Implementation of the Standard EN ISO 19036 for the estimation of measurement uncertainty associated with the enumeration of *Campylobacter*, coagulase positive staphylococci and *Listeria monocytogenes* in the food chain

Questions and Answers

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This is a list of questions collected by the EURL-*Campylobacter*, EURL-Coagulase Positive Staphylococci and EURL-*Listeria monocytogenes* from their respective NRL networks at different training sessions or workshops, or directly posed to each EURL. The answers to these questions were prepared by the EURL working group on Measurement Uncertainty (MU), including the same EURLs and Bertrand Lombard, ANSES expert on MU. References to EN ISO 19036 (link), the EURLs 'Guide for implementation of the Standard EN ISO 19036:2019 for the estimation of measurement uncertainty associated with the enumeration of *Campylobacter*, coagulase positive staphylococci and *Listeria monocytogenes* in the food chain' (link) and other International Standards are listed beneath each answer.

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General

Sampling uncertainty
Since our lab does not do sampling, we do not need to calculate the uncertainty related to sampling, is this correct? Do we consider 0?

> **Reply**: Yes, and do not give value on the uncertainty related sampling, not even 0. This topic is not covered by EN ISO 19036 nor the EURL 'Guide for implementation of the Standard EN ISO 19036:2019'.

Calculation of technical and matrix uncertainties (ISO 19036, clauses 5.2.2.3.1 and 6.3)
What it the reason for the limit of 30 counted colonies for calculations of intralaboratory reproducibility (technical uncertainty) and repeatability (matrix uncertainty)?

> **Reply**: Above this limit, the Poisson uncertainty can be considered as negligible.

Calculation of technical and matrix uncertainty for MPN methods
Is it necessary to $\log_{10}$ transform the results before calculating the intralaboratory reproducibility (technical uncertainty) or the repeatability (matrix uncertainty) for MPN methods?

> **Reply**: Yes, the $\log_{10}$ transformation is appropriate and the standard (clause 5.2.2.3.1) does not exclude MPN from this transformation.

Technical uncertainty

Experimental design for the estimation of intralaboratory reproducibility

Intralaboratory reproducibility is the preferred option for estimating the technical uncertainty. A difference exists between EN ISO 16140-3 on method verification (clause 6.1.2), which requires that (at least) 10 laboratory samples belong to the same (food) item, and EN ISO 19036 (clause 5.2.2.2.2), which states that it is not necessary to repeat the estimation of intralaboratory reproducibility for different matrices and this estimate may be based on a single matrix. What to do to conduct one study applicable to both standards?

> **Reply**: Given the current version of EN ISO 16140-3, the 10 laboratory samples have to belong to the same (food) item (matrix). Once this standard will be revised, an alignment with EN ISO 19036 will be considered, that is to allow using different items.

Experimental design for the estimation of intralaboratory reproducibility: case of solid matrices

Is it possible to take the test portions from the initial suspension prepared from the solid matrix (sample)? It would allow an easier and better homogenization, using in particular a stomacher, than homogenization of the sample without diluent (as prescribed in EN ISO 19036, Figure 2).

> **Reply**: ISO/TC 34/SC 9/WG 2 did not agree to take the test portion from the initial suspension since it would exclude the technical uncertainty associated to the preparation of the initial suspension. WG 2 recommended, as to avoid the risk of partial homogenization of solid matrices, to use liquid or easy to homogenize matrices, i.e. belonging to the 2 following categories, mentioned in clause 5.2.2.2.4 of EN ISO 19036:
>
> - 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).

Experimental design for the estimation of intralaboratory reproducibility: case of more than 2 sets of conditions

What should we do when more than two sets of conditions are used in the experimental design for the estimation of intralaboratory reproducibility (e.g. more than 2 operators, 2 incubators)?

> **Reply**: Use more test portions according to Annex A of EN ISO 19036. The Excel tool associated to the standard conducts calculations for more than 2 test portions.

Experimental design for estimation of intralaboratory reproducibility: measurement conditions

How much do you need to vary when setting up the experimental protocol for estimation of intralaboratory reproducibility?

> **Reply**: The variation should reflect that of when the method is being applied in the laboratory in terms of personnel, equipment, media batch etc. The objective is to maximise the variation between repeated measurements but maintaining a realistic representation of the laboratory's operations (see clause 5.2.2.2.6 of EN ISO 19036).

Estimation of technical uncertainty for Most Probable Number (MPN) methods
Can the experimental design to estimate the technical uncertainty for MPN methods be detailed?

> **Reply**: The experimental design and calculations for the estimation of intralaboratory reproducibility (clause 5.2 of EN ISO 19036) are applicable to MPN-based methods. The Excel calculation tool can also be used.

Do we have to subtract matrix uncertainty for a homogenized matrix (log 0.1) when estimating technical uncertainty?

> **Reply:** In the EURLs Guide on MU, it is recommended that the matrix uncertainty is not being taken into account in the calculations for estimation of technical uncertainty to facilitate calculations. In addition, for a homogeneous/homogenized matrix, matrix uncertainty has a small value of little impact on the value of technical uncertainty.

Frequency of re-estimating technical uncertainty
How often should technical uncertainty be re-estimated?

> **Reply:** It does not need to be re-estimated at a fixed frequency, but when there are changes in the laboratory with a potential impact on the method, new staff for example (see clause 5.1.4 of EN ISO 19036).

Can you advice how to calculate the technical uncertainty from the results of all participants in the PT? I know this is not best best choice but in this case I don't have other intralaboratory comparative results that I can use to estimate the technical uncertainty. The EURLs guide on MU and EN ISO 19036 state this third option, but do not provide any specific information on the calculation.

