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

**AMENDMENT 1: Measurement uncertainty
for low counts**

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

AMENDEMENT 1: Incertitude de mesure sur les faibles taux



PDF disclaimer

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The ISO Central Secretariat accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies. In the unlikely event that a problem relating to it is found, please inform the Central Secretariat at the address given below.



COPYRIGHT PROTECTED DOCUMENT

© ISO 2009

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Foreword

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

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

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

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

- an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;
- an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

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

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

Amendment 1 to ISO/TS 19036:2006 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

STANDARDSISO.COM : Click to view the full PDF of ISO/TS 19036 Amd 1:2009

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

AMENDMENT 1: Measurement uncertainty for low counts

Page 1, Clause 1, paragraphs 3 and 4

Delete paragraphs 3 and 4 and insert,

“This Technical Specification is not applicable to enumeration using a most probable number technique.

In this Technical Specification, MU is estimated using a simplified approach taking into account the Poisson distribution and is then applicable to any result, including “low” counts and/or “low” numbers of organisms.”

Page 4, 4.1, paragraph 2

Delete “(4.2).” and insert “(4.2), combined with a component due to Poisson distribution.”

Page 4, 4.3

Delete “ $= 2s_R$ ” from the equation.

Page 5, 5.2.1, paragraph 4, sentence 1

Delete “given that low levels are not considered here” and insert “provided that results based on low counts are not used in the calculations (see 5.3)”.

Page 7, 5.3

Add at the beginning of the subclause:

“Experiments should be performed to ensure that sufficiently large numbers of counted colonies can be used for the calculations. Enumeration results based on less than 10 counted colonies should be excluded. Enumerations results with 10 to 30 counted colonies may be included only if the standard deviation of reproducibility, s_R , that is being estimated is expected to be higher than $0,2 \log_{10}$ (cfu/g) or $0,2 \log_{10}$ (cfu/ml).

NOTE 1 This limit of 10 (or 30) colonies applies to the sum of the total numbers of counted colonies on all plates, ΣC .

NOTE 2 This limit only relates to the specific case of this experimental protocol for the intralaboratory standard deviation of reproducibility (i.e. experiments aiming specifically to assess the uncertainty) and not the use of this standard deviation to assess the measurement uncertainty for new samples (see Clause 8).”

After Clause 7, insert a new Clause 8,

“8 Calculation of expanded uncertainty

8.1 Introduction

It is assumed that the number of colony-forming units in Petri dishes follows a Poisson distribution. This random error is taken into account in the estimation of the expanded uncertainty described in 8.2.

NOTE The calculations described for the estimation of the intralaboratory standard deviation of reproducibility (see 5.3) neglect the random error due to Poisson distribution, which means that they should exclude enumeration results based on low numbers of counted colonies.

8.2 Calculation

8.2.1 General case

Denoting the test result $y = \log_{10}x$, then the expanded uncertainty, U , with a coverage factor of 2 (corresponding approximately to a confidence level of 95 %) can be calculated using Equation (1):

$$U = 2 \sqrt{s_R^2 + \frac{0,188\ 61}{\sum C}} \quad (1)$$

where

s_R is the standard deviation of reproducibility;

$0,188\ 61/\sum C$ is the variance component due to the Poisson distribution, in which $\sum C$ is the sum of the total numbers of colonies counted on all plates.

NOTE The numerator is derived by using a theoretical property of the Poisson distribution (equality of the expectation and the variance, which immediately leads to an estimated Poisson component coefficient of variation, $CV = 1/\sqrt{\sum C}$), and the approximation that the Poisson variance component on a logarithmic scale is approximately equal to the coefficient of variation squared, $(CV)^2$, when a natural logarithmic scale is used, and therefore to $(\log_{10} e)^2 = 0,188\ 61 \times (CV)^2$ when a decimal logarithmic scale is used.

