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Measurement uncertainty in quantitative food microbiology: revision of ISO/TS 19036

Workshop EURL/NRLs *Campylobacter*, 10 October 2018, Uppsala

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Outline

- Basic aspects
- Standardization approach to measurement uncertainty in quantitative food microbiology
- Revision of ISO/TS 19036
- Interpretation of measurement uncertainty

INTRODUCTION

Why?

- Significance of microbiolgical analysis = direct hazard for the consumers' health
- Quantitative methods (enumeration) in food microbiology
  = highly variable (0,1-1 $\log_{10}$)
  ➔ Need to quantify this variability

Why? (foll.)

- Lab accreditation
  - Requirements EN ISO 17 025 (2017), § 7.6
    - To identify contributions to measurement uncertainty (MU)
    - To evaluate test MU
      - To take into account the main contributions
    - If the test method precludes rigourous MU evaluation
      ↳ estimation based on understanding of theoritical principles or practical experience of method performance
    - Note 1: If the method specifies
      - Limits for the values of the major MU sources
      - Form of presentation of calculated results

      ↳ MU evaluation requirements = respected
    - Note 2
      - For a method where
        - MU associated to results has been established and verified
        - Critical factors are under control

      ↳ no need to evaluate MU for each result
    - Note 3: Refer to Guide ISO 98-3, ISO 21748 & ISO 5725 series

Why ? (foll.)

- Lab accreditation (foll.)
  - Implementation in microbiology: Guide EA-04/10
    - Metrologically rigorous and statistically valid MU estimation generally not possible
    - MU estimated on the basis of precision data + ideally bias
    - Individual components
      - To be identified and demonstrated to be under control
      - Some can be measured (pipetting, weighting and dilution effects) and evaluated (negligible/total MU)

BASIC ASPECTS

Definition

- According to GUM (1995)/Guide ISO-CEI 98-3 (2008)
- *Parameter, associated with the result of a measurement, which characterizes the dispersion of the values which could reasonably be attributed to the measurand*
- + 3 notes

I. Decomposition approach

- Decomposition/step-by-step/bottom-up approach

1. To estimate the individual MU components
   = individual sources of variability (variances) which contribute to the uncertainty in the measurement process
2. To derive MU using formal principle of uncertainty propagation by combination (addition) of variances

II. Global approach

- Global/top-down approach
- Defined in ISO 21 748 (2017)

  Guidelines for use of the estimations of repeatability, reproducibility and trueness in the estimation of measurement uncertainty

- If absence of a comprehensive model of the measurement process (MU decomposition)
  ↳ MU based on trueness & precision of a method of analysis
  (inter-lab study according to ISO 5725)
- With conditions

STANDARDIZATION APPROACH TO MEASUREMENT UNCERTAINTY IN QUANTITATIVE FOOD MICROBIOLOGY

Standardization in food microbiology

- Conducted by
  - ISO/TC 34/SC 9
    - SC 9 *Microbiology of* TC 34 *Food products of* ISO
    - Chair: Jacques-Antoine HENNEKINNE (Anses)
    - Secretary: Gwénola HARDOUIN (AFNOR)
  - CEN/TC 275/WG 6
    - WG 6 *Microbiology of the food chain* of TC 275 *Food analysis-Horizontal methods* of CEN
    - Convenor: Alexandre LECLERCQ (Institut Pasteur)
    - Secretary: Gwénola HARDOUIN (AFNOR)

Quantitative MU in food microbiology

- « Half-global » approach (revision of ISO 19036)

1. Technical uncertainty (ISO/TS 19036)
   - Experimental reproducibility standard-deviation
     - On final measurement result
   - Advantages/GUM decomposition
     - Less risk to under-estimate MU
       - ⇦ type of matrix, sub-sampling of test portion taken into account
     - No need to estimate each MU component
     - A priori less heavy to implement
2. + Matrix uncertainty (revision of ISO 19036)
   - ⇦ Distribution of bacteria in the sample matrix
3. + Distribution uncertainties (mostly revision of ISO 19036)
   - ⇦ Depending on the principle of the method used

ISO/TS 19036

- ISO/TS 19036 (2006) + Amendment 1 (2009)
  Microbiology of foods and animal feeding stuffs–Guide on estimation of measurement uncertainty for quantitative determinations
- Developped by a group of ISO/TC 34/SC 9
- Technical Specification
  - For a new topic
  - For 3 years
  - Users' feedback requested
- Scope
  - Mainly bacteria quantification
  - Colony-count techniques
    Incl. low numbers (Amendment 1)
  - Alternative (instrumental) techniques