> **Reply:** To estimate technical uncertainty, EN ISO 19036 recommends three options, the third one being to estimate the reproducibility standard deviation from PT.
> The reproducibility standard deviation is calculated by the PT provider with the results of PT participants assessed as satisfactory and having used the same method. Apart from the limitations mentioned in EN ISO 19036 (clause 5.2.3.2), this option may conduct to an overestimation of the technical uncertainty for a given laboratory.

Matrix uncertainty

When estimating matrix uncertainty from different samples, can they be analysed by different personnel?

> **Reply:** To estimate matrix uncertainty, at least two portions need to be tested for each laboratory sample. The test portions from the same laboratory sample needs to be analysed under repeatability conditions, thus with a minimum of technical deviation. However, different personnel can take part in the analysis as long as the same person performs the same step of the method. On the other hand, in the analysis of different laboratory samples, conditions may vary.

Using the EURL database with values for matrix uncertainty, does it need to match my matrix exactly or can I use a value for a matrix similar to mine?

> **Reply:** EN ISO 19036 (clause 6.4) states that it is possible to use known values for samples expected to have a similar matrix uncertainty. This is why the database contains detailed information about each matrix for the user to be able to determine if one value can be applied to similar matrices. For example, cheeses may vary a lot in composition and in processing and it may not be relevant to use a matrix uncertainty value estimated for one type of cheese on other cheeses.

Distributional uncertainties

Uncertainty related to Poisson

Can the distributionnal uncertainties (for example Poisson) be calculated in advance (such as for technical or matrix uncertainty)?

> **Reply**: No, the distributionnal uncertainties, such as Poisson, must be calculated for each result, depending on the number of colonies counted on the dishes, unlike the technical uncertainty evaluated for each analytical method and the matrix uncertainty calculated for each matrix.

If two plates are used for confirmation, and five colonies are analysed for confirmation on each plate, should the distributional uncertainty be calculated separately or combined?

> **Reply:** All colonies combined analysed for confirmation should be used in the calculation for distributional uncertainty.

Expression of MU values in the test reports

When do you have to report the MU with the test result?

> **Reply:** Laboratories accredited according to ISO 17025 report MU depending on customer requirements. Regarding samples included in official control, it is up to the competent authority of each MS. But MU can always be included with the test result even if it is not required.

MU expression for results below the limit of quantification (LOQ)

Clause 9.2.1 of EN ISO 19036 deals with results below LOQ and the example in 9.2.2 gives a value of 9,091 cfu/g for the LOQ of a colony-count technique, which is different from the revised version of ISO 7218 (ISO/DIS 7218, 2022, clause 11.2.6.4), which takes a value of 10 cfu/g for the limit of determination (corresponding to LOQ) and asks to express results as estimated for less than 10 counted colonies. Which standard should be followed to express low numbers?

> **Reply**: Follow the latest version of EN ISO 7218. This aspect of EN ISO 19036 should be aligned with EN ISO 7218, when it will be amended or revised.

Uncertainty on estimated counts

In a case where you get a *Listeria monocyt*ogenes enumeration result less than 100 cfu/g but close enough, so that if the upper limit of uncertainty is taken into account, the upper limit of the result range is >100 cfu/g. The problem is that, almost invariably, this result would be an estimated count according to EN ISO 7218 (e.g. 9 colonies on plates from initial suspension). Would it be acceptable to apply the uncertainty value on an estimated count?

> **Reply**: Yes, one of the MU components covered by EN ISO 19036 is the Poisson uncertainty, one of the distributional uncertainties for colony-count techniques, including the one of EN ISO 11290-2. Poisson uncertainty is calculated from a total of colonies counted (see Table 2, clause 7.2). Then total MU is estimated by adding the technical, matrix and distributional (including Poisson) uncertainties.

Uncertainty on low count

Clause 9.2 of the standard gives how to report the uncertainty for results below the limit of quantification (<xLOQ in cfu/g). In Example 9.2.2.c, it is stated that the result in real numbers with limit values can be expressed as: <9.1 cfu/g [0.0; 79.2]. In the official food control, the result that we express as in accordance with EN ISO 7218 "Less than 1 / Vd cfu/g" should be presented with uncertainty, as given in the example above. Such presentation of the results would raise many doubts among customers, especially in relation to *Listeria monocytogenes*, where the allowed number must not exceed 100 cfu/g, and the upper uncertainty limit for a result of 0 was calculated at 79.2 cfu/g?

> **Reply**: Expressing a result as, in the example of clause 9.2.2: “below < 9,1 cfu/g” [0,0; 79,2]”, doesn’t seem to be a problem for the customers and for official controls, since the higher limit of the uncertainty range, 79,2 cfu/g, is still lower than the regulatory limit of 100 cfu/g, thus the result indicates conformity with the regulatory limit.

Should the lower value or the upper value of expanded uncertainty be taken into account for interpretation of results (Decision Rule)?

> **Reply:** Given that a decision rule is not defined in EC Regulation 2073/2005, it is a duty of the national competent authorities to decide the interpretation of the results in terms of conformity with legal limits.

After calculating the expanded uncertainty for *Listeria monocytogenes* enumeration, is it possible that the difference between the enumeration result and the upper or lower value (after considering the expanded uncertainty) is about 70%?

> **Reply**: According to the EURL 'Guide for implementation of the Standard EN ISO 19036:2019' (2022), we recommend referring to the acceptable values in Chapter 3.

Can the estimated values of each type of uncertainty be rounded?

> **Reply:** No, according to clause 9.1 of EN ISO 19036, the full values should be used, and rounding down to two decimals is only done on the final result (combined standard uncertainty or expanded uncertainty).