade name of a product supplied by Microgen. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

under conditions as similar as possible, i.e. under “repeatability conditions”. Conditions may vary between laboratory samples.

Use naturally contaminated samples, since artificial contamination is unlikely to reflect real matrix uncertainty. Because matrix uncertainty is regarded as independent of measurand and of test method used, measurands should be chosen for which naturally contaminated samples are likely to be found. Total mesophilic aerobic count, *Enterobacteriaceae* or thermophilic spore-forming microorganisms can all be good choices as the test to be applied.

All the test portions may come from one laboratory sample. This can be especially appropriate when a new matrix is analysed, i.e. a matrix not of the same type as those assessed previously. See [6.4](#) for guidance when considering matrices of the same type.

Alternatively, test portions may come from multiple laboratory samples, which may be analysed over a period of time so as to give a more generally applicable estimate of matrix uncertainty.

In all cases, at least two test portions shall be taken from each laboratory sample, and the total number of test portions shall be at least ten more than the number of laboratory samples. For example:

- 2 test portions are taken from each of 10 laboratory samples; or
- 11 test portions are taken from 1 laboratory sample.

Refer to [5.2.2.3.1](#) for defining acceptable results. In brief:

- colony count techniques; at least 30 counted colonies;
- methods including partial confirmation; at least half of tested colonies confirmed;
- MPN-based methods; at least five positive test results.

These restrictions may influence the choice of measurand and test method discussed above.

Calculate the repeatability standard deviation in accordance with [Annex A](#). For a single laboratory sample, this is equivalent to the standard deviation of the \log_{10} transformed data. For multiple laboratory samples, it is equivalent to a one-way ANOVA by sample on the \log_{10} transformed data.

NOTE The repeatability standard deviation inevitably includes any of the technical and distributional components relevant to the repeatability data. If uncertainty of a test result is calculated by combining this repeatability standard deviation with technical and distributional components relevant to the test result, there will be an overestimate of uncertainty. The laboratory can choose to avoid this overestimation, at the expense of more complicated calculations, by subtracting any of the relevant distributional uncertainty components from the repeatability standard deviation. This optional alternative approach to give a corrected standard deviation, $s_{r,corr}$ is described in [Annex D](#).

6.4 Known characteristic of the matrix

The laboratory may be able to judge, from prior knowledge, the matrix uncertainty to be expected of a given laboratory sample. This may rely on previous analyses of multiple test portions (see [6.3](#)) from laboratory samples expected to have a similar matrix uncertainty (matrix homogeneity).

When assessing whether laboratory samples can be expected to have a similar matrix uncertainty, the laboratory may consider ISO 16140-3. Examples of items for different categories and types are given in ISO 16140-3:—, Annex A³⁾.

Matrix uncertainty values obtained in one laboratory may be used by another laboratory for laboratory samples expected to have a similar matrix uncertainty.

3) Under preparation. Stage at the time of publication: ISO/DIS 16140-3:2018.

7 Distributional uncertainties

7.1 General aspects

Even for homogeneous material, irreducible minimum uncertainty components arise from the random distribution of microorganisms in the test material, usually modelled by the Poisson distribution (see 7.2). Similar perfect mixing assumptions underlie confirmation uncertainty of certain methods based on a colony-count technique (see 7.3) and most probable number uncertainty (see 7.4).

In this document, uncertainties arising from such fully random distribution of particles are termed distributional uncertainties. According to the features of the analytical method, calculate these distributional uncertainties from values underlying each individual result, in accordance with 7.2 to 7.4.

7.2 Colony-count technique — Poisson uncertainty

For methods based on a colony-count technique, there is a minimum distributional uncertainty contribution depending on the total number of counted colonies used in the calculation of the result, ΣC (see ISO 7218).

Table 2 gives the values of the Poisson standard uncertainty, u_{Poisson} , in units of \log_{10} , for values of counts (ΣC) from 1 to 40.

If $\Sigma C = 0$, that is no colonies are counted, $u_{\text{Poisson}} = 0,434$.

Table 2 — Values of u_{Poisson} for values of ΣC from 1 to 40

ΣC	u_{Poisson}	ΣC	u_{Poisson}	ΣC	u_{Poisson}	ΣC	u_{Poisson}
1	0,434	11	0,131	21	0,095	31	0,078
2	0,307	12	0,125	22	0,093	32	0,077
3	0,251	13	0,120	23	0,091	33	0,076
4	0,217	14	0,116	24	0,089	34	0,074
5	0,194	15	0,112	25	0,087	35	0,073
6	0,177	16	0,109	26	0,085	36	0,072
7	0,164	17	0,105	27	0,084	37	0,071
8	0,154	18	0,102	28	0,082	38	0,070
9	0,145	19	0,100	29	0,081	39	0,070
10	0,137	20	0,097	30	0,079	40	0,069

u_{Poisson} for other values of ΣC can be calculated using Formula (2):

$$u_{\text{Poisson}} = \frac{1/\ln(10)}{\sqrt{\Sigma C}} = \frac{0,4343}{\sqrt{\Sigma C}} \quad (2)$$

So, for example, if $\Sigma C = 100$, $u_{\text{Poisson}} = \frac{0,4343}{\sqrt{100}} = \frac{0,4343}{10} = 0,04343$.

For large values of ΣC , the Poisson uncertainty component may be negligible if other uncertainty components are large (see 8.1.2).

7.3 Colony-count technique — Confirmation uncertainty

Some methods based on a colony-count technique give presumptive numbers of organisms. Confirmation tests are then used to correct the presumptive count by estimating the proportion of a selected number of colonies that is confirmed as the target organism using appropriate tests. It is reasonable to regard the colonies as being evenly distributed and the binomial distribution is used to calculate the corresponding distributional uncertainty specific to an individual result.

Suppose presumptive colonies, n_p , are tested and a number of them are confirmed, n_c , the relative number of successes n_c/n_p is used as the multiplier to convert the presumptive count into the confirmed count. This has the effect of adding a correction, $\log_{10} n_c/n_p$, to the \log_{10} cfu/g or ml value based on the presumptive count. [Table 3](#) shows values of u_{conf} in units of \log_{10} , for selected values of n_p and n_c .

