

*A Simple Microbiologist's View on
Estimation of Uncertainty of
Measurement*



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Objectives

- ☛ To understand what uncertainty of measurement means in simple language
- ☛ To review and understand the implications of SCC/CAEAL's policy and position on uncertainty of measurement, particularly for environmental microbiology laboratories
- ☛ To suggest procedures that environmental microbiology laboratories can use for estimating the uncertainty of measurement (MU)
- ☛ To open a dialogue on the application of MU

Uncertainty of Measurement

(VIM 3.9)

- ☛ Parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand
- ☛ Degree of Confidence in an analytical result
- ☛ Variance or Standard Deviation of the result
- ☛ Precision of the result

Excerpts from CAEAL Policy on Uncertainty P19 Rev 1.4

- 1 Laboratories accredited under the joint SCC-CAEAL Accreditation Program for Environmental Laboratories shall fulfil the requirements of CAN-P-4D (ISO/IEC 17025) with respect to the estimation of uncertainty of measurement associated with environmental testing for those tests which produce numerical results.
 - ☛ i.e. Does not apply to qualitative analyses (yes/no outcomes)

Excerpts from CAEAL Policy on Uncertainty P19 Rev 1.4

- Beginning with the 2003 assessment year, laboratories shall demonstrate their implemented use of adequate procedures for their estimation of the uncertainty of measurement associated with their accredited tests and shall have begun reporting the estimates (as expanded standard uncertainties) in accordance with the requirements of CAN-P-4D

Requirement from CAN-P-4D

5.4.6 Estimation of uncertainty of measurement

- ☛ 5.4.6.2 Testing laboratories shall have and shall apply procedures for estimating uncertainty of measurement.*

Requirement from CAN-P-4D

5.4.6 Estimation of uncertainty of measurement (5.4.6.2 cont'd)

- ☛ In certain cases the nature of the test method may preclude rigorous, metrologically and statistically valid, calculation of uncertainty of measurement. In these cases the laboratory shall at least attempt to identify all the components of uncertainty and make a reasonable estimation, and shall ensure that the form of reporting of the result does not give a wrong impression of the uncertainty.*

*Factors that Influence the Precision of
Quantitative Microbiological Results and
Control Points*

SAMPLING

- C Source of sample
- C Method of sampling
- C Transportation Time and Temperature of sample
- ☛ Storage Time and Temperature of sample after receipt until analysis

*Factors that Influence the Precision of
Quantitative Microbiological Results and
Control Points*

METHOD OF ANALYSIS

- C Source (SMEWW, AOAC, ASTM, in-house)
- C Level of Performance Verification Or Validation

*Factors that Influence the Precision of
Quantitative Microbiological Results and
Control Points*

CULTURE MEDIA AND REAGENTS

- C Formulation specifications
- C Preparation protocols
- C Water quality
- C Performance verification
- C Storage conditions and shelf-life

*Factors that Influence the Precision of
Quantitative Microbiological Results and
Control Points*

ANALYTICAL PROCEDURE

- C Sample Homogenization/mixing
- C Subsampling
- C Preparing And Dispensing Dilutions
- C Inoculation Technique e.g. Filtration Technique
- C Incubation Conditions
- ☛ Reading, Interpreting And Reporting Results
- ☛ Impact of Microbial Density

*Factors that Influence the Precision of
Quantitative Microbiological Results and
Control Points*

EQUIPMENT

- C Maintenance
- C Calibration
- C Repair

*Factors that Influence the Precision of
Quantitative Microbiological Results and
Control Points*

PERSONNEL

C Hiring

☛ Validating & Maintaining Competency

Requirement from CAN-P-4D

☛ 5.4.6 Estimation of uncertainty of measurement (5.4.6.2 cont'd)

☛ Reasonable estimation shall be based on knowledge of the performance of the method and on the measurement scope and shall make use of, for example, previous experience and validation data.