Measurement uncertainty according to Equation (1) depends both on the reproducibility standard deviation estimated from an experiment with high counts, s_R , and on the total plate count for the sample under investigation, $\sum C$. It is recommended, for the sake of simplicity, to use Equation (1) wherever possible.

8.2.2 Differentiation between low and high counts (optional)

For high counts, the second term under the square root, the Poisson term depending on $\sum C$, can be ignored and Equation (1) simplifies to:

$$U = 2s_R \quad (2)$$

Given the limit value, C_{lim} :

$$C_{\text{lim}} = \frac{(\log_{10} e)^2}{s_R^2 \times ((1 - 0,05)^{-2} - 1)} \approx \frac{1,75}{s_R^2} \quad (3)$$

For all cases where $\sum C > C_{\text{lim}}$, the difference between U calculated by Equations (1) and (2) is negligible (< 5 %).

Once s_R has been estimated, C_{lim} can be either calculated from Equation (3) or taken from Table B.1.

Two cases can be differentiated:

if $\sum C > C_{lim}$ use Equation (2) to derive U ;

if $\sum C \leq C_{lim}$ use Equation (1) to derive U .

NOTE Calculation of C_{lim} is not necessary when Equation (1) is used in all cases."

Page 10

Delete Clause 8, and insert,

"9 Expression of measurement uncertainty in the test reports

Once the measurement uncertainty has been derived as explained in Clause 8, it may be expressed in the report, together with the test result, as an interval on the decimal logarithmic scale (see Note to 5.3) or as natural values (cfu per gram or cfu per millilitre), or as a percentage, as illustrated by the following possibilities.

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

a) interval for log result:

$$y \pm U [\log_{10} (\text{cfu/g})] \text{ or}$$

$$y \pm U [\log_{10} (\text{cfu/ml})];$$

b) decimal logarithmic result estimate with limits:

$$y [\log_{10} (\text{cfu/g})] \quad [y - U, y + U] \text{ or}$$

$$y [\log_{10} (\text{cfu/ml})] \quad [y - U, y + U];$$

c) result estimate with absolute limits:

$$x \text{ cfu/g} \quad [10^{y-U}, 10^{y+U}] \text{ or}$$

$$x \text{ cfu/ml} \quad [10^{y-U}, 10^{y+U}];$$

d) result estimate with relative limits:

$$x \text{ cfu/g} \quad [-(1 - 10^{-U}) \times 100 \%, +(-1 + 10^U) \times 100 \%] \text{ or}$$

$$x \text{ cfu/ml} \quad [-(1 - 10^{-U}) \times 100 \%, +(-1 + 10^U) \times 100 \%].$$

NOTE 1 Relative limits depend only on U . Examples of relative limits are found in Table B.1.

NOTE 2 While x has either cfu/g or cfu/ml as a unit, as a logarithm, y is, like pH, dimensionless, and has none. To remind users of the unit of the raw data and the type of logarithm used, $\log_{10} (\text{cfu/g})$ or $\log_{10} (\text{cfu/ml})$ can be added in brackets after the numerical result.

EXAMPLE 1

The standard deviation of reproducibility, s_R , is 0,15 $[\log_{10} (\text{cfu/g})]$.

The test result is 100 000 cfu/g, i.e. $y = 5,00 [\log_{10} (\text{cfu/g})]$, with $\Sigma C = 110$ (dilution –3: 102 colonies; dilution –4: 8 colonies).

Thus the expanded uncertainty, U , with a coverage factor of 2 (95 % confidence level) is, using Equation (1):

$$U = 2 \sqrt{0,15^2 + \frac{0,188\ 61}{110}} = 0,31$$

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

$$5,0 \pm 0,3 [\log_{10} (\text{cfu/g})];$$

$$5,0 [\log_{10} (\text{cfu/g})] [4,7; 5,3];$$

$$1,0 \times 10^5 \text{ cfu/g} [4,9 \times 10^4; 2,0 \times 10^5];$$

$$1,0 \times 10^5 \text{ cfu/g} [-51 \% ; +100 \%].$$

NOTE Applicable only if two formulae are used (see 8.2.2): $C_{\text{lim}} = 78$. Then, as $\Sigma C = 110 > C_{\text{lim}} = 78$, simplified Equation (2) for high counts $U = 2 s_R = 0,30$ could have been used.