Table 3 — Values of confirmation uncertainty (u_{conf}) in \log_{10} for selected values of number colonies tested (n_p) and number of colonies confirmed (n_c)

Number of colonies confirmed (n_c)	Number colonies tested (n_p)			
	5	10	15	20
1	0,355 4	0,430 2	0,460 5	0,476 9
2	0,202 3	0,262 7	0,286 8	0,299 9
3	0,134 9	0,194 6	0,217 7	0,230 0
4	0,088 8	0,154 1	0,177 6	0,190 0
5	0,045 4	0,125 4	0,150 1	0,162 8
6		0,102 7	0,129 3	0,142 7
7		0,083 4	0,112 6	0,126 8
8		0,065 7	0,098 6	0,113 6
9		0,047 8	0,086 2	0,102 4
10		0,026 1	0,075 0	0,092 6
11			0,064 6	0,083 8
12			0,054 4	0,075 7
13			0,044 1	0,068 3
14			0,032 9	0,061 1
15			0,018 3	0,054 3
16				0,047 5
17				0,040 6
18				0,033 3
19				0,025 1
20				0,014 1

u_{conf} for other numbers can be calculated from [Formula \(3\)](#), which is derived from ISO 29201:2012, Formula (E.4):

$$u_{\text{conf}} = \frac{1}{2,303} \sqrt{\frac{(n_c + 0,5)(n_p - n_c + 0,5)n_p^2}{(n_p + 1)^2(n_p + 2)n_c^2}} \tag{3}$$

If $n_c = 0$, calculate u_{conf} as if $n_c = 1$.

7.4 Most probable number uncertainty

The most probable number (MPN) technique derives most probable numbers from multiple detection or non-detection results. This technique includes automated micro-titre plate techniques where many tubes may be assessed as positive or negative. For an MPN technique, the minimum distributional uncertainty is greater than the simple Poisson and depends on the detailed results. Procedures for estimating the corresponding standard uncertainty in \log_{10} , u_{MPN} , are given in [Annex C](#).

Some MPN tests require confirmation of the presence of the target organism in every presumptive positive. In that situation, calculate the MPN and its uncertainty from the number of confirmed positive results.

8 Combined and expanded uncertainty

8.1 Combined standard uncertainty

8.1.1 General considerations

The combined standard uncertainty may be based upon one of the two following options:

- a) a combination (see [8.1.2](#)) of separately estimated:
 - 1) technical standard uncertainty;
 - 2) matrix standard uncertainty;
 - 3) distributional standard uncertainties.
- b) if consistent with laboratory protocols and client requirements, reproducibility standard deviation alone (see [8.1.3](#)).

8.1.2 Combined standard uncertainty based on separate technical, matrix, and distributional standard uncertainties

Technical standard uncertainty, u_{tech} , is estimated in accordance with [Clause 5](#) as a reproducibility standard deviation, which may be corrected for matrix and distributional standard uncertainties, as an optional alternative procedure, in accordance with [Annex D](#):

$$— u_{\text{tech}} = S_R; \text{ OR}$$

$$— u_{\text{tech}} = S_{R:\text{corr}}$$

Matrix standard uncertainty, u_{matrix} , is estimated in accordance with [Clause 6](#). If matrix uncertainty is estimated in accordance with [6.3](#) from multiple test portions of laboratory samples, the matrix standard uncertainty is estimated as the repeatability standard deviation, which may be corrected for distributional standard uncertainties, as an optional alternative procedure, in accordance with [Annex D](#):

$$— u_{\text{matrix}} = S_r; \text{ OR}$$

$$— u_{\text{matrix}} = S_{r:\text{corr}}$$

Any relevant distributional standard uncertainties (u_{Poisson} , u_{conf} , u_{MPN}) are calculated from the data underlying the reported result, in accordance with [Clause 7](#).

Then, calculate the combined standard uncertainty as the square root of the sum of the squares of technical, matrix, and any relevant distributional standard uncertainties. Note that not all terms will be included for a given method, e.g. a method will not include both colony counting (u_{Poisson}) and MPN (u_{MPN}).

EXAMPLE 1 Instrumental methods such as ATP where no colonies or cells are counted: $u_c(y) = \sqrt{u_{\text{tech}}^2 + u_{\text{matrix}}^2}$

EXAMPLE 2 Colony-count methods, without partial confirmation: $u_c(y) = \sqrt{u_{\text{tech}}^2 + u_{\text{matrix}}^2 + u_{\text{Poisson}}^2}$

EXAMPLE 3 Colony-count methods, with partial confirmation: $u_c(y) = \sqrt{u_{\text{tech}}^2 + u_{\text{matrix}}^2 + u_{\text{Poisson}}^2 + u_{\text{conf}}^2}$

EXAMPLE 4 MPN methods: $u_c(y) = \sqrt{u_{\text{tech}}^2 + u_{\text{matrix}}^2 + u_{\text{MPN}}^2}$

NOTE It is generally accepted that the effect of a component is negligible if its standard uncertainty is no greater than one fifth of the magnitude of the largest component standard uncertainty^{[14][16]}. Distributional and matrix uncertainty components that are negligible compared to the technical uncertainty, as shown in the examples in [8.3](#), can be ignored. In the extreme, when all distributional and matrix uncertainty components are negligible compared to the technical uncertainty, the examples above reduce to $u_c(y) = u_{\text{tech}}$.

8.1.3 Combined standard uncertainty based on reproducibility standard deviation alone

Reproducibility standard deviation is calculated by one of the three methods given in [5.2](#).

If consistent with laboratory protocols and client requirements, combined standard uncertainty may be estimated as the reproducibility standard deviation only, without the correction described in [Annex D](#), as shown by [Formula \(4\)](#):

$$u_c(y) = s_R \quad (4)$$

8.2 Expanded uncertainty

Use [Formula \(5\)](#) to derive the expanded uncertainty U from the combined standard uncertainty $u_c(y)$ (see [8.1](#)) with a coverage factor k chosen, in this document, as a value of 2 (to correspond approximately to a confidence level of 95 %):

$$U = 2 u_c(y) \quad (5)$$

8.3 Worked examples

8.3.1 Example 1 — Technical, matrix and Poisson components of uncertainty

Suppose a validated method using 1,0 ml inocula on one plate of each of two successive dilutions has technical uncertainty estimated previously in accordance with [Clause 5](#), as $u_{\text{tech}} = 0,15 \log_{10}$ cfu/g.

Then suppose that the method applied to a homogenous laboratory sample gave the following results: at 10^{-3} dilution, 102 colonies, and at 10^{-4} dilution, 8 colonies.

