Measurement Uncertainty

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INTERNATIONAL WORKSHOP & TRAINING

PROGRAM ON GOOD FOOD LABORATORY PRACTICES

15-19 November 2016

Conference Hall, Pilot Test House (PTH), Export Inspection Agency, Mumbai
What is Measurement Uncertainty?

“A parameter, associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand”

(ISO Guide) (Measurand in other terms is referred as Analyte.)

The number after the ±
Expressing Uncertainty of Measurement

How to express uncertainty of measurement

For example:

We might say that the length of a certain stick measures 20 centimetres plus or minus 1 centimetre, at the 95 percent confidence level.

This result could be written:

\[20 \text{ cm } \pm 1 \text{ cm}, \text{ at a level of confidence of 95\%.}\]

The statement says that we are 95 percent sure that the stick is between 19 centimetres and 21 centimetres long.
What is NOT a measurement/ calculation?

Concept

- **Mistakes** made by operators are not measurement uncertainties. They should not be counted as contributing to uncertainty. They should be avoided by working carefully and by checking work.

- **Tolerances** are not uncertainties. They are acceptance limits which are chosen for a process or a product.

- **Specifications** are not uncertainties. A specification tells you what you can expect from a product.

- **Accuracy (or rather inaccuracy)** is not the same as uncertainty. Unfortunately, usage of these words is often confused. Correctly speaking, ‘accuracy’ is a qualitative term (e.g. you could say that a measurement was ‘accurate’ or ‘not accurate’).
Why Uncertainty calculations? ----Requirement of ISO/IEC 17025

Clause 5.4.6

Clause 5.4.6 on “Measurement Uncertainty” as per ISO/IEC-17025:2005

5.4.6.1 A calibration laboratory, or a testing laboratory performing its own calibrations, shall have and shall apply a procedure to estimate the uncertainty of measurement for all calibrations and types of calibrations.
Testing laboratories must document and implement procedures for estimating uncertainty of measurement (refer to NABL document No. 141 for application of this clause).

All uncertainty components which are of importance in the given situation must be taken into account using appropriate methods of analysis when estimating the uncertainty of measurement.
Every measurement has an uncertainty associated with it, unless it is an exact, counted integer, such as the number of trials performed.

Every calculated result also has an uncertainty, related to the uncertainty in the measured data used to calculate it. This uncertainty should be reported either as an explicit ± value or as an implicit uncertainty, by using the appropriate number of significant figures.

The numerical value of a ± uncertainty value tells you the range of the result. For example a result reported as 1.23 ± 0.05 means that the experimenter has some degree of confidence that the true value falls in between 1.18 and 1.28.
Uncertainty of Measurement

- It is a parameter associated with the result of a measurement, that characterizes the dispersion of true values, which could reasonably be attributed to the measurand.

- **UNCERTAINTY IS ALWAYS WITH US**
- **ERRORS MAY BE CORRECTED**
- **UNCERTAINTY IS VITAL IN INTERPRETATING RESULTS OR DECIDING IF THEY ARE FIT FOR THE PURPOSE**
- **HOW CAN WE QUANTIFY UNCERTAINTY?**
The relationship of accuracy and precision may be illustrated by the familiar example of firing a rifle at a target where the black dots below represent hits on the target.

Good precision does not necessarily imply good accuracy. However, if an instrument is well calibrated, the precision or reproducibility of the result is a good measure of its accuracy.
The **error** of an observation is the difference between the observation and the actual or **true value** of the quantity observed. Returning to our target analogy, error is how far away a given shot is from the bull's eye. Since the true value, or bull's eye position, is not generally known, the exact error is also unknowable.

**Types of Errors**

Errors are often classified into two types: **systematic** and **random**

Systematic errors may be caused by fundamental flaws in either the equipment, the observer, or the use of the equipment. For example, a balance may always read 0.001 g too light because it was zeroed incorrectly. In a similar vein, an experimenter may consistently overshoot the endpoint of a titration because he/she is wearing tinted glasses and cannot see the first color change of the indicator.
Errors

• **Random errors** arise from unpredictable variations, for example:
  - Uncontrolled environmental conditions
  - Inherent instability of the measuring equipment
  - Personal judgment of the operator or observer

• **Systematic errors** are:
  - Reported in calibration certificate of instrument and of reference standards, purity and COA values.
    These can be isolated and minimized.