EXAMPLE 2

The standard deviation of reproducibility, s_R , is 0,25 $[\log_{10} (\text{cfu/g})]$.

The test result is 280 cfu/g, i.e. $y = 2,45 [\log_{10} (\text{cfu/g})]$, with $\Sigma C = 31$ (dilution –1, 1 ml on three plates: 9 + 9 + 9 colonies; dilution –2: 4 colonies).

Thus the expanded uncertainty, U , with a coverage factor of 2 (95 % confidence level) is, using Equation (1):

$$U = 2 \sqrt{0,25^2 + \frac{0,188\ 61}{31}} = 0,52$$

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

$$2,4 \pm 0,5 [\log_{10} (\text{cfu/g})];$$

$$2,4 [\log_{10} (\text{cfu/g})] [1,9; 3,0];$$

$$280 \text{ cfu/g} [85; 930];$$

$$280 \text{ cfu/g} [-70 \% ; +230 \%].$$

NOTE Applicable only if two formulae are used (see 8.2.2): $C_{\text{lim}} = 28$. Then, as $\Sigma C = 31 > C_{\text{lim}} = 28$, simplified Equation (2) for high counts $U = 2 s_R = 0,50$ could have been used.

EXAMPLE 3

The standard deviation of reproducibility, s_R , is 0,11 $[\log_{10} (\text{cfu/g})]$.

The test result is 100 cfu/g, i.e. $y = 2,00 [\log_{10} (\text{cfu/g})]$, with $\Sigma C = 11$ (dilution –1: 9 colonies; dilution –2: 2 colonies).

Thus the expanded uncertainty, U , with a coverage factor of 2 (95 % confidence level) is, using Equation (1):

$$U = 2 \sqrt{0,11^2 + \frac{0,188\ 61}{11}} = 0,34$$

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

$$2,0 \pm 0,3 [\log_{10} (\text{cfu/g})];$$

$$2,0 [\log_{10} (\text{cfu/g})] [1,7; 2,3];$$

$$100 \text{ cfu/g} [46; 220];$$

$$100 \text{ cfu/g} [-54 \% ; +120 \%].$$

NOTE Applicable only if two formulae are used (see 8.2.2): $C_{\text{lim}} = 144$. Then, as $\Sigma C = 11 < C_{\text{lim}} = 144$, simplified Equation (2) for high counts cannot be used.

EXAMPLE 4 (applicable only if two formulae are used)

A standard deviation of reproducibility, s_R , of 0,22 [$\log_{10} (\text{cfu/g})$] has been found.

$C_{\text{lim}} = 36$. Then, if $\Sigma C > C_{\text{lim}} = 36$, simplified Equation (2) for high counts, $U = 2 \times 0,22 = 0,44$ applies.

A general rule, applicable only for results with $\Sigma C > 36$, may be stated according to one of the following possibilities:

$$\log \text{ result} \pm 0,44 [\log_{10} (\text{cfu/g})];$$

$$\log \text{ result} [\log_{10} (\text{cfu/g})] [\log \text{ result} - 0,44; \log \text{ result} + 0,44];$$

$$\text{result cfu/g} [10^{\log \text{ result} - 0,44}; 10^{\log \text{ result} + 0,44}];$$

$$\text{result cfu/g} [-64 \% ; +175 \%].$$

If $\Sigma C \leq 36$, or if a single universally applicable equation is preferred, use Equation (1)."

Page 16

Add Annex B (overleaf) before the bibliography.