Then the weighted mean colony count is $\frac{(102+8)}{1,1} \times 10^3 = 1,0 \times 10^5$ cfu/g, for which the \log_{10} colony count is $5,0 \log_{10}$ cfu/g.

The distributional standard uncertainty u_{Poisson} is determined from the total number of colonies $\Sigma C = 110$; hence,

$$u_{\text{Poisson}} = \frac{0,4343}{\sqrt{110}} = \frac{0,4343}{10,49} = 0,0414 \log_{10} \text{ cfu/g [see Formula (2)].}$$

The ratio $u_{\text{Poisson}}/u_{\text{tech}} = 0,0414/0,15 = 0,276$, which is greater than 0,20 so u_{Poisson} cannot be neglected (see NOTE in [8.1.2](#)).

For a homogeneous matrix, the matrix standard uncertainty $u_{\text{matrix}} = 0,1 \log_{10}$ cfu/g (see [6.2](#)).

— The combined standard uncertainty (see [8.1.2](#)) is:

$$u_c(y) = \sqrt{0,15^2 + 0,10^2 + 0,0414^2} = \sqrt{0,03421} = 0,185$$

— This is multiplied by the coverage factor (k) of 2 to give $U = 0,37 \log_{10}$ cfu/g (to two significant figures).

So the colony count and its expanded uncertainty is $5,0 \pm 0,37 \log_{10}$ cfu/g.

8.3.2 Example 2 — Poisson component negligible

As example 1, except the technical standard uncertainty, u_{tech} , is $0,25 \log_{10}$ cfu/g.

Since $u_{\text{Poisson}}/u_{\text{tech}} = 0,0414/0,25 = 0,166$, which is less than 0,2, u_{Poisson} can be ignored (see NOTE in [8.1.2](#)).

There are no other distributional components, but the matrix uncertainty remains, so the combined standard uncertainty (see 8.1.2) is:

$$u_c(y) = \sqrt{0,25^2 + 0,10^2} = \sqrt{0,0725} = 0,269$$

This is multiplied by the coverage factor (k) of 2 to give $U = 0,54 \log_{10}$ cfu/g (to two significant figures).

8.3.3 Example 3 — Poisson, matrix and confirmation components

As example 1, except the results shown are for typical colonies counted on a differential medium, which needed to be confirmed.

$u_{\text{Poisson}} = 0,0414$ and $u_{\text{Poisson}}/u_{\text{tech}} = 0,0414/0,15 = 0,276$, which is greater than 0,2 so u_{Poisson} cannot be neglected (see NOTE in 8.1.2).

Confirmation: five typical colonies were tested of which four were confirmed as being the target organism. This leads to revised results, as shown in Table 4.

Table 4 — Example of calculation for Poisson, matrix and confirmation components

	Presumptive	Confirmed
ΣC	110	
result; x ; cfu/g	100 000	$100\,000 \times 4/5 = 80\,000$
y ; \log_{10} cfu/g	5,0	4,903

From 7.3: for $n_p = 5$ and $n_c = 4$, $u_{\text{conf}} = 0,0888$. $u_{\text{conf}}/u_{\text{tech}} = 0,0888/0,15 = 0,592$, which is greater than 0,2 so u_{conf} cannot be neglected (see NOTE in 8.1.2).

There are no other distributional components so the combined standard uncertainty (see 8.1.2) is:

$$u_c(y) = \sqrt{0,15^2 + 0,10^2 + 0,0414^2 + 0,0888^2} = \sqrt{0,0421} = 0,205$$

This is multiplied by a coverage factor (k) of 2 to give $U = 0,41 \log_{10}$ cfu/g (to two significant figures).

NOTE The result of the confirmed count is $4,90 \pm 0,41 \log_{10}$ cfu/g whereas that of the presumptive count (see example 1) was $5,0 \pm 0,37 \log_{10}$ cfu/g.

8.3.4 Example 4 — Technical, matrix and most probable number components

A laboratory estimated the presumptive level of an organism (e.g. coliform bacteria) in a liquid sample using a 5-tube/3-dilution level MPN procedure for which the technical standard uncertainty, u_{tech} , taken as equal to the reproducibility standard deviation, s_{JR} , derived from an interlaboratory method validation study was determined to be $0,49 \log_{10}$ MPN/ml, based on 20 replicate sample tests.

For a homogenous liquid laboratory sample, the MPN estimation was based on five inoculated tubes with 1,0 ml of a dilution of the sample at each of three dilution levels, 10^{-2} , 10^{-3} and 10^{-4} , for a total of 15 tubes. After incubation, the number of positive cultures at each dilution were 4, 2 and 1, respectively.

From the spreadsheet of Reference [19] the following values were obtained: MPN 260/ml; \log_{10} MPN = 2,42; u_{MPN} = standard deviation = $0,19 \log_{10}$ MPN.

The ratio $u_{\text{MPN}}/u_{\text{tech}} = 0,19/0,49 = 0,39 > 0,20$, hence u_{MPN} cannot be ignored (see NOTE in 8.1.2).

For a homogeneous liquid, the matrix standard uncertainty can be taken as $u_{\text{matrix}} = 0,1 \log_{10}$ (see 6.2). The ratio $u_{\text{matrix}}/u_{\text{tech}} = 0,10/0,49 = 0,204 > 0,20$, hence the matrix standard uncertainty cannot be ignored (see NOTE in 8.1.2).

These components are combined (see [8.1.2](#)) to give the combined standard uncertainty:

$$u_c(y) = \sqrt{0,49^2 + 0,1^2 + 0,19^2} = \sqrt{0,286} = 0,535 \text{ (to three decimal places).}$$

This is multiplied by a coverage (k) of 2 to give $U = 1,1 \log_{10} \text{ cfu/g}$ (to two significant figures).

So the MPN estimate gives a presumptive contamination level of $2,4 \pm 1,1 \log_{10} \text{ MPN/ml}$.

NOTE 1 The combined standard uncertainty estimate would have been the same for a \log_{10} MPN estimate of 0,42 or even 6,42.

NOTE 2 Some MPN tests require confirmation of the presence of the target organism. In that situation, calculation of the MPN and its uncertainty are determined from the number of confirmed positive results.

9 Expression of measurement uncertainty in the test reports

9.1 General aspects

MU should be reported in the same unit as the test result.