Quantification of both are possible.
DEFINITION: VARIATION

The spread or scatter of data around the average.
Accuracy, Precision & Bias

Analytical Measurement Signal

Accuracy = Measured value – Standard value

Mean value

Standard value

Measured values at fixed times
Understanding Variability

TWO TYPES OF VARIATION

1. Random (Common Causes)
   - Inherent in all processes
   - Unpredictable
   - To reduce must change the process
   - Vary in a completely non reproducible way from measurement to measurement
   - Can be treated statistically

2. Non-random (Special Causes)
   - Due to special or assignable causes
   - Predictable
   - Simpler to eliminate
   - Frequently difficult to discover
   - Can result in high precision, but poor accuracy
Related Terms and Phrases in MU calculations

- Accepted Reference Value- A value that serves as an agreed upon reference for comparison
- Accuracy of Measurement- The closeness of agreement between a test result and accepted reference value
- Arithmetic Mean- The sum of values divided by number of values
- Measurand- A quantity subject to measurement (analyte)
Continued

• Repeatability- Test results are obtained by the same method on identical test items in the same lab by same operator by same equipment with in short interval of time

• Reproducibility- test results are obtained with the same method on identical test items in different labs by different operators using different equipments
Repeatability

• Closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement (Test results are obtained by same method on identical test items in the same lab by same operator by same equipment with in short interval of time)
Reproducibility

• Closeness of the agreement between the results of measurements of the same measurand carried out under changed conditions of measurement (test results are obtained with the same method on identical test items in different labs by different operators using different equipments)
ISO Guide approach on MU calculations

• Specify the measurand
  – including complete equation

• Quantify significant uncertainties in all parameters
  – A: from statistics of repeated experiment
  – B: by any other means (theory, certificates, judgement...)

• Express as standard deviation

• Combine according to stated principles
• Standard uncertainty- Uncertainty of the result of a measurement expressed as a standard deviation
• Type A Uncertainty- Uncertainty evaluated by the statistical analysis of series of observations
• Type B Uncertainty- Uncertainty evaluated by means other than statistical analysis of series of observations
Standard deviations: Square root of Variance $V = S^2$

$$S^2 = \frac{\sum (x_i - \bar{x})^2}{n - 1}$$

Histogram
Standard Uncertainty

- Standard uncertainty is defined as one standard deviation.

\[ S_{X} = \frac{S}{\sqrt{n}} \]

Type A Uncertainty = From repeated results: Uniform distribution
Normal distribution also standard uncertainty

Type B Uncertainty = From certificates and literature.
- Rectangular: Divisor: \( \sqrt{3} \)
- Triangular: Divisor: \( \sqrt{6} \)

Coverage factor: 95% : Factor = 2
Types of Probability distributions associated with variables

• Normal Distribution:- is used when an estimate is made from repeated observations of a randomly varying process. An uncertainty is then given in the form of a Standard Deviation; Standard deviation of the mean (s/x mean), Relative standard- deviation (RSD), or coefficient of variation\%. An uncertainty for a variable with given 95% (or other) confidence interval is given as:
  \[ u(x) = \frac{k}{2} \] (for 95% probability)
  \[ u(x) = \frac{k}{3} \] (for 99.7% probability).
Types of Probability distributions associated with variables

- (B) Rectangular Distribution:- is used when the information is taken from a certificate or specifications, which gives associated uncertainty without specifying the level of confidence.

Example 1:- Concentration of the calibration standard is quoted in the certificate as $= 1000 \pm 2 \text{ mg/l}$.

Example 2:- purity of cadmium is quoted as $99.99 \pm 0.1 \%$.

These are cases of uniform or rectangular distribution. Here the distributions are such that individual units (purity) probability are more likely to be near extremes.

Therefore, an estimate is made by applying rectangular distribution. Hence, assumed standard uncertainty $= \frac{\text{half width}}{\text{Sq . root of 3}}$. 
Types of Probability distributions associated with variables

• ( C) Triangular Distribution: is used, when the distribution is such that occurrence of mean value from range is most probable. Therefore, an estimate is made by applying triangular distribution.

