Evaluating uncertainty for microbiological methods

According to ISO 29201 Water quality — The variability of test results and the uncertainty of measurement of microbiological enumeration methods

Reporting results Uncertainty

Presented by Bertil Magnusson at Eurachem workshop May 2022
QUALITY ASSURANCE CHALLENGES OF MEASUREMENTS FROM FIELD TO LABORATORY WITH A FOCUS ON ISO/IEC 17025:2017 REQUIREMENTS
Analytical chemist trying to present UNCERTAINTY in microbiology …
Content

- Microbiology analytical steps
- Reporting results
- Uncertainty
  - Approaches
  - Symbols and units used
  - Distributional (Poisson)
  - Determine operational
  - Expanded
- Reporting uncertainty interval
- Additional components
- Summary reporting
- Eurachem guide

Focussing on water matrix

For e.g. food, feed and pharmaceutical same approach but higher U due to additional componets
Microbiological analytical steps

Product → Lab. sample → Homogenate → Dilution series

0: Product
1: Lab. sample
2: Homogenate
3: Dilution series
4: Reading
5: Confirmation
6: Result & Uncertainty?

CFU

Trollboken AB

MU Microbiology
### Reporting results

<table>
<thead>
<tr>
<th>CFU*</th>
<th>Report</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>Value and text</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Result is an estimate</td>
</tr>
<tr>
<td>≥ 10</td>
<td>Value &amp; Uncertainty</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Value and asymmetric confidence interval (95%)</td>
<td>20 [12,34]</td>
</tr>
</tbody>
</table>

*CFU = colony forming units

**CFU ≥ 10**

1) Uncertainty
   or

1) Give an interval
ISO standards say this

<table>
<thead>
<tr>
<th>Counted colonies</th>
<th>Reporting of results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ISO 8199 Water matrix</td>
</tr>
<tr>
<td>0</td>
<td>Not detected or &lt; 1</td>
</tr>
<tr>
<td>1-2</td>
<td>Microorganisms are present</td>
</tr>
<tr>
<td>3</td>
<td>Report results as an estimate</td>
</tr>
<tr>
<td>4 - 9</td>
<td>Report results as an estimate</td>
</tr>
<tr>
<td>≥ 10</td>
<td>Report results</td>
</tr>
</tbody>
</table>

NOTE 1 Legislation may require different ways of reporting.

NOTE 2 Eventual dilution must be considered, e.g. 3 CFU obtained in a food sample diluted 10 times will be reported as: microorganisms present but < 40 CFU.

Results ≥ 10 CFU measurement uncertainty is needed
Content

- Microbiology analytical steps
- Reporting results
- Uncertainty
  - Approaches
  - Symbols and units used
  - Distributional (Poisson)
  - Determine operational
  - Expanded
- Reporting uncertainty interval
- Additional components
- Summary reporting
- Eurachem guide

Focus on water matrix

For e.g. food, feed and pharmaceutical same approach but with more uncertainty components
Uncertainty approaches

**GUM principles**

- Definition of the measurand
- List of uncertainty components

**Intralaboratory**

**Interlaboratory**

**based on ...**

- Modelling
- Single lab validation/Global
- Interlaboratory validation
- Proficiency testing

**Experimental approaches**
ISO 29201 Water quality

Variability of test results and the uncertainty of measurement of microbiological enumeration methods

- All variants of methods of
  - colony counts
  - most probable number (MPN)
- Two approaches
  - component
  - global
- Not sampling, but subsampling
- Refers to Nordtest 537 for handling bias and proficiency testing

ISO 29201 is a general standard for uncertainty in ubio
Many many other ISO standards e.g. ISO 19036
## Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n_c$</td>
<td>Number of counts</td>
</tr>
<tr>
<td>$u$</td>
<td>Standard uncertainty</td>
</tr>
<tr>
<td>$u_{SIR}$</td>
<td>Intra (within) laboratory reproducibility</td>
</tr>
<tr>
<td>$u_R$</td>
<td>Between laboratory reproducibility</td>
</tr>
<tr>
<td>$u_o$</td>
<td>Operational or technical uncertainty</td>
</tr>
<tr>
<td>$u_d$</td>
<td>Distributional or Poisson uncertainty</td>
</tr>
<tr>
<td>$u_{\text{matrix}}$</td>
<td>Uncertainty arising from imperfect mixing of the laboratory sample</td>
</tr>
<tr>
<td>$u_c$</td>
<td>Combined uncertainty</td>
</tr>
<tr>
<td>$U$</td>
<td>Expanded uncertainty</td>
</tr>
</tbody>
</table>

### Expanded uncertainty

$$ U = 2 \times u_c $$

Coverage factor $k=2$
Units for uncertainty

Several units are used for uncertainty in microbiology. Example with standard uncertainty for 15 CFU.