As indicated in [8.1.1](#), the reported MU may be based on one of the two following options:

- reproducibility standard deviation and separate estimations of matrix and any relevant distributional uncertainties; or
- reproducibility standard deviation alone.

When an estimate of MU is required in the test report, include in the report an explicit statement that the indicated MU is an expanded uncertainty, together with a statement of the confidence level and an indication that the MU has been estimated in accordance with this document. For example:

- “The reported expanded measurement uncertainty has been estimated in accordance with ISO 19036 and is based on a standard uncertainty multiplied by a coverage factor of $k = 2$, providing a level of confidence of approximately 95 %.”

If the MU is based on reproducibility standard deviation alone, this shall be made clear in the test report. For example:

- “The reported expanded measurement uncertainty has been estimated in accordance with ISO 19036 and is based on a standard uncertainty multiplied by a coverage factor of $k = 2$, providing a level of confidence of approximately 95 %. Combined standard uncertainty has been taken as equal to the intralaboratory reproducibility standard deviation.”

The number of figures in a reported MU should always reflect practical measurement capability. In view of the process for estimating uncertainties, it is seldom justified to report MU to more than two significant figures. It is therefore recommended that the expanded uncertainty be rounded to two significant figures, using the normal rules of rounding in accordance with ISO 7218. The numerical value of the measurement result in the test report should normally be rounded to the least significant figure in the value of the expanded uncertainty assigned to the measurement result. Rounding should always be carried out at the end of the process to avoid the effect of cumulative rounding errors, see ISO/IEC Guide 98-3.

Once the expanded MU has been derived, as explained in [8.2](#), it may be expressed in the test report, together with the test result, as an interval on the \log_{10} scale or as natural values (cfu/g or cfu/ml), as illustrated by the following alternative examples:

- a) \log_{10} result with $\pm U$: $y \pm U \log_{10} \text{ cfu/g}$ or cfu/ml ;
e.g. $5,00 \pm 0,31 \log_{10} \text{ cfu/g}$;

- b) \log_{10} result with limits: $y \log_{10}$ cfu/g or cfu/ml [$y - U; y + U$];
e.g. $5,00 \log_{10}$ cfu/g [4,69; 5,31];
- c) natural result value with limits: x cfu/g or cfu/ml [$10^{y-U}; 10^{y+U}$];
e.g. $1,0 \times 10^5$ cfu/g [$4,9 \times 10^4; 2,0 \times 10^5$].

9.2 Results below the limit of quantification

9.2.1 General aspects

Results below the limit of quantification (LOQ) can arise, for example:

- for a colony-count method, when the number of counted colonies is zero, $\Sigma C = 0$;
- for a colony-count method with partial confirmation, when the number of confirmed colonies is zero, $n_c = 0$;
- for an MPN method, when there are no detection results, $x_i = 0$ for all i .

Although such results could be interpreted as zero, they are often expressed as “ $< x_{\text{LOQ}}$ ” where x_{LOQ} is the LOQ in cfu/g or ml.

Relevant sections (see [7.2](#), [7.3](#) and [Annex C](#)) include calculation of distributional standard uncertainty in such circumstances so that combined standard uncertainty and expanded uncertainty, $u_c(y)$ and U , can be calculated in \log_{10} units in accordance with [Clause 8](#).

However, the result is consistent with a measurand value of zero cfu/g or ml. When expressing the result as a natural value with limits, option c) in [9.1](#), calculate the upper limit as if the result was equal to the LOQ and take the lower limit at zero.

However, log zero is undefined and when expressing the result as \log_{10} result with limits, express the lower limit as “less than”; $< (\log_{10} x_{\text{LOQ}}) - U$.

9.2.2 Example

Subclause [8.3.1](#) is an example of a colony-count method with $u_{\text{tech}} = 0,15 \log_{10}$ cfu/g and $u_{\text{matrix}} = 0,1 \log_{10}$ cfu/g.

If a laboratory sample gave zero counted colonies at 10^{-1} dilution and at 10^{-2} dilution, then $\Sigma C = 0$ and [7.2](#) gives $u_{\text{Poisson}} = 0,434 \log_{10}$ cfu/g.

Combined standard uncertainty and expanded uncertainty can be calculated in accordance with [8.1](#) and [8.2](#):

$$u_c(y) = \sqrt{0,15^2 + 0,10^2 + 0,434^2} = \sqrt{0,2209} = 0,470 \log_{10} \text{ cfu/g}$$

$$U = 2 \times 0,470 = 0,940 \log_{10} \text{ cfu/g}$$

The limit of quantification (x_{LOQ}) corresponds to a single counted colony, $\Sigma C = 1$:

$$x_{\text{LOQ}} = 10^{d_1} \frac{\Sigma C}{1,1} = 10^1 \frac{1}{1,1} = 9,091 \text{ cfu/g}$$

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$$y_{\text{LOQ}} = \log_{10}(9,091) = 0,959 \log_{10} \text{ cfu/g}$$

Limits on the uncertainty intervals for results equal to the LOQ are:

$$y_{\text{LOQ}} + U = 0,959 + 0,940 = 1,899 \log_{10} \text{ cfu/g}$$

$$y_{\text{LOQ}} - U = 0,959 - 0,940 = 0,019 \log_{10} \text{ cfu/g}$$

$$10^{y_{\text{LOQ}}+U} = 10^{1,899} = 79,16 \text{ cfu/g}$$

$$10^{y_{\text{LOQ}}-U} = 10^{0,019} = 1,044 \text{ cfu/g}$$

Note that $y_{\text{LOQ}} - U$ can be negative but $10^{y_{\text{LOQ}} - U}$ is always positive.

With appropriate rounding, the result with its uncertainty can be expressed as:

- a) \log_{10} result with $\pm U$: $< y_{\text{LOQ}} \pm U \log_{10} \text{ cfu/g}$;
e.g. $< 0,96 \pm 0,94 \log_{10} \text{ cfu/g}$;
- b) \log_{10} result with limits: $< y_{\text{LOQ}} \log_{10} \text{ cfu/g} [< y_{\text{LOQ}} - U; y_{\text{LOQ}} + U]$;
e.g. $< 0,96 \log_{10} \text{ cfu/g} [< 0,02; 1,90]$;
- c) natural result value with limits: $< x_{\text{LOQ}} \text{ cfu/g} [0; 10^{y_{\text{LOQ}}+U}]$;
e.g. $< 9,1 \text{ cfu/g} [0,0; 79,2]$.