Example: - Manufacturer quoted volume of Volumetric flask of 100ml
   As 100 ± 0.1 ml (Tolerance of 0.1ml)
   In this case a nominal value is most probable.
   Therefore, an estimate is made by applying triangular Distribution.
   Hence, assumed standard uncertainty = half width/ Sq . root of 6.
Combined Uncertainty

• The component uncertainties are combined to produce an overall uncertainty.
• Some of the uncertainties may cancel each other out.
• Some may be interdependent.
• Type A and Type B Uncertainty factors.
• When combining all factors need to be converted into similar unit of measurement eg. %; gm; ml; °C; unit less.

\[ U_c = \sqrt{U_A^2 + U_B^2} \]
Combining uncertainties (ISO)

- Standard deviations
- Established error propagation theory

\[\sqrt{u_1^2 + u_2^2}\]
Expanded Uncertainty

- In some cases, the combined standard uncertainty needs to be multiplied by the appropriate coverage factor.

\[ U_E = k \cdot U_c \]

Where, \( k \) is normally 1.96 or 2 for 95% confidence level
Sources of Uncertainty in Chemical testing

- Sampling: the sample measured may not represent the defined measurand
- Incomplete extraction and/or pre-concentration of the measurand, contamination of the measurement sample, interferences and matrix effects
- Inadequate knowledge of the effects of environmental conditions on the measurement procedure or imperfect measurement of environmental conditions
- Cross-contamination or contamination of reagents or blanks
- Type B factors: Inadequate information
Continued

- Personal bias in reading analogue instruments
- Uncertainty of weights and volumetric equipment
- Instrument resolution or discrimination threshold
- Values assigned to measurement standards and reference materials
- Values of constants and other parameters obtained from external sources and used in the data-reduction algorithm
- Approximations and assumptions incorporated in the measurement method and procedure
- Variations in repeated observations of the measurand under apparently identical conditions.
Sources of Uncertainty in Recovery Estimation

- Repeatability of the recovery experiment
- Uncertainties in reference material values
- Uncertainties in added spike quantity
- Poor representation of native analyte by the added spike
- Poor or restricted match between experimental matrix and the full range of sample matrices encountered
- Effect of analyte/spike level on recovery and imperfect match of spike or reference material analyte level and analyte level in samples
Uncertainty Components

Determination of Acesulfam-K

- Method recovery
- Sample recovery
- Precision
- Calibration
- Stock concentration
- Sample volume
- Final volume
- TOTAL

Contribution to Uncertainty (RSD)
Method of Stating Test Results

• When reporting the test results and its uncertainty, the use of excessive number of digits should be avoided.

• Unless otherwise specified, the test result should be reported together with the expanded uncertainty at 95% level of confidence in the following manner:

Measured value: 100.1 (Units)
Uncertainty of measurement: +/- 0.1 (Units)
Step by step Process of MU calculations

• 1. Define the analyte which is being calculated with the formula for calculation.
• Look for all probable causes of MU sources.
  • Repeat/ Reproduce analysis data: Type A
  • Data From other sources involved: Type B
• Create a Fish Bone diagram to understand the probable sources and sub sources for MU.
• Generate Data on both Type A and Type B.
FISH BONE DIAGRAM

- Stock Purity
- Standards/ CRM
- Volumetric flasks
- Pipettes
- Volumetrics
- Uncertainty of Pesticides
- Sample preparation
- Precision
- Weighment
- Standard Calibration
Type A calculations

• Perform repeat/ reproducible analysis on parameter being evaluated.
  – $S_1; S_2; S_3; S_4; S_5; S_6; S_7; S_8; S_9; S_{10}$
  – Calculate Average of above, $Av = \Sigma (S_1 + S_2 + \ldots + S_{10} / n)$
  – Calculate Standard deviation, $SD = STDV(S_1 + S_2 + \ldots + S_{10})$
  – Calculate Standard uncertainty, $SU = SD / \sqrt{n}$
  – Calculate Relative standard uncertainty, $RSU$ (repeatability); $UA_1 = SU / Av$; This is a unit less entity. Why ????

Also called as relative Type A uncertainty.
Calculation of Type B uncertainties

Example Electronic Balance

• From calibration certificate: External agencies
  Value for specific range (weights) M, @95 % CL = x units.
  \[ U_B = \frac{x}{2}, \text{ or } 1.96 \text{ units} \]
  \[ UB_1 = \text{RSD} = \frac{x}{2} \times M \text{ unitless.} \]

• From balance accuracy: say 0.12 % (Supplier manual)
  Error = \( \frac{0.12}{100} = 0.0012 \text{ unitless.} \)
  \[ UB_2: \text{Assuming rectangular distribution:} \frac{0.0012}{\text{SQRT}(3)} \]

• From Resolution factor: Say 0.1 mg
  Assuming rectangular distribution: \( \frac{0.1}{\text{SQRT}(3)} \)
  \[ UB_3: \text{Relative Uncertainty} = \frac{0.1}{\text{SQRT}(3)} \times M \text{ in mg}. \text{ unitless.} \]

