anses

EURL Lm
European Union Reference Laboratory for
Listeria monocytogenes
http://eurl-listeria.anses.fr

MEASUREMENT UNCERTAINTY FOR QUANTITATIVE ANALYSES: MATRIX UNCERTAINTY

Joint EURL Training on **MEASUREMENT UNCERTAINTY**

Léna BARRE
Project Leader; EURL Lm
ANSES
Lena.barre@anses.fr

INVESTIGATE, EVALUATE, PROTECT

Definition of Matrix Uncertainty according to ISO 19036:2019

Matrix uncertainty - uncertainty resulting from the extent to which the test portion is not truly representative of the laboratory sample

General information: Matrix uncertainty; u_{matrix}

- arises from imperfect mixing of the laboratory sample
- estimated for each matrix/food item
- independent of analytical method used
- can be large for solid matrices and multi-component food products (e.g., pizza)

Practical approaches to estimate Matrix Uncertainty

Guide on the 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

Version 1-23/06/2022

Working group of the EURLs:

- *Campylobacter* (SVA, Uppsala, Sweden): Helena Höök, Elina Lahti and Hanna Skarin – eurl-campylobacter@sva.se
- Coagulase Positive Staphylococci (ANSES, Laboratory for Food Safety, Maisons-Alfort, France): Abakabir-Mahamat Abdelrahim - eurl-staphylococci@anses.fr
- *Listeria monocytogenes* (ANSES, Laboratory for Food Safety, Maisons-Alfort, France): Adrien Asséré, Léna Barre and Ludivine Bonanno - eurl-listeria@anses.fr
- Expert: Bertrand Lombard (ANSES, Strategy & Programmes Department, Maisons-Alfort, France) bertrand.lombard@anses.fr

2.3 Matrix uncertainty

Matrix uncertainty corresponds to the uncertainty associated with taking the test portion from the laboratory sample; it is therefore different from the uncertainty linked to sampling (taking a sample from a batch of production). It is linked to the degree of the spatial heterogeneity of the microorganisms within the matrix. It is thus expected to be large for solid or multi-component matrices, and low for homogeneous matrices. It is assumed in the standard as being independent of the analytical method used (including target bacteria) and of the laboratory estimating it.

The standard allows using values of matrix uncertainty already obtained for laboratory samples expected to have a similar matrix uncertainty (matrix homogeneity), see clause 6.4. We thus suggest using values of matrix uncertainty, per matrix type, obtained in a collaborative study organised (i) by the three EURLs *Campylobacter,* CPS, *Lm*, together with the associated NRLs networks, as well as (ii) at French level (AFNOR committee on food microbiology and NRLs CPS, *Lm, Salmonella*). For the EURLs study, see: https://sitesv2.anses.fr/en/minisite/listeria-monocytogenes/measurement-uncertainty

Based on an experimental study[6], EN ISO 19036 (clause 6.2) recommends using a fixed value of 0,1 $\log_{10}$ for liquids (thin, non-viscous fluids, e.g. milk and beverages) and powders as well as when the whole laboratory sample can be made homogeneous before taking the test portion. Details on homogenization techniques are provided in the ISO 6887 series, in particular in EN ISO 6887-part 1 (clause 9.1), which is referred to in clause 6.2 of ISO 19036. For homogeneous

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

[6] Ah Soon C., Cornu M. Report of 2003/2004, ISO Trials about uncertainty measurement, June 2004, AFSSA, Maisons Alfort, France. Freely available for download at http:// standards .iso .org/ iso/ 19036

Matrix Uncertainty

Approaches to estimate matrix uncertainty:

A. Use of fixed value - for homogenous or well-mixed laboratory sample, a minimum fixed value can be used

B. Repeatability experiments - analysing multiple test portions from laboratory samples to determine the within-sample variance

C. Already known - relevant characteristics of the matrix well known and matrix uncertainty estimated from prior knowledge

Matrix Uncertainty

A. Homogeneous matrix or homogenised laboratory sample

- Experience indicates that **liquids** and **powders** are regarded as being homogeneous => a relatively low matrix uncertainty, typically u_{matrix} = 0,1 $\log_{10}$ cfu/g or ml

- If the whole laboratory sample can be homogenised before taking the test portion, then the matrix uncertainty can be taken at a fixed value of u_{matrix} = 0,1 $\log_{10}$

