It's Not That Scary:
Measurement Uncertainty, Validation and Verification for Micro

Disclaimer:
This presentation is intended to introduce you to general principles based on current guidance and suggested practices from government agencies and industry groups. As with any overview program, these materials and our guidance are general, and you should always consult your own advisors as appropriate for your circumstances.

Thank you – To Gil Dichter for his help in producing this presentation.

Measurement Uncertainty

Measurement Uncertainty (MU) in Micro

What is Measurement Uncertainty?

How confident are you about the accuracy of the measurement you've made?

What factors could have influenced your measured quantity? How do you control for those factors?

How do you numerically express Measurement Uncertainty?

Definition of Measurement Uncertainty

No measurement is exact.
When a quantity is measured, the outcome depends on the measuring system, the measurement procedure, the skill of the operator, the environment, and other effects. Even if the quantity were to be measured several times, in the same way and in the same circumstances, a different measured value would in general be obtained each time, assuming the measuring system has sufficient resolution to distinguish between the values.

https://en.wikipedia.org/wiki/Measurement_uncertainty

Factors that affect Measurement Uncertainty (MU) in Micro

- Method used
- Media supply variation
- Sample collection process
- Incubation temperature
- Pre-processing samples (filtration, dilution)
- Not following SOP
- Media storage

- Others factors?

- Some of these things we have partial control over, others we do not!.

Addressing Measurement uncertainty factors

- Method used
  - Insure the use of Standardized or 3rd party validated methods
- Media supply variation
  - Use appropriate Control Charts to track media performance
- Sample collection process
  - Train collectors, explicit COC forms/instructions, provide gloves
- Incubation temperature
  - Calibrate incubators with certified company
- Pre-processing samples (filtration, dilution etc.)
  - Avoid contamination, training, on-going DOC
- Not following SOP
  - On-going DOC, internal audit checks
- Media storage
  - Insure SOPs are followed, training, internal audit checks

Use of Standard Deviation to express MU

- Take several readings of your sample, more is always better
- Perform calculation to determine the standard deviation (expressed uncertainty, see reference)
- Your specific measurement for each measurement, using example calculation to the right, is then:
  - 16 ± 2.1, result could be 13.9 – 18.1
  - 19 ± 2.1, result could be 17.9 – 21.1
  - 18 ± 2.1, result could be 15.9 – 20.1

etc….

Example 2. Calculating the estimated standard deviation of a set of values

It is rarely convenient to calculate standard deviations by hand, with pen and paper alone. But it can be done as follows:

Suppose you have a set of n readings (let's use the same set of 10 as above). Start by finding the average:

For the set of readings we used before, 16, 19, 18, 16, 17, 19, 20, 15, 17 and 13, the average is 17.

Next, find the difference between each reading and the average,

i.e. -1 +2 +1 -1 0 +2 +3 -2 0 -4,

and square each of these,

i.e. 1 4 1 1 0 4 9 4 0 16.

Next, find the total and divide by n-1 (in this case n is 10, so n-1 is 9), i.e.

$$\frac{1+4+1+1+0+4+9+4+0+16}{9} = \frac{40}{9} = 4.44.$$

The estimated standard deviation, s, is found by taking the square root of the total, i.e.

$$s = \sqrt{4.44} = 2.1$$

(correct to one decimal place).

http://publications.npl.co.uk/npl_web/pdf/mgpg11.pdf

Use of Confidence Interval tables to express MU

Pre-determined tables of calculated confidence intervals when using IDEXX Quanti-Tray

51-Well Quanti-Tray® MPN Table

Number of wells giving positive reaction per 100 mL sample	Most Probable Number (MPN)	95% Confidence Limits	
		Lower	Upper
0	<1	0.0	3.7
1	1.0	0.3	5.6
2	2.0	0.6	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5

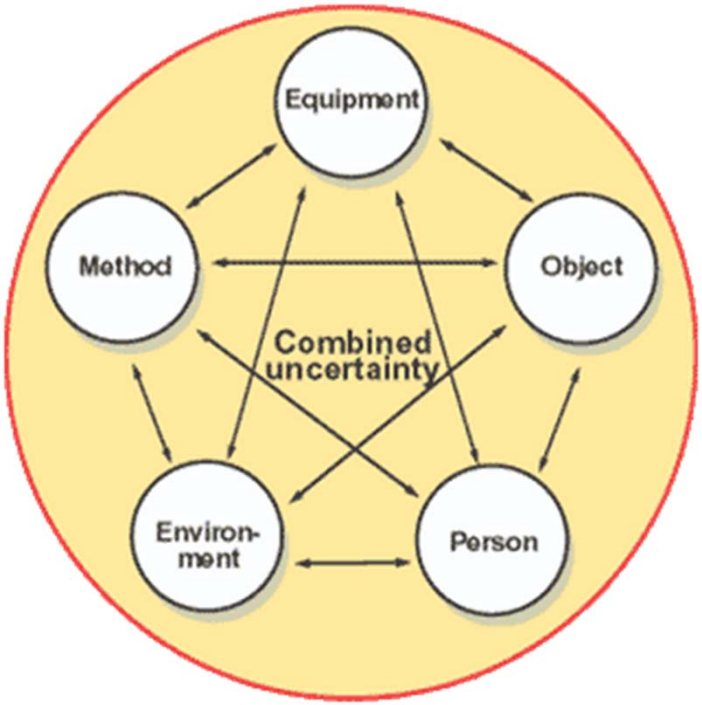
Pre-determined tables of calculated confidence intervals when using *Standard Methods* 9221