REVISION OF ISO/TS 19036

- Conducted by WG 2 *Statistics* of ISO/TC 34/SC 9
- Project Leader: Basil JARVIS (UK)
  - Co-Project Leaders
    - Keith JEWEL (UK)
    - Paul IN'T VELD (NL)
- Objectives of revision
  - Transformation into a CEN/ISO Standard
  - Feedback on implementation of ISO/TS 19036
  - Inclusion of MPN technique
  - Harmonisation with water microbiology (ISO 29201)
- ISO/DIS Vote – CEN Enquiry
  - 17/05 ➔ 09/08/2018
  - 100 % approval (28 ISO Members, 19 CEN Members)
  - Follow-up to comments ➔ final vote

Sources of uncertainty

- Trueness/bias not taken into account
  - Empirical nature of bacterial enumerations
  - True/reference values generally not available
- 3 uncertainty components
  - Technical uncertainty
    - Major component
  - Matrix uncertainty
  - Distribution uncertainties
- MU surveillance
  - New estimation required if a critical factor modified
    - Ex: source & type of culture media & other reagents, dilution/inoculation/incubation mode, counting technique, change of operator
  - For accredited labs, requirement verified

1. Technical uncertainty

- MU sources covered or not

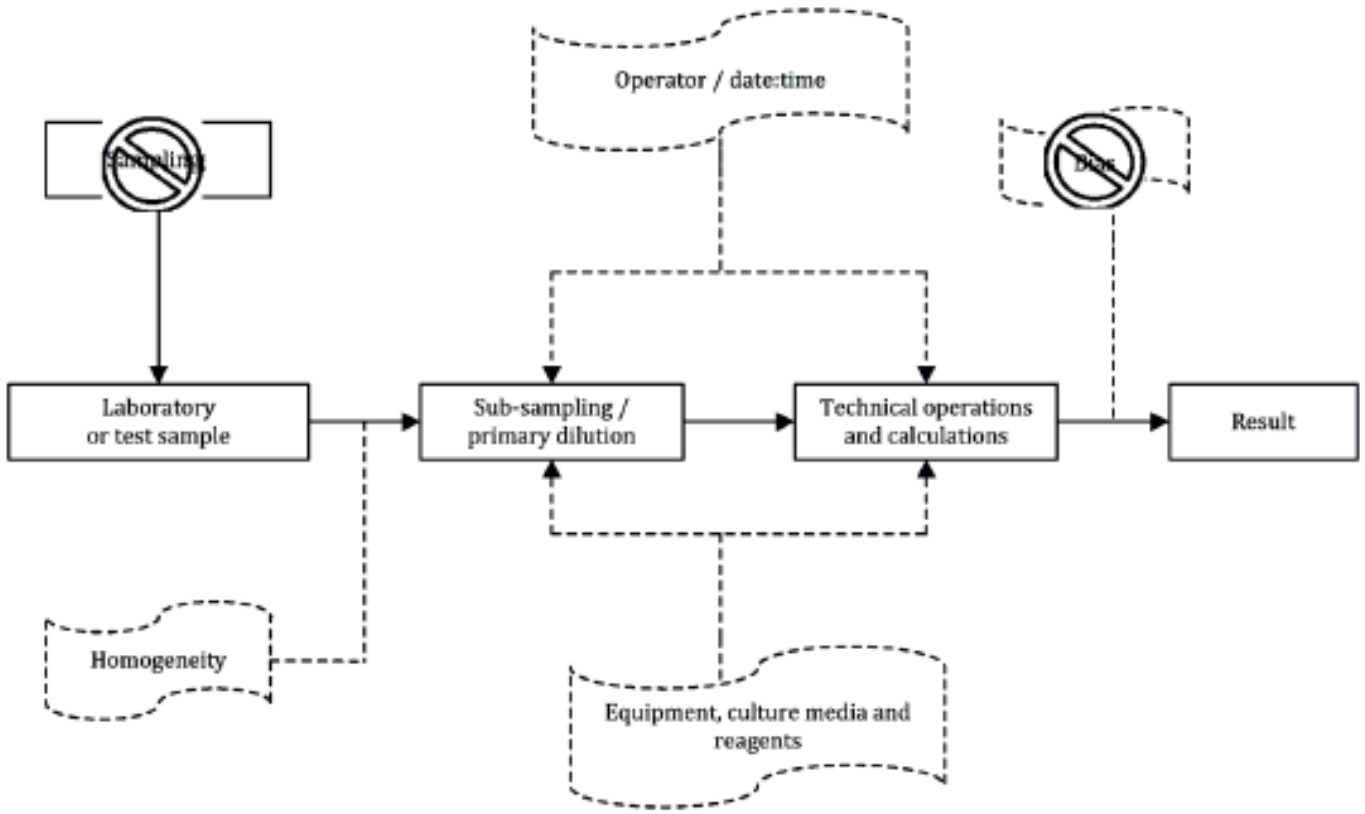


Figure 1 — Diagram of the main sources of uncertainty in food chain microbiology covered in this International Standard. Solid lines indicate the sequential procedures and dotted lines the factors that affect uncertainty estimation. The symbol ⌀ indicates that these factors are not covered by this International Standard.

1. Technical uncertainty

- Estimated by reproducibility standard-deviation
- 3 options
  a) Intralaboratory
  b) Interlaboratory
     ⇘ interlab studies of method validation
  c) Interlaboratory
     ⇘ proficiency tests

1.a) intralaboratory s_R

- Experimental design

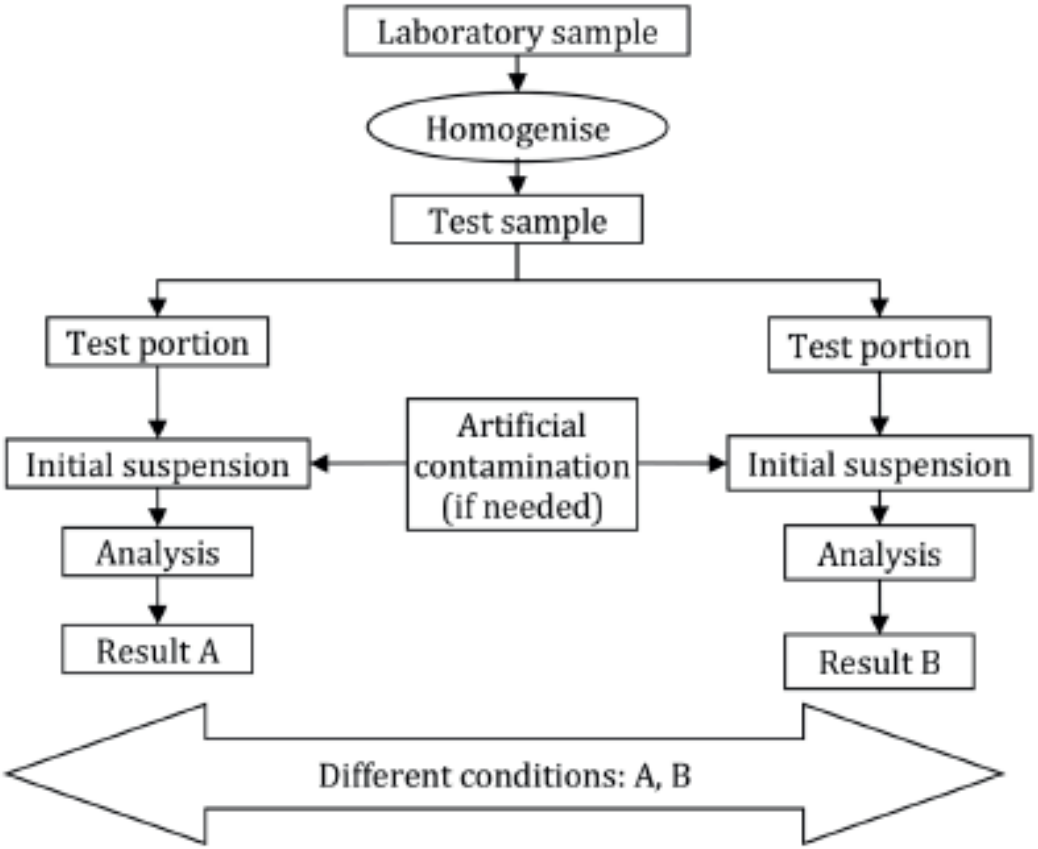


Figure 2 — Experimental protocol for estimation of intralaboratory reproducibility; two determinations on each laboratory sample