Annex A (informative)

Calculation of standard deviations with two or more than two test portions (intralaboratory reproducibility standard deviation and matrix uncertainty standard deviation)

Subclauses [5.2.2.2](#) and [5.2.2.3](#) describe the experimental protocol and calculations for estimation of reproducibility standard deviation from intralaboratory experiments, with exactly two test portions from each laboratory sample. As shown in [Figure A.1](#), the experimental protocol can be extended to more than two test portions from each sample and/or differing numbers of test portions for different samples.

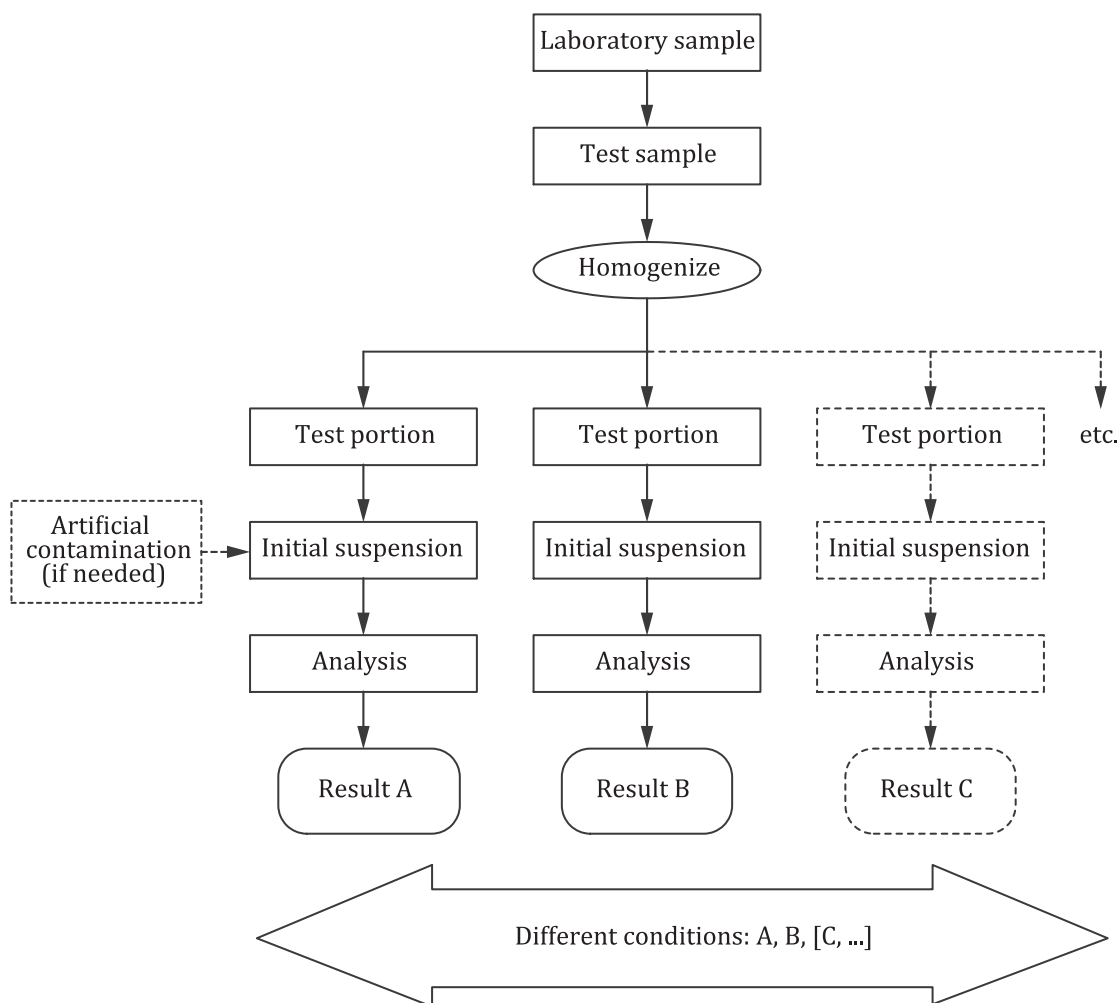


Figure A.1 — Experimental protocol for estimation of intralaboratory reproducibility — Two or more test portions from each laboratory sample

This protocol is very similar to that used to assess repeatability standard deviation from multiple test portions from laboratory samples (see [6.3](#)) and the calculations are identical.

Calculate the standard deviation as follows. In each case, there are n laboratory samples with p_i ($i = 1, 2, \dots, n$) test portions for sample i , resulting in value x_{ij} cfu/g or ml for test portion j of sample i . Calculate the standard deviation, s_{IR} or s_r , as in [Formula \(A.1\)](#):

$$s_{IR} \text{ or } s_r = \sqrt{\frac{\sum_{i=1}^n \sum_{j=1}^{p_i} (y_{ij} - \bar{y}_i)^2}{\sum_{i=1}^n (p_i - 1)}} \tag{A.1}$$

where

i is the index of the laboratory sample ($i = 1, 2, \dots, n$);

j is the index of the value of test portion within the sample i ($j = 1, 2, \dots, p_i$);

$y_{ij} = \log_{10} x_{ij}$.

$$\bar{y}_i = \frac{\sum_{j=1}^{p_i} y_{ij}}{p_i}$$

For a single sample, $n = 1$, which can occur when assessing matrix uncertainty, this is simply the standard deviation of the \log_{10} transformed data.

[Table A.1](#) shows the manual calculation on a data set similar to that in [Table 1](#). Calculations were performed in Excel^{®1}) from values shown for the dilution factors (d) and colonies counted (C). Derived values have been rounded for display.