TABLE 9221:III. MPN INDEX AND 95% CONFIDENCE LIMITS FOR ALL COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN TEN 10-mL PORTIONS ARE USED

No. of Tubes Giving Positive Reaction Out of 10 (10 mL Each)	MPN Index/ 100 mL	95% Confidence Limits (Exact)	
		Lower	Upper
0	<1.1	—	3.4
1	1.1	0.051	5.9
2	2.2	0.37	8.2
3	3.6	0.91	9.7
4	5.1	1.6	13
5	6.9	2.5	15
6	9.2	3.3	19
7	12	4.8	24
8	16	5.8	34
9	23	8.1	53
10	>23	13	—

Summary
Mitigating Measurement Uncertainty

1. Every measurement has uncertainty
2. Understand the factors that can affect MU and do your best to address them
3. No test provides 100% accuracy
4. Leverage training, competent/accredited calibration services
5. Use Standard Deviation or 95% CI tables to demonstrate and communicate MU



Method Validation

Validation
Why validation is done and by whom

- Validation is performed by the method developer
  - Whether it's a company or home-brew test developed in a laboratory
- Validation is performed by a 3rd party, *not* the method developer
- Validation results confirm the performance characteristics of a method, including, but not necessarily limited to

1. Sensitivity
2. Specificity
3. False positives and false negatives
4. Repeatability
5. Reproducibility
6. Measurement of Uncertainty
7. Statistical analysis of data

Validation parameters
Definitions of each according to ISO 13843

1. **Sensitivity:** fraction of the total number of positive cultures or colonies correctly assigned
2. **Specificity**: fraction of the total number of negative cultures or colonies correctly assigned
3. **Selectivity** - ratio of target colonies to the total number of colonies in the sample volume
4. **False positives** and **false negatives**, as determine through confirmatory tests
5. **Repeatability:** agreement in counts obtained by repeated counting by one analyst
6. **Reproducibility:** agreement in counts obtained by repeated counting by two or more analysts
7. **Measurement of Uncertainty**: uncertainty of the result of a measurement, expressed as a standard deviation
8. **Efficiency:** the fraction of wells correctly assigned

Calculating Validation Characteristics of a method

a = number of positive wells found to contain *target* (true positives)

b = number of negative wells found to contain *target* (false negatives)

c = number of positive wells found not to contain *target* (false positives)

d = number of negative wells found not to contain *target* (true negatives)

The sensitivity, specificity, selectivity, false positive rate and false negative rates are calculated as follows:

$$\text{Sensitivity} = a / (a+b)$$
$$\text{Specificity} = d / (c+d)$$
$$\text{Selectivity} = \log_{10} [(a+c) / (a+b+c+d)]$$
$$\text{False positive rate} = c / (a+c)$$
$$\text{False negative rate} = b / (b+d)$$
$$\text{Efficiency} = (a+d) / (a+b+c+d)$$

Calculating Validation Characteristics of a method

ISO/TR 13843 provides guidance on the assessment of counting repeatability and reproducibility using relative standard deviations of repeated counts.

It also recommends that, when using pure cultures, RSDs should ideally be less than 0.02 (i.e. not more that 2 % deviation).

Sensitivity	98%
Specificity	> 99%
Selectivity	- 0.18
False positive rate	< 0.01%
False negative rate	4.2%
Efficiency	> 99%
Repeatability	< 0.01
Reproducibility	< 0.01

Example data from ISO 13843 report using Legiolert™

Validation Summary

- Validation is a characterization of a method's performance
- It is performed by a 3rd party on behalf of the method developer
- The characteristics to be determined should include: sensitivity, specificity, selectivity, FP/FN, R&r, efficiency
- Resulting data are complied in a report, which should be made available to those who want to adopt the method, to regulatory bodies for inclusion in regulations or to standard method compendia organizations for inclusion in standards

Method Verification

Verification isn't the same as method validation

- Verification of a method's performance is performed when a laboratory is changing or adding a method

- The goal of verification is a one-time determination by the laboratory to confirm (verify) a subset of the method's validation performance outcomes

Laboratory verification parameters

Comparison of a new method vs. an approved method could include these checks:

a. Parallel evaluation of results between the current and proposed methods, typically 10 – 20 split samples

b. QC samples - range from low, mid and high

c. Repeatability, as described in this presentation

d. Reproducibility, as described in this presentation

e. Analysis of data into a report that is kept by QA

Verification Summary

- Verification is performed by the laboratory to confirm the performance characteristics of the method (its' Validation data)
- It is done when a new method is being brought into the laboratory for use
- Choose a few performance characteristics to verify (Specificity, selectivity, repeatability etc.)
- Split samples can be field (natural) samples or spiked with QC strains
- Assess the data, compare to method Validation data
- Prepare a report that is kept by QA