<table>
<thead>
<tr>
<th>Unit</th>
<th>u</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU</td>
<td>5 CFU</td>
</tr>
<tr>
<td>%</td>
<td>30 %</td>
</tr>
<tr>
<td>( \ln )</td>
<td>( \ln 0.30 )</td>
</tr>
<tr>
<td>( \log_{10} )</td>
<td>( \log_{10} 0.13 )</td>
</tr>
</tbody>
</table>

NOTE The uncertainty given can be recalculated to other units.
**Uncertainty from in-house validation data**

<table>
<thead>
<tr>
<th>Component</th>
<th>General</th>
<th>ubio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision Between days</td>
<td>Intermediate precision</td>
<td>Operational + Distributional</td>
</tr>
<tr>
<td>Confirmation</td>
<td>-</td>
<td>Uncertainty of confirmation</td>
</tr>
<tr>
<td>Inhomogeneity</td>
<td>Repeatability</td>
<td>Matrix uncertainty</td>
</tr>
</tbody>
</table>

\[
u_c = \sqrt{u_o^2 + u_d^2}\]
Main components for uncertainty

Operational $u_o$ (technical)

Distributional $u_d$ (Poisson)

$$u_c = \sqrt{u_o^2 + u_d^2}$$

Only distributional uncertainty is needed for CFU < 10
Uncertainty due to subsampling

Poisson – distributional

(3) Fundamental variability

1 kg sample

10 subsamples

10 subsamples à 100 ml

possible distribution of contaminated particles in 10 subsamples

Distributional

\[ u_d = \sqrt{\frac{1}{n_c}} \times 100 \]

CFU = 1

\[ u_d = 100\% \]

CFU = 1

Contaminated particles
Standard uncertainty

Combined uncertainty (blue), Poisson (green) and operational (red) vs CFU
Main components for uncertainty

Operational $u_o$ (technical)

Distributional $u_d$

$$u_c = \sqrt{u_o^2 + u_d^2}$$
Estimate $u_0$ in %

Operational uncertainty is estimated as a difference

$u_0 = \sqrt{u_{SIR}^2 - u_d^2}$

$u_{SIR}$ Intra (within) laboratory reproducibility
Estimate $u_o$ in %

When operational uncertainty is small we can often not estimate it

\[
u_o = \sqrt{u_R^2 - u_d^2}
\]

$u_R$ Intra (within) laboratory reproducibility
Operational uncertainty - experiment

conditionally A

Sample portion 1

Initial suspension 1

(Homogenate)

Steps of analysis 1

conditionally B

Sample portion 2

Initial suspension 2

(Homogenate)

Steps of analysis 2

Laboratory sample

"Global approach"

Large differences condition A and B

30 duplicates are suitable

Calculate $u_R$ (sd) for $n_{c1}$ and $n_{c2}$
When operational uncertainty is small we can often not estimate it.
"Global", ISO 29201 (Example in $\log_{10}$ units)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>$C_1$</th>
<th>$C_2$</th>
<th>$\log(C_1)$</th>
<th>$\log(C_2)$</th>
<th>$s^2_R$</th>
<th>$u^2_d$</th>
<th>$u^2_o$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-4</td>
<td>5</td>
<td>8</td>
<td>0.6990</td>
<td>0.9031</td>
<td>0.0208</td>
<td>0.0290</td>
<td>-0.0082</td>
</tr>
<tr>
<td>2</td>
<td>-3</td>
<td>15</td>
<td>11</td>
<td>1.1761</td>
<td>1.0414</td>
<td>0.0091</td>
<td>0.0145</td>
<td>-0.0054</td>
</tr>
<tr>
<td>3</td>
<td>-4</td>
<td>11</td>
<td>19</td>
<td>1.0414</td>
<td>1.2788</td>
<td>0.0282</td>
<td>0.0126</td>
<td>0.0156</td>
</tr>
<tr>
<td>4</td>
<td>-6</td>
<td>21</td>
<td>39</td>
<td>1.3222</td>
<td>1.5911</td>
<td>0.0361</td>
<td>0.0063</td>
<td>0.0299</td>
</tr>
<tr>
<td>5</td>
<td>-5</td>
<td>68</td>
<td>45</td>
<td>1.8325</td>
<td>1.6532</td>
<td>0.0161</td>
<td>0.0033</td>
<td>0.0127</td>
</tr>
<tr>
<td>6</td>
<td>-4</td>
<td>151</td>
<td>203</td>
<td>2.1790</td>
<td>2.3075</td>
<td>0.0083</td>
<td>0.0011</td>
<td>0.0072</td>
</tr>
</tbody>
</table>