1.a) intralaboratory s_R

- Experimental design (foll.)
  - ≥ 10 samples
  - Possibility to use proficiency test samples
- Calculations
  - Results' acceptability
    - Colony-count technique: ≥ 30 counted colonies
    - MPN: ≥ 5 + tubes
  - Log_{10} transformation
  - Calculation
    - Formula

$$s_{IR} = \sqrt{\frac{1}{2p}\sum_{i=1}^{p}(y_{iA} - y_{iB})^2}$$

    - Or ANOVA, ≥ 2 replicates: Annex A

1.b) method validation interlaboratory s_R

- Restrictive conditions to estimate technical MU
  - Repeatability & reproductibility estimated in the lab ≤ corresponding values in interlab study
    - See ISO 21748
  - Sub-sampling & preparation of initial suspension = included in interlab study?
  - Artificial conditions of interlab study samples: matrices, strains, stress,…

    ➔Risk to under-estimate MU
- Interlab repeatability & reproductibility not available for all methods

1.c) Proficiency test interlaboratory s_R

- Restrictive conditions to estimate technical MU
  - ↳See option 2
  - \+ the same method
  - to be used by all proficiency test participants (or a sufficient number)
  - with satisfactory results

2. Matrix uncertainty (s_{matrix})

- Important if matrix heterogeneously contaminated
  - Ex: solid food, multiple ingredients
- Estimation: 3 possible approaches

a. Use of a fixed value
  - 2 cases
    a) Homogeneous matrices: liquids, non-viscous fluids
    b) Lab sample can be well homogenised
  - $S_{matrix} = 0{,}1 \log_{10}$
    - Trials organised by FR, 2003/04

2. Matrix uncertainty (s_{matrix})

b. Analysis of several test portions (TP)

- From one/several lab samples, naturally contaminated only
- 11 TP/1 sample, or 2 TP/sample from 10 samples