Excerpts from CAEAL Policy on Uncertainty P19 Rev 1.4

- ☛ CAEAL supports the determination of estimates uncertainties through the use of experimental data such as that from routine laboratory QA/QC work (duplicates, reference material usage, method validation studies, and proficiency testing (PT) and other interlaboratory programs, for example).
- ☛ Referred to as a Type A

CAEAL Guidelines for Using the Type A Approach

- ☛ Using the method SOP and the final result-calculation equation, identify and list all potential sources of uncertainty.
- ☛ Identify and compile recent laboratory repeat analysis and PT data that is available.
- ☛ Match each repeat data set with those sources of uncertainty that are likely to have varied during the collection of the repeat data and identify double counted sources of uncertainty.
- ☛ Estimate the magnitude of any source of uncertainty that is not varied during the collection of any of the repeat data sets.

CAEAL Guidelines for Using the Type A Approach (cont'd)

- ☛ Tabulate each source of uncertainty and its associated SD, and/or relative SD (RSD) derived from the repeat data set(s) matched to it, or from the estimate made. Eliminate double counted sources.
- ☛ Using only those SDs that are 1/3 or more the size of the largest individual SD, calculate the combined standard uncertainty using standard propagation of error rules (the square root of the sums of squares of SDs known as the “root sum of squares” - RSS).

CAEAL Guidelines for Using the Type A Approach (cont'd)

- ☛ Apply the appropriate coverage factor 'k'.
- ☛ Report the result with the expanded uncertainty and with a description of how the uncertainty was calculated.
- ☛ Measurement uncertainty shall be expressed as a combined Standard Deviation (SD) with the same units as those of the measurand.

Excerpts from CAEAL Policy on Uncertainty P19 Rev 1.4

- ☛ The expanded standard uncertainty shall be calculated to give a confidence level of 95% using an expansion factor 'k' of:
 - $k = 2$ when n is 30 or more (n = number of observations from which the SD is calculated).
 - k = the appropriate (95% confidence level) Student distribution 't' (two tailed) factor for $n < 30$ for $n-1$ degrees of freedom.
- ☛ Now isn't that simple to understand and implement?

Measures of Spread or Dispersion

☛ Variance = S^2

$$\text{☛ } S^2 = \frac{(X_1 - \bar{X})^2 + (X_2 - \bar{X})^2 + \dots + (X_n - \bar{X})^2}{n - 1}$$

☛ Standard Deviation = $S = \sqrt{S^2}$

☛ Relative Standard Deviation (CV%) = $S/\bar{X} \times 100$

☛ 95% confidence interval for \bar{X} (population mean):

$\bar{X} \pm (k \times s/\sqrt{n})$ (estimate of the uncertainty of measurement)

Estimating Measurement of Uncertainty

- ☛ Dataset 1: 100, 90, 110, 80, 120
- ☛ Calculate the Mean, Standard Deviation and the estimate of the uncertainty of measurement (95% confidence interval of the mean)

Dataset 1

☛ Mean: $(100+90+110+80+120)/5 = 100$

☛ Variance:

- $(100-100)^2 = 0$

- $(100-90)^2 = 100$

- $(100-110)^2 = 100$

- $(100-80)^2 = 400$

- $(100-120)^2 = 400$

☛ Total = 1000

☛ $S^2 = 1000/(5-1) = 250$

☛ Standard Deviation (S) = 15.8

Data Set 1
Calculation of the Uncertainty of
Measurement and Relative Standard
Deviation

☛ Mean = 100

☛ n = 5

☛ S = 15.8

β MU $0 \pm (k \times s//n)$

☛ MU $100 \pm (2.77 \times 15.8//5) = 100 \pm 19.5$

☛ Relative Standard Deviation (RSD) or CV =
 $15.8/100 \times 100 = 15.8\%$

☛ Note: 2.77 is from t distribution with (n-1) 4 df at 95% confidence

LOG Transformation

- ☛ Most statistical tests require constant variance and normality.
- ☛ This presents a problem since bacterial counts often have a Poisson distribution and variance is not constant.
- ☛ Solution: make a log transformation of the bacterial count.
- ☛ $\text{Log}_{10} X$ (where X is the original bacterial count) will have approximately constant variance and will be approximately normally distributed.