Mean: $0.0198$ $0.0111$ $0.0086$

$u_o^2(\log_{10}) = 0.0086$.
This can be converted to %, in this case $u_o = 21\%$. 
Reporting results

Operational $u_o$ is 21 %

Distributional $u_d$ depends on CFU

$$u_c = \sqrt{u_o^2 + u_d^2}$$

Confidence interval

$$U_{min} = n / \exp \left( \frac{2u_c}{100} \right)$$

$$U_{max} = n \times \exp \left( \frac{2u_c}{100} \right)$$

Operational is needed for CFU $\geq 10$
Calculation of interval
Example with operation. 15 %

Here is calculated an asymmetric interval (95 %)
Similar for MPN

<table>
<thead>
<tr>
<th>Count CFU</th>
<th>$u_o$ %</th>
<th>$u_d$ %</th>
<th>$u_c$ %</th>
<th>U %</th>
<th>$U_{min}$ CFU</th>
<th>$U_{max}$ CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>15</td>
<td>32</td>
<td>35</td>
<td>70</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>26</td>
<td>30</td>
<td>60</td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
<td>22</td>
<td>27</td>
<td>54</td>
<td>12</td>
<td>34</td>
</tr>
<tr>
<td>30</td>
<td>15</td>
<td>18</td>
<td>23</td>
<td>46</td>
<td>19</td>
<td>48</td>
</tr>
<tr>
<td>40</td>
<td>15</td>
<td>16</td>
<td>22</td>
<td>44</td>
<td>26</td>
<td>62</td>
</tr>
<tr>
<td>50</td>
<td>15</td>
<td>14</td>
<td>21</td>
<td>42</td>
<td>33</td>
<td>76</td>
</tr>
<tr>
<td>75</td>
<td>15</td>
<td>12</td>
<td>19</td>
<td>38</td>
<td>51</td>
<td>110</td>
</tr>
<tr>
<td>100</td>
<td>15</td>
<td>10</td>
<td>18</td>
<td>36</td>
<td>70</td>
<td>143</td>
</tr>
</tbody>
</table>
Additional uncertainty components

\[ u_c = \sqrt{u_d^2 + u_o^2 + u_{conf}^2 + u_{matrix}^2} \]

Take into account when \( > 1/3 \) of \( u_c \)

When laboratory perform the sampling also \( u_{samp} \)

For solids and viscous liquids

\[ u_{matrix} \]

Often uncertainty over 100 %

Use unit \( \log_{10} \)
Uncertainty approaches

GUM principles

- Definition of the measurand
- List of uncertainty components

Intralaboratory

Interlaboratory

Modelling

- Single lab validation/
  Global
- Interlaboratory validation
- Proficiency testing

Experimental approaches

based on …
Estimating $u_R$ ($s_R$ from proficiency testing) (NMKL 86)

The aerobic plate count* (APC) is intended to indicate the level of microorganism in a product.

Pooled $u_R = 0.18 \log_{10}$ or 41%
Reporting uncertainty

- Test results = 180 cfu/g
- $u_R = 41\%$
- $u_d$ – 20 CFU counted

- $\Sigma C = 20$ (dilution -1, 1 ml on 3 plates: 3 +8+7 colonies, dilution -2, 2 colonies)

- $u_d = \sqrt{\frac{1}{n_c}} \times 100 = \sqrt{\frac{1}{20}} \times 100 = 22\%$

$$u_c = \sqrt{u_d^2 + u_R^2} = \sqrt{22^2 + 41^2} = 47\%$$
Reporting result with confidence interval

180 cfu/g [82,395]

Where 82 – 395 is the asymmetric confidence interval
### Summary - reporting results

<table>
<thead>
<tr>
<th>CFU*</th>
<th>Report</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>Value and text</td>
<td>5 CFU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Result is an estimate</td>
</tr>
<tr>
<td>≥ 10</td>
<td>Value &amp; Uncertainty</td>
<td>20 CFU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>U = 54 %</td>
</tr>
<tr>
<td></td>
<td>Value and asymmetric confidence interval (95%)</td>
<td>20 CFU [12,34]</td>
</tr>
</tbody>
</table>

*CFU = colony forming units

**CFU ≥ 10**

1) Inform client

1) Uncertainty

or

1) Give an interval
New version of the Guide late 2022