Matrix Uncertainty

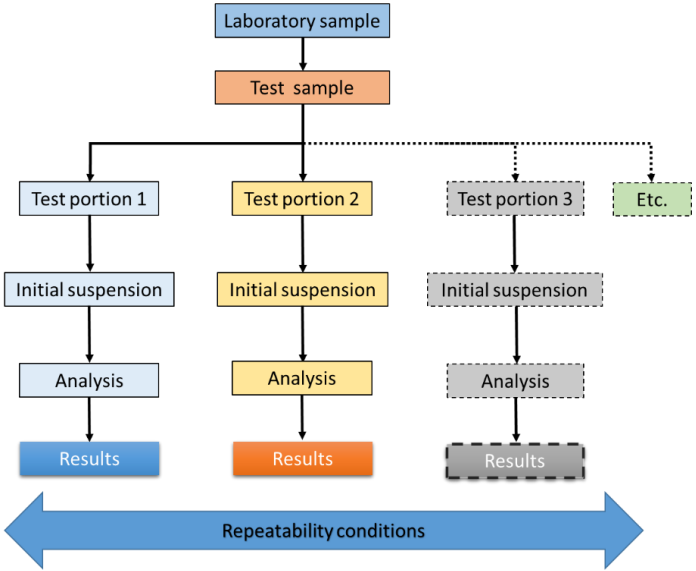
B. Experiment - Multiple test portions from laboratory samples

- 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.
- Repeated measurements **on a single laboratory sample** are made under same conditions (i.e., same time, same operator, same equipment, same media batches, same equipment etc).
- Repeated measurements **from multiple laboratory samples**, may be analysed over a period of time to give a more generally applicable estimate of matrix uncertainty.

Matrix Uncertainty – Repeatability Experiment

- Repeat design for each laboratory sample
  - do not homogenise
  - Use naturally contaminated samples, i.e. do not artificially contaminate
- Matrix uncertainty is regarded as independent of target microorganism and test method used

- chose target microorganisms for which naturally contaminated samples are likely to be found (e.g. total mesophilic aerobic count, *Enterobacteriaceae* etc.)



Matrix Uncertainty

C. Already known

- Matrix uncertainty values obtained in one laboratory may be used by another laboratory for laboratory samples expected to have a similar matrix uncertainty.
- We suggest to use the estimated values of MaU obtained from the collaborative study of the three networks of EURLs (presented next)

anses

EURL Lm
European Union Reference Laboratory for
Listeria monocytogenes
http://eurl-listeria.anses.fr

INTER-EURLS WORKING GROUP ON MEASUREMENT UNCERTAINTY: STUDY OF MATRIX UNCERTAINTY

Léna BARRE

INVESTIGATE, EVALUATE, PROTECT

Study to generate values of matrix uncertainty

EURL Lm

- **Recall of EN ISO 19036 (2019)**
  - 3 main MU components: technical uncertainty, **matrix uncertainty** & distributional uncertainty
  - Matrix uncertainty (clause 6)
    - Assumed to be independent from the laboratory determining it and the target bacteria chosen
    - Possibility to share values generated by a lab, that can be used by other labs

- **Study to generate values of matrix uncertainty**
  - Per type of matrix
  - Co-organised by the inter-EURLs WG MU for the 3 NRLs networks

Study to generate values of matrix uncertainty

- **Recall of EN ISO 19036 (2019)**
  - 3 main MU components: technical uncertainty, **matrix uncertainty** & distributional uncertainty
  - Matrix uncertainty (clause 6)
    - Assumed to be independent from the laboratory determining it and the target bacteria chosen
    - Possibility to share values generated by a lab, that can be used by other labs

- **Study to generate values of matrix uncertainty**
  - Per type of matrix
  - Co-organised by the inter-EURLs WG MU for the 3 NRLs networks

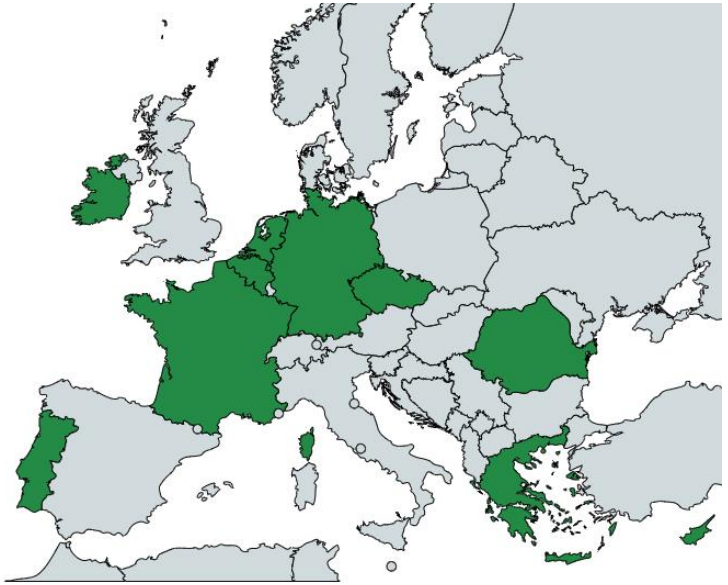
Study to generate values of matrix uncertainty: organisation

EURL Lm

1. **Invitation to participate to this study**
   - Participating NRLs were requested to propose matrices that they are able to study
2. **Inter-EURLs WG MU: allocation of matrices between participants**
3. **NRLs/EURLs: experiments, July-October 21**
4. **Inter-EURLs WG MU: data analysis 22**
5. **Matrix uncertainty values obtained to be shared publicly on EURL websites**

Study to generate values of matrix uncertainty: Results

Results were provided by 12 NRLs from Belgium, Cyprus (3 NRLs), Czech Republic, Germany, Greece (2 NRLs), Ireland, Portugal, Romania, The Netherland and EURLs *Campylobacter*, CPS and *Lm*.



