Agence fédérale pour la Sécurité de la Chaîne alimentaire
(Federal Agency for the Safety of the Food Chain – FASFC)

Procedure

MICROBIOLOGY – ESTIMATING MEASUREMENT UNCERTAINTY

Version	2
Comes into force on	**15/11/2009**
Service in charge	**Quality officers**
Addressees	**All laboratories approved by the FASFC for microbiological parameters**

	Name – function / service	Date	Signature
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List of revisions

Revision by / date*	Reason of revision	Amended part of text / importance of revision
22/09/2009	Extension for the estimation of the measurement uncertainty for low counts Retention of two modes of expression	2/3/5.2/5.3 5.4

* the time interval between the date of coming into force and the present date must not exceed 5 years.

Adjust the number of revision and the year (when relevant). Upon approval, adjust the date of coming into force (taking into account the time needed to inform the staff members involved).

Addressees

- Central services of the Laboratories administration
- Laboratories approved by the FASFC for microbiological parameters

This procedure is available on the website of the FASFC (*www.afsca.be* > Business Sectors > Laboratories > Appoved laboratories > Office circular). For staff of the FASFC the documents are also available on the central server. Only versions of group A are considered valid.

The modifications compared to the previous version are shown by activation of the Word-function "track changes":
New text: red colour
Deleted text: blue colour and crossed out

Key words : measurement uncertainty
microbiological analyses

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1. Aim

This document contains instructions for estimating and expressing the measurement uncertainty associated with the results of quantitative microbiological analyses.

2. Scope

- Products intended for human consumption
- Products intended for animal consumption
- Environment samples taken in connection with the production and the storage of foodstuffs

The instruction applies to:
- The quantitative analyses conducted according to the plate count technique

The instruction does not apply to:
- The quantitative analyses conducted according to the Most Probable Number technique
- ~~The determination of low numbers of micro-organisms (< 15 colonies on the lowest countable dilution)~~

~~**Note: for low numbers, the laboratory may refer to the table in Annex 2 : confidence intervals for estimating low numbers of colonies.**~~

3. References

ISO 19036:2006 « Microbiology of food and animal feeding stuffs – Guidelines for the estimation of measurement uncertainty for quantitative determinations ».

ISO 19036:2009 – Amendment 1: Measurement uncertainty for low counts.

4. Definitions and abbreviations

Measurement uncertainty: a parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand.

Standard uncertainty $u(x_i)$: the uncertainty of the result of a measurement expressed as a standard deviation.

Combined standard uncertainty $u_c(y)$: the uncertainty of the result of a measurement in which are combined the contributions of all relevant sources of uncertainty according to an appropriate statistical model

Expanded measurement 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. The expanded uncertainty U is calculated from a combined standard uncertainty $u_c(y)$ and a coverage factor k :
$U=k.u_c(y)$.

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Coverage factor *k* : numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty. In general, the value of k varies between 2 and 3. It equals 2 in this instruction, which approximately corresponds to a 95 % confidence interval.

Systematic error : **:** the difference between the mean value of the measurements and the reference value.

5. Estimating the measurement uncertainty

5.1. Principle

This instruction is based upon the overall approach of the estimation of the measurement uncertainty ; it relies upon the total variability of the analysis process.
This total variability includes only the precision (random error) and not the systematic error, given the empirical nature of the way in which the number of micro-organisms is determined.
The overall approach is based upon an experimental estimation of the standard deviation of the reproducibility of a final result that has undergone the entire process. This standard deviation corresponds to the combined standard uncertainty.

The overall approach may be considered as a 'black box'. In food microbiology, the main sources of uncertainty are:

a) sampling
b) the laboratory sample
c) the matrix
d) equipment, growing media and reagents
e) additional random errors
f) the taking of subsamples – primary dilution
g) the analyst, time
h) the systematic error

The experimental protocol described in the instruction does not take into account the sampling and the systematic error as sources of uncertainty.

