Report on the matrix uncertainty study organised by the three EURLs with the NRL networks of *Campylobacter*, Coagulase Positive Staphylococci and *Listeria monocytogenes*

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1. Introduction and scope

Matrix uncertainty, as described in the Standard EN ISO 19036[1], is the effects of microbial distribution in a given matrix, i.e. the variation between analytical results from different test portions taken from the same laboratory sample when analysed under repeatability conditions.

The matrix uncertainty is expected to be large for solid or multi-component matrices, and low for homogeneous matrices such as liquids and powders. It is assumed in EN ISO 19036 (clauses 6.3 and 6.4) as being independent of the analytical method used (including target bacteria) and of the laboratory estimating it.

A collaborative study was organised in 2021 by the three European Union reference laboratories (EURLs) for *Campylobacter,* Coagulase Positive Staphylococci (CPS) and *Listeria monocytogenes (Lm),* together with the associated networks of national reference laboratories (NRLs) to determine matrix uncertainty for certain routinely analysed matrices.

2. Method

Matrix uncertainty was estimated in this study according to EN ISO 19036 (clause 6.3) as the within-laboratory-sample repeatability standard deviation, by analysing multiple test portions under repeatability conditions from one or more laboratory samples, using the experimental design shown in Figure 1.
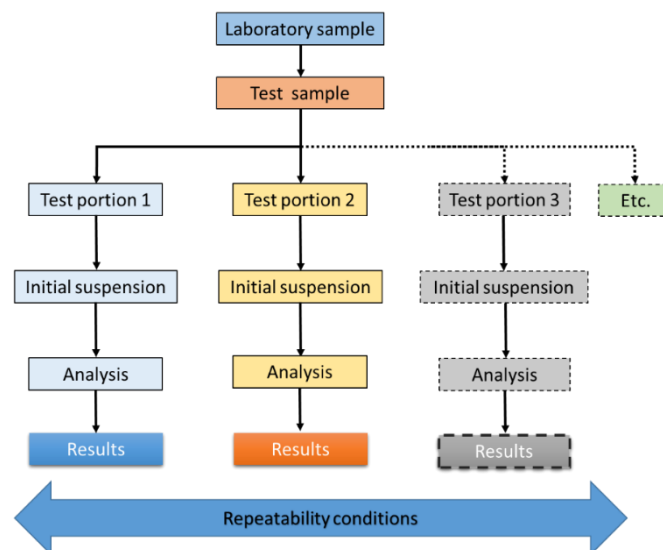

Figure 1: Experimental design to estimate matrix uncertainty from at least two test portions from each laboratory sample — Design for each laboratory sample.

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[1] EN ISO 19036: 2019 Microbiology of the food chain — Estimation of measurement uncertainty for quantitative determinations

Only naturally contaminated samples were used in the study, since artificial contamination is unlikely to reflect real matrix uncertainty. The measurand was selected by each participant per matrix with the aim to find samples with levels of natural contamination high enough to permit valid analysis of matrix uncertainty. Total mesophilic aerobic count and *Enterobacteriaceae* were the main measurands used in the study, except one laboratory used *Listeria monocytogenes* and another *Escherichia coli*. The methods chosen was in all cases already implemented in the laboratories and carried out according to the CEN/ISO standardised reference method or to an alternative (commercial) method validated against this reference method. All laboratories, except two, were accredited for the methods applied.

For multi-component products, which contain pieces of different foods, the test portion was sampled by taking amounts of each component representative of their proportions in the initial product.

All the test portions either came from one laboratory sample, or alternatively, from multiple laboratory samples collected over a period of time.

In all cases, at least two test portions were taken from each laboratory sample. The total number of test portions was at least ten more than the number of laboratory samples. For example:
—2 test portions from each of 10 laboratory samples; or
—11 test portions from 1 laboratory sample.

The size of each test portion was fixed at 10 g.

The analyses were repeated from each laboratory sample under conditions as similar as possible, i.e. under repeatability conditions (same technician, same batch of media, same equipment, etc.). Conditions could vary between laboratory samples. Technician could vary between different steps of analysis as long as the same technician was performing the same step for all test portions from the same laboratory sample.

