Estimation of Measurement Uncertainty for Quantitative Determinations in Microbiological Testing

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OUTLINE

- General principles
- Global approach for MU estimation
- Intralaboratory standard deviation of reproducibility
- Expression of measurement uncertainty
- MU estimation for Campylobacter enumeration in broiler carcasses

- Technical Specification ISO/TS 19036, Microbiology of food and animal feeding stuffs – Guidelines for the estimation of measurement uncertainty for quantitative determinations

- Gives guidance for the estimation of MU in microbiological testing associated with quantitative results gathered by e.g. a colony-count technique

- Is not applicable to the analysis of low levels of microorganisms (<10 colonies counted on at least one plate)
GENERAL PRINCIPLES

MEASUREMENT UNCERTAINTY (MU)

- The bacterial count obtained in an enumeration test is only an approximation of the actual count.

- **Measurement uncertainty (MU):** a parameter, associated with the result of a measurement, that characterizes the dispersions of the values that could reasonably be attributed to the measurand (e.g. count/g).

- MU demonstrates how well the result represents the value of the quantity being measured in the test portion.

- MU allows an assessment of the reliability of the result.
GLOBAL APPROACH FOR ESTIMATION OF MU


- Is based on the **overall variability** of the analytical process.
In microbiological testing the greatest sources of uncertainty arise from sampling and the non-homogeneous distribution of microorganisms in the sample.
GLOBAL APPROACH FOR MU ESTIMATION

MAIN SOURCES OF UNCERTAINTY IN FOOD MICROBIOLOGY

- Sampling
  - Equipment, culture media, reagents
  - Sub-sampling/primary dilution

- Laboratory sample

- Matrix
- Residual random errors

- Bias
- Operator/time

- Result
GLOBAL APPROACH FOR ESTIMATION OF MU

- Is based on the overall variability of the analytical process.
- Is derived from an experimental estimation of the standard deviation of reproducibility of the final result of the complete measurement process.
GLOBAL APPROACH FOR MU ESTIMATION

ESTIMATION OF THE STANDARD DEVIATION OF REPRODUCIBILITY ($S_R$)

- Three different possibilities:
  
  a) Intralaboratory standard deviation of reproducibility
     preferred option for deriving MU,
     MU value can be attached to the laboratory results
  
  b) Interlaboratory study
  
  c) Interlaboratory proficiency trial
GENERAL RULES FOR ESTIMATION OF $S_R$

- For each target microorganism (or consistent groups)
- For each type of matrix (or consistent groups)
- At least 10 samples
- Repetition of protocol on different days
- Naturally contaminated samples should be preferred
- Samples should be chosen as to cover the concentration range in routine testing
Various sources of uncertainty (sub-sampling, nature of matrix, residual random errors, operator/time, etc. are considered simultaneously but not separately.
EXPERIMENTAL PROTOCOL

Food sample

Test portion A

1<sup>st</sup> operator(s) (condition A)

Initial suspension A

Analysis

Test portion B

2<sup>nd</sup> operator(s) (condition B)

Initial suspension B

Analysis

Different conditions
INTRALABORATORY STANDARD DEVIATION OF REPRODUCIBILITY

CALCULATION OF $S_R$

<table>
<thead>
<tr>
<th>$i$</th>
<th>$x_A$</th>
<th>$x_B$</th>
<th>$y_A = \log_{10}(x_A)$</th>
<th>$y_B = \log_{10}(x_A)$</th>
<th>$(y_A - y_B)^2 / 2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$6,7 \times 10^4$</td>
<td>$8,7 \times 10^4$</td>
<td>4,83</td>
<td>4,94</td>
<td>0,0064</td>
</tr>
<tr>
<td>2</td>
<td>$7,1 \times 10^6$</td>
<td>$6,2 \times 10^6$</td>
<td>6,85</td>
<td>6,79</td>
<td>0,0017</td>
</tr>
<tr>
<td>3</td>
<td>$3,5 \times 10^5$</td>
<td>$4,4 \times 10^5$</td>
<td>5,54</td>
<td>5,64</td>
<td>0,0049</td>
</tr>
<tr>
<td>10</td>
<td>$1,1 \times 10^8$</td>
<td>$2,2 \times 10^8$</td>
<td>8,04</td>
<td>8,34</td>
<td>0,0453</td>
</tr>
</tbody>
</table>

$$S_R = \sqrt{\frac{(0,0064 + 0,0017 + \ldots + 0,0453)}{10}} = 0,15 \text{ (log}_{10}\text{) cfu/g}$$
Expanded Uncertainty \((U) = k \cdot u_c(y)\)

combined standard uncertainty \(u_c(y)\) = standard deviation of reproducibility

coverage factor \(k\) (value of 2 approx. corresponds to confidence level of 95%)

\[ U = 2 \ S_R \]

Test result:

\(x\) cfu/g \([10^{y-2SR}, 10^{y+2SR}]\)  

E.g. \(10^5\) cfu/g \([5 \times 10^4, 2 \times 10^5]\)
Based on ISO/TS 19036

- MU is estimated in a global approach based on the overall variability of the measurement process.
- Determination of the intralaboratory standard deviation of reproducibility is recommended for MU estimation.
- Reassessment of MU estimation is necessary following changes to critical factors (e.g. culture media and/or other reagents, operators, counting techniques...).
MU estimation for quantification of *Campylobacter* spp. in broiler carcasses

- Estimation of MU derived from the intralaboratory standard deviation of reproducibility for each laboratory

- Experimental setting:
  - 1 matrix
  - 12 samples (collected from May to September)
  - Analysis of samples in duplicate (conditions A and B) during routine laboratory operation
    (different operators, different media and reagents batches, different pipetting and mixing sets, different incubators etc.)

- Calculation of $S_R$ and expanded uncertainty