Table A.1 — Manual calculations of standard deviation for analytical results on multiple samples

Laboratory sample <i>i</i>	Test Portion <i>j</i>	Dilution factors (<i>d</i>) colonies counted (<i>C</i>)				Total colonies counted ΣC_{ij}	Colony count result in cfu/g or ml weighted mean (ISO 7218) x_{ij}	\log_{10} cfu/g or ml $y_{ij} = \log_{10} x_{ij}$	Mean \log_{10} count	Squared differences from mean	Degrees of freedom $n_i - 1$
		d_1	C_1	d_2	C_2						
1	A	3	102	4	8	110	$1,00 \times 10^5$	5,000 0	5,052 0	0,002 70	2
1	B	3	59	4	4	63	$5,73 \times 10^4$	4,757 9		0,086 44	
1	C	3	248	4	27	275	$2,50 \times 10^5$	5,397 9		0,119 70	
2	A	5	61	6	6	67	$6,09 \times 10^6$	6,784 7	6,806 3	0,000 47	1
2	B	5	66	6	8	74	$6,73 \times 10^6$	6,827 8		0,000 47	
3	A	4	168	5	18	186	$1,69 \times 10^6$	6,228 1	6,119 1	0,011 88	3
3	B	4	86	5	7	93	$8,45 \times 10^5$	5,927 1		0,036 88	
3	C	4	95	5	12	107	$9,73 \times 10^5$	5,988 0		0,017 20	
3	D	4	216	5	21	237	$2,15 \times 10^6$	6,333 4		0,045 89	
4	A	5	266	6	25	291	$2,65 \times 10^7$	7,422 5	7,287 1	0,018 33	1
4	B	5	140	6	16	156	$1,42 \times 10^7$	7,151 7		0,018 33	
5	A	6	45	7	5	50	$4,55 \times 10^7$	7,657 6	7,387 3	0,073 06	1
5	B	5	129	6	15	144	$1,31 \times 10^7$	7,117 0		0,073 06	
6	A	4	129	5	12	141	$1,28 \times 10^6$	6,107 8	6,085 1	0,000 52	1
6	B	4	117	5	10	127	$1,15 \times 10^6$	6,062 4		0,000 52	
7	A	2	92	3	8	100	$9,09 \times 10^3$	3,958 6	4,083 0	0,015 48	2
7	B	2	131	3	13	144	$1,31 \times 10^4$	4,117 0		0,001 15	
7	C	2	149	3	15	164	$1,49 \times 10^4$	4,173 5		0,008 18	
8	A	3	139	4	13	152	$1,38 \times 10^5$	5,140 5	5,148 9	0,000 07	1
8	B	3	143	4	15	158	$1,44 \times 10^5$	5,157 3		0,000 07	
9	A	1	49	2	5	54	$4,91 \times 10^2$	2,691 0	2,981 2	0,084 20	3
9	B	1	129	2	13	142	$1,29 \times 10^3$	3,110 9		0,016 83	
9	C	1	88	2	7	95	$8,64 \times 10^2$	2,936 3		0,002 01	
9	D	1	151	2	18	169	$1,54 \times 10^3$	3,186 5		0,042 15	
10	A	4	142	5	13	155	$1,41 \times 10^6$	6,148 9	5,755 3	0,154 93	1
10	B	3	227	4	26	253	$2,30 \times 10^5$	5,361 7		0,154 93	

Table A.1 (continued)

Laboratory sample <i>i</i>	Test Portion <i>j</i>	Dilution factors (<i>d</i>) colonies counted (<i>C</i>)				Total colonies counted ΣC_{ij}	Colony count result in cfu/g or ml weighted mean (ISO 7218) x_{ij}	\log_{10} cfu/g or ml $y_{ij} = \log_{10} x_{ij}$	Mean \log_{10} count	Squared differences from mean	Degrees of freedom $n_i - 1$
		d_1	C_1	d_2	C_2						
								sums	0,985 44	16	
								$0,985\ 44/16 =$	0,061 59		
								$s_{IR} = \sqrt{0,061\ 59} =$	0,248 17		

As an alternative to manual calculation, the standard deviation can conveniently be calculated using any tool capable of one-way ANOVA, when the required standard deviation is the square root of the within-groups mean square. For example, Excel's^{®1)} single factor ANOVA on the y_{ij} data above gives this result, where the within-groups mean square value is 0,06159, then:

$$s_{IR} = \sqrt{0,06159} = 0,24817$$

See [Table A.2](#).

NOTE The ANOVA approach can also be used in cases of exactly two values from each laboratory sample.

Table A.2 — Calculations of intralaboratory reproducibility standard deviation for analytical results on multiple samples, using a tool with one-way analysis of variance

Anova: Single factor						
SUMMARY						
Groups	Count	Sum	Average	Variance		
Row 1	3	15,155 89	5,051 963	0,104 423		
Row 2	2	13,612 52	6,806 261	0,000 931		
Row 3	4	24,476 56	6,119 139	0,037 283		
Row 4	2	14,574 23	7,287 116	0,036 658		
Row 5	2	14,774 55	7,387 274	0,146 128		
Row 6	2	12,170 24	6,085 119	0,001 031		
Row 7	3	12,249 03	4,083 009	0,012 404		
Row 8	2	10,297 72	5,148 858	0,000 141		
Row 9	4	11,924 72	2,981 18	0,048 398		
Row 10	2	11,510 67	5,755 333	0,309 851		
ANOVA						
Source of variation	SS	df	MS	F	P-value	F crit
Between groups	51,327 08	9	5,703 009	92,596 53	4,36102E-12	2,537 667
Within groups	0,985 438	16	0,061 59			
Total	52,312 52	25				
Key						
SS: sum of squares, df: degrees of freedom, MS: mean of squares, F: F-distribution variable, P-value: significance level, F-crit: critical value of F-distribution variable						

Annex B (informative)

Matrix effect and matrix uncertainty

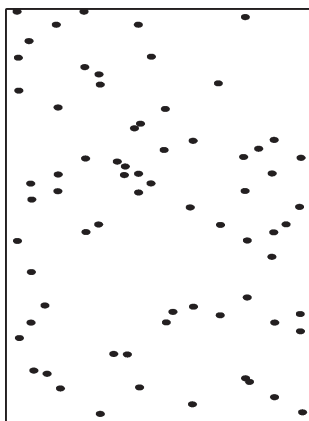
Bacterial or yeast cells in a liquid matrix (e.g. milk, water) generally conform to a random (Poisson) distribution [see [Figure \(B.1\)](#), case A], although both individual cells and small clusters of cells can form colonies when plated on agar. Solid foods such as cheese contain cells and clusters of microorganisms distributed within and between the original particles that form the product, but they are generally not distributed randomly and most often occur as a contagious distribution [see [Figure \(B.1\)](#), case B].

Solid foods, such as meats and vegetables, are generally contaminated randomly on the surface but not in deep tissues. Growth of some cells results in an overall surface distribution of individual cells and microcolonies that is usually contagious [see [Figure \(B.1\)](#), case B].