The standard deviation of the reproducibility is estimated by means of the standard deviation of the in-house reproducibility based upon an experimental protocol and must be estimated for each target group of micro-organisms and for each matrix (or group of matrices) for a given analysis method. It is necessary that the results are obtained by means of well controlled methods.

5.2. Operating mode

For each micro-organism (or group of micro-organisms) and for each category of matrices the experimental protocol, such as described in the diagram (Annex 1) must be applied to at least

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10 samples per category. It is recommendable to repeat the protocol on different dates in order to spread the results in time.
The laboratories with scope “all products” must calculate their measurement uncertainty for at least the 4 categories of matrices mentioned in Table 1.

Table 1 : definition of categories

Categories	Matrix type
category (1) : fluids and powders	milk, milk powder, etc.
category (2) : mixed solid substances	minced meat, mechanically recovered meat, sausage meat, ground meat, whipped cream, etc.
category(3) : small (or very small) solid substances	Dried parsley and mushrooms, shredded carrots & celeriac, lettuce, shrimp, cereals, feedstuffs, chopped nuts, etc.
category(4) : other solid substances	Meat (not ground), cheese, pastry, etc.

Within each category, the matrices must be representative of the type of matrices that are analysed by the laboratory.

Within each category, the laboratory may analyse samples of various matrices. For category 1 the laboratory may e.g. analyse 2 milk samples, 2 milk powder samples and 5 coconut milk samples.

The number of matrix categories that is used to estimate the measurement uncertainty may be adjusted according to the scope of the laboratory and the diversity of the matrices that are analysed by the laboratory as a routine or when the laboratory fears a particular problem with a matrix type (presence of inhibitors, competitive flora, …)

The standard deviations are calculated by means of log transformed data to make sure that the reproducibility variance is independent from the contamination level, knowing that in this protocol ~~instruction does not apply to~~ low contamination levels are not included.
For the estimation of uncertainty of low counts (<10 colonies on the lowest countable dilution) the in-house reproducibility standard deviation is calculated in accordance with this instruction.

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As a consequence, it is not necessary to determine the reproducibility standard deviation for each level of contamination.
Samples and/or dilutions are preferably chosen from the routine analyses.

If it is necessary to use artificial contamination, this must be done in a controlled manner.

2 analysts take an analysis sample of each sample (Annex 1) and prepare a primary dilution that is submitted to a single analysis. Conditions A and B must vary from each other as much as possible (different analysts, different batch numbers of growing media, stomachers, balances, water baths, incubators, analysis times, …).

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5.3. Mode of calculation

5.3.1. Uncertainty of measurement of high counts

The results in **cfu**/g must be converted to log(**cfu**/g).

Calculate the in-house standard deviation s_R for the n samples of a matrix as follows:

$$s_R = \frac{1}{\sqrt{2}} \sqrt{\frac{\sum_{n=1}^{n} (y_{iA} - y_{iB})^2}{n}} = u(y)$$

with
y_{ij} : the log converted data **in $\log_{10}$ (cfu/g)**
i : index of the sample ; i=1 to n **(n≥10)**
j : index of the reproducibility condition; j = A of B

Annex 3 contains a file with an Excel spreadsheet model.

If the analysis result y = log x and the reproducibility standard deviation = s_R, the expanded measurement uncertainty U amounts to $2s_R$

5.3.2. Uncertainty of measurement of low counts

For low counts (<10 colonies on the lowest countable dilution) the measurement uncertainty is estimated as follows:

$$U = 2\sqrt{s_R^2 + \frac{0{,}18861}{\sum C}}$$

Where:

S_R: the in-house reproducibility standard deviation of corresponding matrix calculated in accordance with paragraph 5.3.1.

$\frac{0{,}18861}{\sum C}$: the component due to the Poisson distribution, where $\sum C$ is the sum of the colonies counted on all the plates.