Study to generate values of matrix uncertainty: Results

EURL Lm

Majority of matrices covered : *Sr* available

Above 82 types of matrices listed on Annex A of the standard ISO 16140-3:

Homogeneous matrices have been excluded from this study

THANK YOU !!!

Results were provi
Belgium, Cyprus (3
Germany, Greece (
Romania, The Netherland and EURL *Campylobacter*, CP and *Lm*.

Advertisements

Study to generate values of matrix uncertainty: Results

Matrix uncertainty were generated for:

31 matrices of 20 food types in 11 different categories as defined in ISO 16140-2 Annex A.

Results from the study can be found on the EURL Lm website:

https://sitesv2.anses.fr/en/minisite/listeria-monocytogenes/measurement-uncertainty

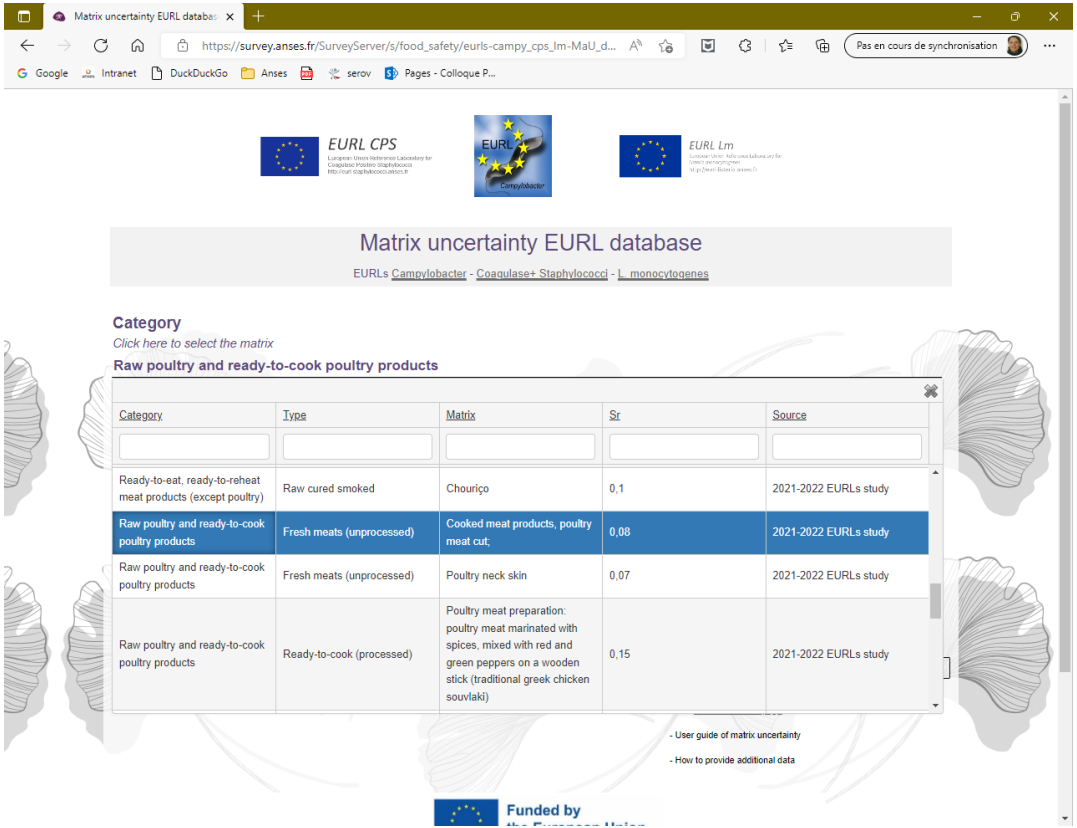
https://sitesv2.anses.fr/en/minisite/listeria-monocytogenes/measurement-uncertainty

EURL Lm website

EURL Lm

EURL Lm website

EURL Lm



Study to generate values of matrix uncertainty: Results

- ❑ **Publication of the list of MU on the 3 EURL websites**
- ❑ **Publication of the list of MU for French value by Afnor (linked to the purchase of NF EN ISO 19036)**

To submit new data, please contact your EURL for Campy/CPS/Lm to receive the file for reporting data.

anses

EURL Lm

Thank you for your attention