If pieces of a solid ingredient (e.g. meat) are mixed with other ingredients (e.g. vegetables and sauces) to form a composite food product, then the surface bacteria from all the solid ingredients become distributed throughout the multi-component product, but the bacteria are not randomly distributed throughout the final food product. The numbers of bacteria that occur in the food matrix reflect the relative levels of contamination of each of the ingredients and the extent to which microcolonies are disrupted during the manufacturing process. The levels of contamination in several test portions of the food product taken for analysis are not consistent. The levels of specific bacteria reflect both the relative quantity and quality of the ingredients and the extent to which the manufacturing process has distributed these bacteria throughout the batch of product. A similar situation occurs if dried milk and other powder products are contaminated by specific organisms in only a small proportion of a product batch [see [Figure \(B.2\)](#)].

Even when the laboratory sample has been made homogeneous prior to analysis, variation in levels of contamination occurs between different test portions, especially for solid food matrices. Such variation is referred to in this document as the “matrix uncertainty”.

For more information on the distribution of microorganisms in foods, see Reference [17].



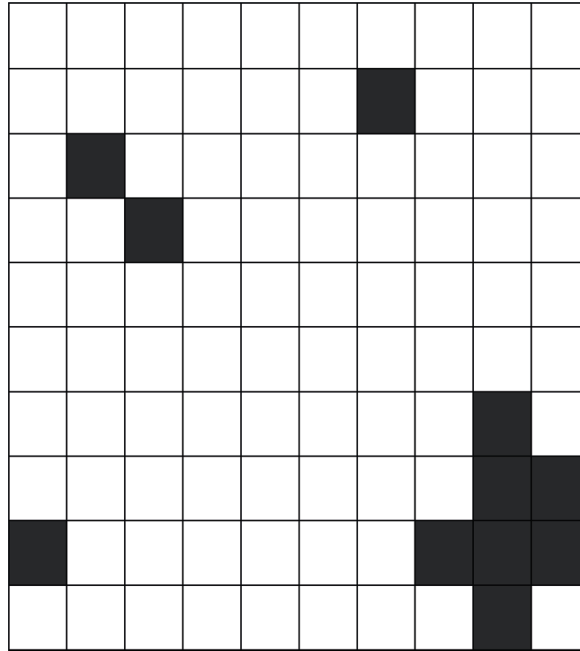
a) Case A: random distribution



b) Case B: contagious distribution showing clumps and microcolonies

NOTE Source: modified from ISO 21748.

Figure B.1 — Hypothetical illustration of the distribution of microorganisms on a surface



NOTE Black squares indicate those samples in which the target organism is detected.

Figure B.2 — Hypothetical distribution of low levels of a specific microorganism in 100 samples of a powder or a complex food (such as a meat and vegetable stew)

Annex C (informative)

Intrinsic variability (standard uncertainty) of most probable number estimates

The most probable number (MPN) technique is described in ISO 7218. In this technique, several inocula from each test portion are inoculated separately into different aliquots of a growth medium. The result from each aliquot is qualitative, i.e. the result is either positive or negative. A number of aliquots at various dilutions may have to be examined to obtain an estimate of the number of microorganisms that is present. The MPN is derived using statistical procedures.

The intrinsic variability of an MPN value is greater than that associated with the Poisson distribution because of an additional element of binomial probability associated with the positive or negative nature of the data. The statistical procedures used to determine the MPN can also give the standard error in \log_{10} MPN/g or MPN/ml. The standard uncertainty, u_{MPN} , is equal to the standard error.

To determine the standard error, ISO 7218 recommends using the Excel^{®1} spreadsheet available at <http://standards.iso.org/iso/7218>, which reports the required standard uncertainty, u_{MPN} , as “SD \log_{10} MPN”.

If an MPN is estimated by means that do not give the standard error, then the uncertainty (u_{MPN}) can be calculated from the MPN value and the experimental conditions used to derive the result, using [Formula \(C.1\)](#) which is taken from Reference [19]:

$$u_{\text{MPN}} = \frac{1/\ln(10)}{\mu \sqrt{\sum_{i=1}^k \frac{x_i \times m_i^2 \exp(-m_i \times \mu)}{(1 - \exp(-m_i \times \mu))^2}}} \quad (\text{C.1})$$

where

- u_{MPN} is the standard uncertainty, in \log_{10} MPN/g or MPN/ml;
- μ is the MPN value /g or ml, not in \log_{10} ;
- k is the number of dilutions;
- m_i is the amount of sample (g or ml) in each aliquot of dilution i ;
- x_i is the number of positive results from dilution i .

Where there are no positive results, that is where $\sum x_i = 0$, then calculate u_{MPN} as if there were one positive result at the most concentrated dilution, that is for the greatest m_i .

For example, when five aliquots each of 1 g, 0,1 g and 0,01 g were used, and if the numbers of positives were 4, 0 and 1, respectively, then the MPN (μ) = 1,7 MPN/g. The standard uncertainty can then be calculated as shown in [Table C.1](#).

Table C.1

Quantity of sample/test	Number of positive results/test	Calculation factor
m_i g	x_i	$\frac{x_i \times m_i^2 \exp(-m_i \times \mu)}{[1 - \exp(-m_i \times \mu)]^2}$
1	4	1,093 9
0,1	0	0,000 0
0,01	1	0,346 0
Σ		1,439 9

$$u_{\text{MPN}} = \frac{1/\ln(10)}{\mu\sqrt{\Sigma}} = \frac{0,4343}{1,7\sqrt{1,4399}} = \frac{0,4343}{2,0399} = 0,2129 \text{ as } \log_{10} \text{ MPN}$$

If there had been zero positive results, the standard uncertainty (but not the result) would have been calculated as if one of the five $m_i = 1$ g aliquots had been positive. This would have given a result of 0,20 MPN/g with a standard uncertainty of 0,44 \log_{10} MPN/g.

Therefore, for a 5 × 3 MPN design when five aliquots each of 1 g, 0,1 g and 0,01 g are used, the result corresponding to zero positives is 0,0 MPN/g with a standard uncertainty of 0,44 \log_{10} MPN/g.

Annex D (informative)

Correction of experimental standard deviations for unwanted uncertainty components

Subclauses [5.2.2](#) and [6.3](#) estimate technical and matrix uncertainty from experimental reproducibility and repeatability standard deviations, respectively. These standard deviations include other uncertainty components associated with the experimental data, leading to overestimates of technical and matrix uncertainty.