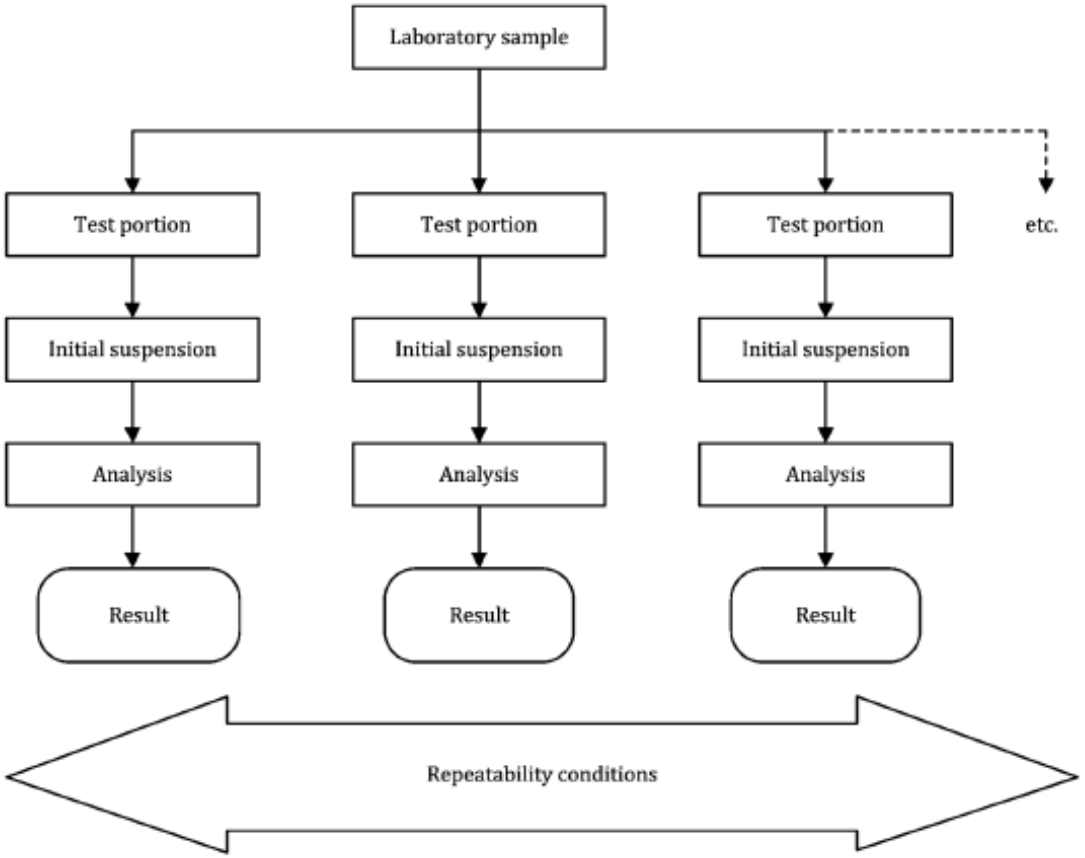


Figure 3 — Experimental design to estimate matrix uncertainty from multiple test portions from laboratory sample – Case of one laboratory sample

2. Matrix uncertainty (s_{matrix})

c. Use of known characteristics of the sample matrix

↳Analysis of several test portions from a lab sample of a similar matrix

- Performed earlier
- By the same lab or another lab

➔possibillity of collaboration

- Between EURLs/their respective NRL network
- To share s_{matrix} values for specific matrices

3. Distribution uncertainties

a. Colony-count technique

a.1 Poisson uncertainty, $s_{Poisson}$

- Already in Amendment 1 to ISO/TS 19036
- Significative for low numbers
- Calculation
  - Table ($\sum C \leq 40$)
  - Or equation

$$S_{Poisson} = \frac{1}{\ln(10)\sqrt{\sum C}} = \frac{0{,}4343}{\sqrt{\sum C}}$$

a.2 Colony-count technique with confirmation step, s_{conf}

- For colony-count techniques with confirmation of presumptive colonies (5 in general) – case of EN ISO 10272-2 for *Campylobacter*
- S_{conf} according to binomial law
- Calculation
  - Table
  - Equation (from ISO 29201)

b. MPN, s_{MPN}

– Calculation

- In Annex C
- Or Excel tool referenced in ISO 7218: http://standards.iso.org/iso/7218

Combined & expanded uncertainty

- Combined uncertainty *u*(y)
  - At least
    - Technical uncertainty
    - Addtionnal relevant uncertainties
  - Calculation
    - Ex for CCT: $u(y) = \sqrt{s_R^2 + s_{Poisson}^2 + s_{conf}^2 + s_{matrix}^2}$
- Expanded uncertainty *U*

$$U = 2\,u(y)$$

- Examples

MU expression in test report

- Two possibilities
  1. MU including technical, matrix & distribution uncertainties
  2. MU restricted to technical uncertainty, with a general value
     - ⇦ Technical uncertainty = major MU component
     - According to lab protocols and if agreed with clients
- 3 possible expressions

a) **interval for $\log_{10}$ result:** $y \pm U \log_{10}$ (cfu/g or /ml); e.g. $5{,}00 \pm 0{,}31 \log_{10}$ (cfu/g)

b) **$\log_{10}$ result with limits:** $y \log_{10}$ cfu/g $[y - U; y + U]$ or $y \log_{10}$ cfu/ml $[y - U; y + U]$;

e.g. $5{,}00 \log_{10}$ (cfu/g) [4,69; 5,31];

c) **absolute result with limits:** x (cfu/g) $[10^{y - U}; 10^{y + U}]$ or x (cfu/ml) $[10^{y - U}; 10^{y + U}]$,

