Estimation of measurement uncertainty in food microbiology: a normative approach

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Outline

• The normative approach for food microbiology
• Measurement uncertainty in quantitative microbiology
• Measurement uncertainty in qualitative microbiology
• Interpretation against legal limits
Introduction
Why?

- **Accreditation** of laboratories
  - Requirements of EN ISO 17025, § 5.4.6
    - To estimate the measurement uncertainty (MU) associated to the results produced by the laboratory
    - If rigorous/statistically valid calculation of MU not possible, to identify the MU components and to make a reasonable estimation of them
  - Implementation in France (COFRAC) for food microbiology
    = delayed (see ISO works)
Why ? (foll.)

• **Significance** of microbiological analysis = direct hazard for the consumer health

• **Quantitative** methods in microbiology = highly variable
  \(\rightarrow\) Need to quantify this variability

• How to express MU for **qualitative** determinations?
The normative approach for food microbiology
The ISO structure

• ISO/TC 34/SC 9
  – TC 34 « Food products »
  – SC 9 « Microbiology »
• Microbiological analysis of foods & feeds
• « Horizontal » approach
Bangkok, December 2002

- MU in **quantitative** microbiology
  = Basic approach adopted
  → Technical Specification (ISO/TS)
    - Quicker publication
    - Users’ review & 2-year revision
  - MU for **qualitative** determinations
    = In a 2\textsuperscript{nd} step
Measurement uncertainty in quantitative microbiology
The approach (1)

• Quantitative determinations
  – Colony-Count Techniques (+ Most Probable Number Techniques)
  – Alternatives methods (instrumental)

• Decision based on a 1st series of ISO trials (2002)

• Broad consensus at ISO meeting
The approach (2)

• GUM decomposition approach not selected
  – MU underestimation?
  – Heavy in food microbiology
The approach (3)

- « Global » approach chosen
  - Reproducibility standard-deviation (s_R) on the final result of the entire measurement process
  - In agreement with
    - Codex Alimentarius (CCMAS)
    - ISO/TS 21 748 : 2004
      - « Guide for use of the estimations of repeatability, reproducibility and trueness in the estimation of measurement uncertainty »
      - Established by ISO/TC 69/SC 6
      - Bridge between GUM & ISO 5725
        - Global approach = special case of type A experimental estimation
The main steps

• 2003: 1\textsuperscript{st} draft of ISO/TS 19036
  «Microbiology of foods and animal feeding stuffs – Guide for the expression of measurement uncertainty of quantitative determinations»

• 2003/2004: 2\textsuperscript{nd} series of ISO trials
  – To quantify MU component linked to
    • Sampling of the test portion
    • Preparation of the initial suspension
  – 78 participants, from 10 countries

• 2004: Decisions for final draft of ISO/TS 19036
  – ISO Project Group
  – ISO/TC 34/SC 9
Guide ISO/TS 19036 - Presentation (1)

• Final Draft
  ↗ Under 3-month vote (→ 11 May) before publication

• Principles
  1. Global approach
  2. Enlarged MU = 2 \( s_R \)
Guide ISO/TS 19036 - Presentation (2)

• Principles (foll.)
  3. \( s_R \) estimated per
     • (consistent group of) microorganism(s)
     • (consistent group of) matrix(ces)

4. 3 options for \( s_R \)
   • Intra-laboratory \( s_R \)
   • Inter-laboratory \( s_R \) (method validation)
   • Inter-laboratory \( s_R \) (proficiency testing)
The « black-box » diagram

- **Excluded** (out of the analytical process)
- **Low levels excluded**
- **Excluded in general** (empirical nature of microbial counts)

**Sampling**

- **Random errors** (repeatability)
- **Bias**

**Sample** ➔ **Black box** ➔ **Test result**

- **Matrix (test portion)**
- **Initial suspension**
- **Operator/ time**
Intra-laboratory $s_R(1)$

- 1st (preferred) option
  - linked to the laboratory *per se*
Intra-laboratory $s_R(2)$

Experimental protocol

Food sample

1$^{st}$ operator (conditions A)

Initial suspension

Analyse

2$^{nd}$ operator (conditions B)

Initial suspension

Analyse

Different conditions
Intra-laboratory $s_R (3)$

- The experimental protocol
  - 10 samples per matrix type
  - Advantage = MU « at large »
    - Heterogeneity of the sample contamination
    - Preparation of the initial suspension
- Drawbacks
  - Need to repeat the protocol for each type of matrix analysed by the laboratory
  - Need to test naturally contaminated samples
Inter-laboratory $s_R$ (method validation) (1)

- **Advantages** = available values
- **Drawbacks**
  
  1. **Conditions to meet** (see ISO/TS 21 748)
     - Laboratory’s bias & precision
       = compatible with the method’s ones
     - All uncertainty sources (incl. test portion, sample preparation)
       = taken into account in the inter-laboratory trial
Inter-laboratory $s_R$ (method validation) (2)

• Drawbacks (foll.)
  2. Inter-laboratory $s_R$ available for a limited number of methods in food microbiology
  3. Difficulty
     ➢ to apply to routine analyses
     ➢ precision data obtained in limited and artificial conditions
     – Combinaisons (matrix, strain)
     – Annex flora (if any)
  4. Risk to under-estimate MU
     (samples homogenized and stabilized)
Inter-laboratory $s_R$ (PT)

- **Advantages**
  - Available values
  - Large number of PT schemes in food microbiology

- **Drawbacks**

  1. **Conditions to meet**
     - Method used by the laboratory in PT
       = the same than in routine
     - Method used by a sufficient number of participants
     - PT samples $\approx$ routine samples

  2. **Samples**
Measurement uncertainty in qualitative microbiology
• Preliminary works at ISO
• 2 possibilities foreseen
  – CI around the Limit of Detection (LoD$_{50}$)
  – From the equivalent of reproducibility for qualitative determinations
Interpretation against legal limits
Legislative frame

• Food hygiene « package »
  ↳ Regulation 852/2004 (« H I »)
• Draft Regulation on microbiological criteria
• A microbiological criterion
  = a qualitative/quantitative limit +...
Food hygiene controls

• For operators’ own checks
  = MU not taken into account

• For official food controls
  = ?

  ➢ the same rule
  ➢ or (Figure 2)
    o case (ii) for indicators
    o case (iv) for pathogens

  ➔ To be precised into EC Guidelines, to come
Figure 2: Uncertainty and compliance limits
Conclusion
• Global approach
  – Pragmatic, adapted to the complexity of
    • Food analysis
    • Microbiological analysis
  – In agreement with
    • GUM principles
    • International references and rules
• MU estimation becoming widespread in food microbiology
  = Towards a more « scientific » analysis ?