• Balance Uncertainty \( U_{Bal} = \text{SQRT} \{ (UB_1)^2 + (UB_2)^2 + (UB_3)^2 \} \)
Type B continued

Example Volumetric Pipette , say 10 ml

*From calibration certificates or repeat analysis*

For Repeat analysis follow Type A calculations. \(UBP_1\)

*From calibration certificates say @ 95 %, follow process for Balance UB\(_1\)*

\[ UB = UB P_1 \text{ (unitless)} \]

**Uncertainty due to tolerance or readability say 0.1 ml.**

*Assuming triangular distribution , RSU, UBP2 = 0.1/SQRT(6)* 10 unitless

**Uncertainty due to temperature, coef. of expansion: 2.1 \times 10^{-4}**

@ 20C. Working temp say 25C ; factor = 5 * 2.1 *10 \(-4\)

*Assuming triangular distribution and 25C working temp. RSD;*

\[ UB P_3 = 5* 2.1 *10^{-4} / 25* SQRT(6). \text{ Unitless} \]

**Pipette Uncertainty U Pip = SQRT \{ (UBP_1)^2 + (UBP_2)^2+ (UBP_3)^2 \}**
Type B uncertainty continued

Example preparation of standards

• Factors: balance uncertainty; volumetric uncertainty, purity of standard. May be temperature of prepn.

• Balance Uncertainty $U_{Bal} = \sqrt{(UB_1)^2 + (UB_2)^2 + (UB_3)^2}$

• Flask (100 ml) Uncertainty $U_{Pip} = \sqrt{(UBP_1)^2 + (UBP_2)^2 + (UBP_3)^2}$

• Standard purity uncertainty:
  – From certificate say @ 95%: 99.5% purity
    $U_{StandUs} = 99.5/100*2$ unit less
  – From purity from COA = 99.5%
    $U_{StandUs} = 99.5/100*\sqrt{3}$

• Standard Reag. $Un = \sqrt{(UBal)^2 + (UVol)^2 + (Us)^2}$
Example sample preparation & recovery

- **Factors:** balance uncertainty; volumetric uncertainty,
  Repeat recovery data. May be temperature of prepn.

- Balance Uncertainty $U_{Bal} = \sqrt{U_{B1}^2 + U_{B2}^2 + U_{B3}^2}$
- Flask (100 ml) Uncertainty $U_{Pip} = \sqrt{U_{P1}^2 + U_{P2}^2 + U_{P3}^2}$
- **Recovery studies from Validation records (repeat analysis):**
  - From recovery studies: 10 determinations say at 50 ppm;
    average value say 48.5 ppm; Stand Dev. = 0.5 ppm
    $U_{Stand} = \frac{0.5}{\sqrt{10}}$
    RSD Uncertainty, $U_{rec} = \frac{0.5}{\sqrt{10}} \times 0.5$

- **Standard recovery** $U_n = \sqrt{U_{Bal}^2 + U_{Vol}^2 + U_{rec}^2}$
Combined (CU) & Expanded (Exp U) Uncertainty

CU = SQRT \{(All \ type \ A \ Uns.)^2 + (All \ type \ B \ Uns.)^2\}

Expanded Uncertainty = CU \times \text{Factor of } 2 \text{ for } 95\% \text{ confidence level}

These are all unit less.

Expanded Un. with unit = Exp Un \times \text{Average}

Result = \text{Average} \pm \text{Ex Un} \text{ in units.}
Measurement of Uncertainty

Microbiological Parameters
References & Guides

- US FDA BAM
- ISO 19036

Presumption: Type B, which is also considered as Bias, is insignificant as compared to repeat/reproduced data (Type A values). Hence this factor is ignored during MU calculations for biological system.
Step 1 – Transform the raw data by taking the log base 10 value (log10) of the data (column 2, 4).

Step 2 – Calculate the overall mean of 40 results in columns 2 and 4, this is 2.2626

Step 3 – Calculate the difference between the transformed replicates (column 5).

Step 4 – Square the differences between the transformed replicates (column 6).

Step 5 – Add the differences together (column 6) and divide by 2n, where n = the total number of pairs of duplicates (for this example n = 20) to get 0.010103.

Step 6 – Take the square root of the result in step 5; this equals the pooled reproducibility standard deviation, which is 0.100514.