e.g. $1{,}00 \times 10^5$ (cfu/g) $[4{,}90 \times 10^4; 2{,}04 \times 10^5]$

Next steps of ISO/TS 19036 revision

- Final ISO/CEN vote: May-June 2019
- Publication: before end of 2019

INTERPRETATION OF MEASUREMENT UNCERTAINTY

Conformity to legal limits

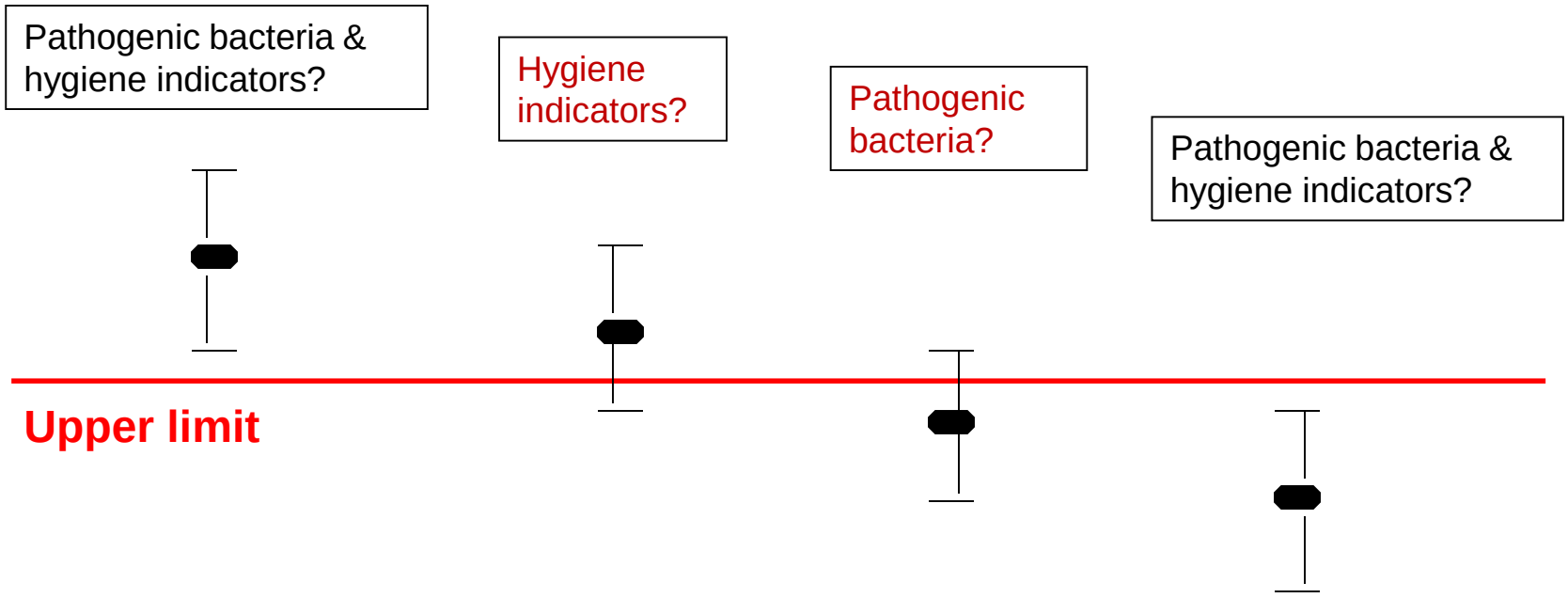
- European regulatory situation
- Own checks
  - EC Regulation 2073/2005: MU not taken into account
- Official controls
  - *Guidance Document on official controls, under Regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs*
    - 13/11/2006
    - https://ec.europa.eu/food/safety/biosafety/food_hygiene/microbiological_criteria_en
  - See next page

Official controls – quantatitive analyses

- MU estimation: refer to ISO/TS 19036
- For pathogenic bacteria/food safety criteria (FSC)
  - Quantitative limit only for *Lm*
  - *Highest acceptable result including MU should be low enough to ensure a high level of human health protection* = result + MU < L ?
  - *Highest acceptable result*: case-by-case basis
- For indicator bacteria/process hygiene criteria (PHC): rules for result interpretation may be less strict/pathogenic bacteria

  ↳??
- Case of *Campylobacter*: pathogenic/PHC ??
- Each lab must calculate its MU and, if requested by CA, report it in the test report

Conformity to a limit with MU: different cases



(i)
Result (X) - MU > Limit (L)
→ Non conformity (nonCF)

(ii)
X > L
But X-MU < L
→ CF/nonCF?

(iii)
X < L
But X+MU > L
→ CF/nonCF?

(iv)
X+MU < L
→ CF

CONCLUSION

- Global/half-global approach
  - Pragmatic
  - Adapted to the complexity of
    - Food analysis
    - Microbiological analysis
- Impact of revision of ISO/TS 19036 for labs having estimated their MU
  - Possibility to use MU values already obtained

    = technical + matrix + Poisson uncertainties
  - To add other distributionnal uncertainties
    - CCT with confirmation step
    - MPN

- Revision of ISO/TS 19036
  - ➔More widespread MU estimation in food microbiology
  - ➔More « scientific » analysis
- Need to precise the legislative frame