5.4. Mode of expression

The analysis result may be expressed in several ways ($y=\log_{10}x$)
- y +/- U (log)
- ~~y log (y-U, y +U)~~
- x cfu/g or x cfu/ml (10^{y-U} ; 10^{y+U})

or $U = 2u_c = 2s_R$

E.g. : a reproducibility standard deviation of 0.15 $\log_{10}$ has been found. The expanded measurement uncertainty, with coverage factor = 2 (95 % confidence interval) amounts to 0.15 x 2 = 0.3 $\log_{10}$. The analysis result is 5.0 $\log_{10}$ cfu/g.
The analysis result may be expressed as follows :

- 5.0 log +/-0.3 log
- ~~5.0 log (4.7 ; 5.3)~~
- 10^5cfu/g ($5x10^4$; $2x10^5$)
- 100.000 cfu/g (50.000 ; 200.000)

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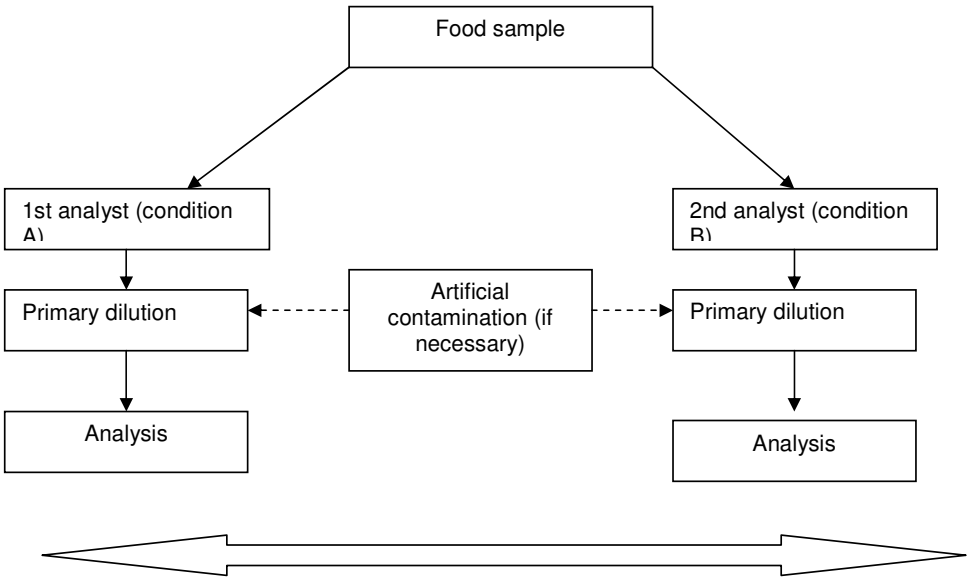
6. Annexes

6.1. Annex 1 – Experimental protocol



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~~6.2. Annex 2 – Confidence intervals for estimating low numbers of colonies~~

~~When less than 15 colonies have been selected, the 95 % confidence intervals amount to the numbers mentioned in the table below.~~

~~Number of micro-organisms~~	~~95 % confidence interval~~	
	~~Lower limit~~	~~Upper limit~~
~~1~~	~~<1~~	~~2~~
~~2~~	~~<1~~	~~4~~
~~3~~	~~<1~~	~~5~~
~~4~~	~~1~~	~~6~~
~~5~~	~~2~~	~~9~~
~~6~~	~~2~~	~~10~~
~~7~~	~~2~~	~~12~~
~~8~~	~~3~~	~~13~~
~~9~~	~~4~~	~~14~~
~~10~~	~~4~~	~~16~~
~~11~~	~~5~~	~~18~~
~~12~~	~~6~~	~~19~~
~~13~~	~~7~~	~~20~~
~~14~~	~~7~~	~~21~~
~~15~~	~~8~~	~~23~~

~~6.3~~6.2. Annex 2

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