Valid enumeration results were based on more than 30 counted colonies (sum of counted colonies on all retained plates) as well as counts below the maximum allowable number per plate (in most cases 300 cfu/plate or lower as specified in the specific standard). For methods including partial confirmation, only results for which more than half of the colonies tested were confirmed were used.

All results obtained by each participating NRL were submitted to the corresponding EURL in a spreadsheet file in Microsoft Excel format provided by the EURLs.

All results were validated by the EURLs to be in line with the method described above, or otherwise excluded.

The matrix uncertainty was estimated as the repeatability standard deviation, without correction for distributional standard uncertainties.

3. Results

Results were provided by 19 laboratories, see Table 1.

Table 1. Participants providing data to the collaborative study for matrix uncertainty.

Network	Function	Institute/City
EURL *Campylobacter*	EURL *Campylobacter*	National Veterinary Institute – Uppsala
	NRL Cyprus	Laboratory for the Control of Food of Animal Origin – Nicosia
	NRL Greece	Veterinary Laboratory of Ioannina – Ioannina
	NRL Romania	Institute for Hygiene and Veterinary Public Health – Bucharest
EURL coagulase positive staphylococci (CPS)	EURL CPS	Anses – Maisons Alfort
	NRL Cyprus	State general laboratory – Nicosia
	NRL Czech Republic	State Veterinary Institute Olomouc – Olomouc
	NRL Germany	German federal Institute for risk assessment – Berlin
	NRL Greece	Department of Food Hygiene of Athens – Athens
	NRL the Netherlands	National Institute for Public Health and the Environment – Bilthoven
EURL *Listeria monocytogenes* (*Lm*)	EURL *Lm*	Anses – Maisons Alfort
	NRL Belgium	Sciensano – Merelbeke
	NRL Cyprus	Laboratory for the Control of Food of Animal Origin, Cyprus Veterinary Services – Nicosia
	NRL Cyprus	State General Laboratory – Nicosia
	NRL Greece	Department of Food Hygiene of Athens – Athens
	NRL Portugal	Instituto Nacional de Investigação Agrária e Veterinária, I. P. – Vairão VCD
	NRL Republic of Ireland	DSL Department of Agriculture, Food and Marine – Co. Kildare
	NRL Romania	Institute for Hygiene and Veterinary Public Health – Bucharest
	NRL the Netherlands	RIVM National Institute for Public Health and the Environment – Bilthoven

Thirty-nine matrices were analysed during the course of the study. Thirty different matrices of 20 types in 11 categories, according to the classification in Annex A of EN ISO 16140-3[2], got valid results.

In one case, two different matrix uncertainty values were obtained when analysing the same matrix with two different methods for different measurands. Considering the values were very close, the mean of the two values was retained, following the proposal by the WG 2 "Statistics" of ISO/TC 34/SC 9 "Food products – Microbiology".

Results from the study can be found both as a flat file for download, and in an online database on the EURL *Lm* website:
https://sitesv2.anses.fr/en/minisite/listeria-monocytogenes/measurement-uncertainty

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[2] EN ISO 16140-3:2021 Microbiology of the food chain — Method validation — Protocol for the verification of reference methods and validated alternative methods in a single laboratory

4. Conclusions

The matrix uncertainty values obtained in the collaborative study organised by the three EURLs *Campylobacter*, CPS and *Lm,* with their corresponding NRL networks, can be used by any laboratory for the calculation of combined uncertainty when analysing the same matrices or matrices expected to have a similar matrix uncertainty.

The aim is to enlarge the list of matrices and corresponding values of matrix uncertainty. To submit new data, please contact any of the three EURLs to receive the experimental design and the file for reporting data. Prerequisites to include new data are that:

- the experimental design followed the setup according to EN ISO 19036, clause 6.3,
- the method as described in this report has been followed,
- valid estimates of matrix uncertainty have been retrieved,
- values correspond to matrices not already included in the list.