Step 7 – Convert this standard deviation into a relative standard deviation by dividing by the mean from Step 2, which produces an RSD of 0.044425.
Step 8 – To provide a higher range of values that cover what is likely to be observed in other samples, apply the coverage factor ($k=2$ for 95% coverage) to the RSD to get the estimate of the expanded uncertainty, $0.08885$ (Note this is a log10 value).

Step 9 – To calculate the uncertainty for any subsequent laboratory result, the result is first converted to the log base 10 value (log10), multiplied by $0.08885$ and then this expanded uncertainty is added and subtracted from the log value.

Step 10 – To estimate the MU of a sample, convert the log value for the sample measurement back to CFU for the reported result. This is accomplished by taking the anti-log of each of the endpoints of the interval (anti-log of $x = 10^x$).

*NOTE: By convention the results are always rounded down” for the value at the lower end of the range and always “rounded up” for the value at the upper end. In this way the minimal 95% coverage is preserved.*

For example, for a result of 150 CFU: $150$ in log10 = 2.1761 and the expanded uncertainty in log counts is 2.1761 times 0.0998 = 0.2172. Adding and subtracting from 2.1761 gives an interval from 1.9589 to 2.3933; transforming back to counts: $10 \times 1.9589 = 90.97$, and $10 \times 2.3933 = 247.33$. Therefore the uncertainty interval is 90 to 248 CFU. Results can also be just mentioned as log values.
Replicates generated under reproducibility conditions

<table>
<thead>
<tr>
<th>Raw Data (actual CFU recovered) – First Replicate</th>
<th>Raw Data (actual CFU recovered) – Second Replicate</th>
<th>Log10 Value</th>
<th>Log10 Value</th>
<th>Difference between Replicates (Log10 Value)</th>
<th>Difference between Replicates Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>131</td>
<td>142</td>
<td>2.1173</td>
<td>2.1523</td>
<td>-0.0350</td>
<td>0.00123</td>
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<td>69</td>
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<td>45</td>
<td>55</td>
<td>1.6021</td>
<td>1.7404</td>
<td>-0.1383</td>
<td>0.01913</td>
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</table>
## Uncertainty for Micro results

<table>
<thead>
<tr>
<th>S No</th>
<th>Counts in replicate</th>
<th>Data in log 10</th>
<th>Counts in replicate</th>
<th>Data in log 10</th>
<th>Difference between replicate</th>
<th>Square the difference</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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<td>2.3617</td>
<td>242</td>
<td>2.3838</td>
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<tr>
<td>2</td>
<td>169</td>
<td>2.2779</td>
<td>190</td>
<td>2.2788</td>
<td>-0.0090</td>
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<td>145</td>
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<td>15</td>
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<td>212</td>
<td>2.3263</td>
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<tr>
<td>16</td>
<td>207</td>
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<td>289</td>
<td>2.4609</td>
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<tr>
<td>17</td>
<td>145</td>
<td>2.1614</td>
<td>162</td>
<td>2.2095</td>
<td>0.00232</td>
<td>2.1761</td>
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<tr>
<td>18</td>
<td>198</td>
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<tr>
<td>19</td>
<td>340</td>
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<td>320</td>
<td>2.5051</td>
<td>0.0263</td>
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<td>20</td>
<td>240</td>
<td>2.3802</td>
<td>220</td>
<td>2.3424</td>
<td>0.0378</td>
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Sum

<table>
<thead>
<tr>
<th></th>
<th>2.2638</th>
<th>2.2614</th>
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<tbody>
<tr>
<td>Ave</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sq root</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooled reproducible Std. deviation</td>
<td>2.2626</td>
<td>0.100514011</td>
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<tr>
<td>RSD</td>
<td>183.0443</td>
<td>150.00</td>
</tr>
<tr>
<td>Expanded Factor 2</td>
<td></td>
<td>0.088849956 0.0998</td>
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</tbody>
</table>

Range of uncertainty

<table>
<thead>
<tr>
<th></th>
<th>2.2626* 0.08884 0.201009 2.1761*0.0998 0.2172</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>183.5533 2.0525 2.4797</td>
</tr>
<tr>
<td>Result</td>
<td>183.0000 149 225</td>
</tr>
</tbody>
</table>
For example, for a result of 150 CFU: 150 in log10 = 2.1761 and the expanded uncertainty in log counts is 2.1761 times 0.0998 = 0.2172. Adding and subtracting from 2.1761 gives an interval from 1.9589 to 2.3933; transforming back to counts: 101.9589 =90.97, and 102.3933 = 247.33. Therefore the uncertainty interval is 90 to 248 CFU. Results can also be just mentioned as log values.