Dataset 1

- ☛ 100, 90, 110, 80, 120
- ☛ Transform the Data into Log_{10}
- ☛ Calculate the Mean Log_{10} , Standard Deviation and Uncertainty of Measurement

Dataset 1

Arithmetic	Log_{10}	$(0-x)^2$
100	2.00	0.00
90	1.95	0.00
110	2.04	0.00
80	1.90	0.01
120	<u>2.08</u>	<u>0.01</u>
Sum:	9.97	0.02
Mean:	1.99	N/A
Variance:		0.005
Standard Deviation:		0.07

Data Set 1
Calculation of Estimate of Uncertainty of
Measurement and Relative Standard
Deviation

☛ $\text{Log}_{10} \text{ Mean} = 1.99$

☛ $n = 5$

☛ $S = 0.07$

$\beta \text{ MU } 0 \pm (k \times s//n)$

☛ $\text{MU } 1.99 \pm (2.77 \times 0.07//5) = 1.99 \pm 0.087$

☛ Relative Standard Deviation (RSD) or CV =
 $0.07/1.99 \times 100 = 3.5\%$

☛ Note: 2.77 is from t distribution with (n-1) 4 df at 95% confidence

Estimate of Uncertainty of Measurement
Pour Plate Method -
Calculating colonies/ml

	Dilution (10 ^r)		
	-1	-2	-3
	—	—	—
Rep 1	260	22	4
Rep 2	200	32	3

1. SMEDP Rules:

$$\text{Colonies/ml} = (2600 + 2000 + 2200 + 3200)/4 = 2500$$

Estimate of Uncertainty of Measurement Pour Plate Method

2. Convert to logs:

- Mean Log_{10} colonies/ml = $(3.41 + 3.30 + 3.34 + 3.51)/4 = 3.390$
- Standard deviation = 0.092
- Take antilog = $10^{**} 3.390 = 2455$ colonies/ml
- This is called the Geometric Mean.

Estimate of Uncertainty of Measurement

Pour Plate Method

$$\bar{O} = 3.390$$

$$S = 0.092$$

☛ 95% confidence interval for $\bar{\mu}$ (population mean):

$$\bar{O} \pm (t \text{ stat.} \times s//n) = 3.390 \pm 3.18 * (0.092//4) = 3.390 \pm 0.146 = (3.244, 3.536)$$

- Note: 3.18 is from t distribution with 3 df

☛ Take antilogs:

$$\text{☛ Lower 95\% limit} = 10^{**} 3.244 = 1754$$

$$\text{☛ Upper 95\% limit} = 10^{**} 3.536 = 3435$$

$$\text{Relative Standard Deviation (RSD}_r\text{) or (CV) = } 0.092/3.390 \times 100 = 2.7\%$$

Performance Characteristics

Definitions

Precision can be measured by:

- ☛ repeatability - measures random error of the method for replicate tests performed under identical conditions
- ☛ reproducibility - measures error under changed conditions of measurement.
“ruggedness of the test”

Repeatability

Sample No.	Rep 1 Plate Count	Rep 2 Plate Count	Rep 1 Log ₁₀ Count	Rep 2 Log ₁₀ Count
1	12	15	1.08	1.18
2	52	63	1.72	1.80
3	118	90	2.07	1.95
4	60	44	1.78	1.64
5	220	220	2.34	2.30

Calculation Details

☛ Replicate 1 Log Count = 1.08

☛ Replicate 2 Log Count = 1.18

☛ Mean = $(1.08 + 1.18) / 2 = 1.13$

☛ Between Replicate Variance =

$$\frac{(1.08 - 1.13)^2 + (1.18 - 1.13)^2}{(2-1)} = 0.0050$$

☛ Repeatability Variance = j Between
Replicate Variances/n

- n = # of samples

Repeatability Calculations

Sample No.	Rep 1 Log Count	Rep 2 Log Count	Between-Replicates Variance
1	1.08	1.18	0.0050
2	1.72	1.80	0.0032
3	2.07	1.95	0.0072
4	1.78	1.64	0.0098
5	2.34	2.30	0.0008
Repeatability Variance =			0.0052

Repeatability Calculations

☛ Repeatability Standard Deviation (S_r) = $\sqrt{0.0052} = 0.0721$

☛ Measurement uncertainty (MU) = $0 \pm (k \times s/\sqrt{n}) = 1.786^* \pm (2.26 \times 0.0721/\sqrt{10}) = 1.786 \pm 0.052$