The experimental designs reduce the unwanted components:

- [5.2.2](#): estimating u_{tech} reduces u_{matrix} by making the laboratory sample homogeneous and reduces u_{distrib} by restricting acceptable results;
- [6.3](#): estimating u_{matrix} reduces u_{tech} by working under repeatability conditions and reduces u_{distrib} by restricting acceptable results.

Nevertheless, the standard deviations, s_{IR} and s_r , are overestimates of the relevant standard uncertainties, u_{tech} and u_{matrix} .

If MU of a reported result is based on reproducibility standard deviation alone (see [8.1.3](#)), then s_{IR} is probably an underestimate of the combined standard uncertainty. The matrix and distributional uncertainties of the reproducibility data are probably lower than those of the reported result.

However, if the MU of a reported result includes separately estimated matrix and distributional uncertainties of the reported result (see [8.1.2](#)), then the overestimates of u_{tech} and u_{matrix} lead to an overestimate of the combined standard uncertainty. To reduce such overestimation, the unwanted components of the reproducibility and repeatability data sets may be subtracted from the standard deviations to give corrected standard deviations. See [Formulae \(D.1\)](#) and [\(D.2\)](#):

$$s_{IR:\text{corr}} = \sqrt{s_{IR}^2 - (u_{\text{matrix}}^2 + u_{\text{distrib}}^2)} \quad (\text{D.1})$$

where u_{matrix} and u_{distrib} are of the reproducibility data.

$$s_{r:\text{corr}} = \sqrt{s_r^2 - u_{\text{distrib}}^2} \quad (\text{D.2})$$

where u_{distrib} is of the repeatability data.

As an optional alternative to the procedure described in [5.2.2](#) and [6.3](#) (no correction of the standard deviations), the procedure to correct a standard deviation for unwanted components is as follows.

- Calculate the values of the unwanted components for each result in the data set underlying the standard deviation:
 - for both s_{IR} and s_r , this will include any relevant distributional components; u_{Poisson} , u_{conf} , u_{MPN} , see [7.2](#), [7.3](#) and [7.4](#) as appropriate;
 - for s_{IR} , it will also include matrix uncertainty, s_{matrix} , which will often be taken as 0,1 because laboratory samples have been made homogeneous, see [5.2.2.2.4](#) and [6.2](#).
- Square each value and add them to give the unwanted sum of squares, S_{unwanted} .

- Divide by the number of results, N , to give the square of the combined standard uncertainty of the unwanted components, u_{unwanted}^2 .
- Subtract this from the square of the standard deviation to give the square of the corrected standard deviation, $s_{IR:\text{corr}}^2 = s_{IR}^2 - u_{\text{unwanted}}^2$ or $s_{r:\text{corr}}^2 = s_r^2 - u_{\text{unwanted}}^2$.
- Take the square root to give the corrected standard deviation, $s_{IR:\text{corr}} = \sqrt{s_{IR:\text{corr}}^2}$ or $s_{r:\text{corr}} = \sqrt{s_{r:\text{corr}}^2}$.

Table D.1 shows the calculations for the example data in Table 1. The only relevant distributional component in this example is the Poisson (see 7.3). The distributional uncertainty for confirmation (see 7.4) is zero but is shown for illustration.

Table D.1 — Calculation of corrected reproducibility standard deviation, example of Table 1

Laboratory sample	Test portion	Dilution factors (d) Colonies counted (C)				Total colonies counted ΣC_{ij}	Distributional uncertainty			Matrix uncertainty u_{matrix}^2
		d_1	C_1	d_2	C_2		u_{Poisson}	u_{Poisson}^2	u_{conf}^2	
1	A	3	102	4	8	110	0,041 41	0,001 71	0	0,01
1	B	3	59	4	4	63	0,054 72	0,002 99		0,01
2	A	5	61	6	6	67	0,053 06	0,002 82		0,01
2	B	5	66	6	8	74	0,050 49	0,002 55		0,01
3	A	4	168	5	18	186	0,031 84	0,001 01		0,01
3	B	4	86	5	7	93	0,045 03	0,002 03		0,01
4	A	5	266	6	25	291	0,025 46	0,000 65		0,01
4	B	5	140	6	16	156	0,034 77	0,001 21		0,01
5	A	6	45	7	5	50	0,061 42	0,003 77		0,01
5	B	5	129	6	15	144	0,036 19	0,001 31		0,01
6	A	4	129	5	12	141	0,036 57	0,001 34		0,01
6	B	4	117	5	10	127	0,038 54	0,001 49		0,01
7	A	2	92	3	8	100	0,043 43	0,001 89		0,01
7	B	2	131	3	13	144	0,036 19	0,001 31		0,01
8	A	3	139	4	13	152	0,035 23	0,001 24		0,01
8	B	3	143	4	15	158	0,034 55	0,001 19		0,01
9	A	1	49	2	5	54	0,059 10	0,003 49		0,01
9	B	1	129	2	13	142	0,036 45	0,001 33		0,01
10	A	4	142	5	13	155	0,034 88	0,001 22		0,01
10	B	3	227	4	26	253	0,027 30	0,000 75		0,01
S_{unwanted}							0,235 29			
$N = \text{number of test portions}$							20			
$u_{\text{unwanted}}^2 = S_{\text{unwanted}}/N$							0,011 76			
s_{IR}^2 (from Table 1)							0,067 00			
$s_{IR:\text{corr}}^2 = s_{IR}^2 - u_{\text{unwanted}}^2$							0,055 24			
$s_{IR:\text{corr}} = \sqrt{s_{IR:\text{corr}}^2}$							0,235 03			

NOTE 1 The divisor, N , is equal to the number of results underlying the standard deviation, that is the number of test portions. This can be less than the number of values in the sum of squares, S_{unwanted} .

NOTE 2 The calculation and subtraction of S_{unwanted} is the same when there are differing numbers of results per sample.

Theoretically, s_{IR}^2 or s_r^2 should never be less than u_{unwanted}^2 , which would lead to a negative $s_{IR:\text{corr}}^2$ or $s_{r:\text{corr}}^2$, respectively. However, this can happen when s_{IR} or s_r is based on a small data set. If the calculated s_{IR}^2 or s_r^2 is less than u_{unwanted}^2 , this indicates that the corresponding standard uncertainty, u_{tech} or u_{matrix} , is small compared to the unwanted standard uncertainties and can be taken as zero. If this very unusual event occurs, its cause should be carefully investigated.

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