*Mean \log_{10} Count

☛ Relative Standard Deviation (RSD_r) or (CV) = $\frac{0.0721}{1.786} \times 100 = 4.0\%$

Use of Reproducibility Relative Standard Deviation

- ☛ Can use RSD_R as estimate of MU-- **IF**:
- ☛ All uncertainty factors are included
- ☛ Lab has acceptable bias
- ☛ Lab has acceptable repeatability

Reproducibility Calculations for Estimate of Uncertainty of Measurement

- ☛ Duplicate analyses using the same SOP
- ☛ By different analysts over an extended period of time (e.g. 1 year)
- ☛ Would encompass the influence of all elements that impact on measurement of uncertainty
- ☛ Pool the data and use the same calculation procedure as for repeatability (SD_r) to calculate the SD_R

Most Probable Number Methods (MPN)

- ☛ The Draft APLAC MU Guidance accepts the data in the McCrady's tables as reasonable estimates of MU for MPNs.
- ☛ For the purposes of CAEAL's Policy, they can be used as estimates of MU for a test, provided the laboratory reviews the results of unusual combinations and flags or rejects such data. `

Potential Sources of Experimental Data for Policy Compliance in Microbiology

- ☛ Proficiency Testing (PT) programs
- ☛ Reference Samples
- ☛ Spike Recovery
- ☛ Method Validation Replicate
- ☛ Sample Duplicates

Proficiency Testing Programs

- ✿ Pooling of data derived by different methods diminishes the usefulness of PT information for estimating measurement of uncertainty
- ✿ Split PT samples between analysts can be used to calculate SD_r (repeatability SD), SD_L (between analyst reproducibility) and (Reproducibility SD).
- ✿ By collecting split PT data over time laboratory will be able to calculate reproducibility SD (SD_R) that includes both intra- and inter-analyst sources of uncertainty.

Reference Sample

- general lack of reference materials available for routine use in microbiological methods.

Spike Samples

- ☛ Time consuming, but a reasonable method to measure repeatability by individual analysts and reproducibility (analytical variability over time)
- ☛ Similar in approach to and could be combined with split PT samples results

Validation Data from Collaborative Method Studies

- Provides reference values for reproducibility standard deviation (SD_R) and repeatability (SD_r)
- Laboratories can evaluate the acceptability of their overall RSD_R and individual analyst repeatability performance (RSD_r) for a given method
 - e.g. Intra-analyst counting variance #7.7%
 - e.g. Inter-analyst counting variance #18.2%

Sample Duplicates

- ☛ In drinking water analysis, this is not a very productive approach for capturing useful data
- ☛ Most results are 0/100 mL

Evaluation of results against a microbiological guideline

☛ e.g. HPC#500/mL

- β For a result to be considered as having exceeded a guideline, the lower limit for the confidence interval (MU) is required to be above this value
- β Alternatively a χ^2 (chi-square test) can be used

Chi Squared test

☛ $\chi^2 = (C-L)^2/L$

☛ $\chi^2 \neq 4C^2/L$ (95% confidence limits)

☛ C = colony count

☛ L = Limit value

☛ The microbial guideline will be exceeded if either $\chi^2 > 4$ or if the count is $> L + 2\sqrt{L}$

☛ For a guideline of 500, the # of CFUs in the sample would have to be > 545 to be statistically in excess of the guideline

Qualitative Methods

False Negative/Positive Rates

Truth or Standard Method

+

-

Test Method	+	a	b
	-	c	d

+ or - : possible outcome of the test

False Negative Rate = $c/a+c$

False Positive Rate = $b/b+d$

Excerpts from CAEAL Policy on Uncertainty P19 Rev 1.4

- 2 They shall report the expanded uncertainty estimate as part of the reported result when the reporting of the estimate of measurement uncertainty is
 - ⟨ Required by the client, or
 - ⟨ Required to establish that the data is 'fit-for-purpose', or
 - ⟨ Required because the data is being used to establish compliance (of the body being represented by the

Excerpts from CAEAL Policy on Uncertainty P19 Rev 1.4

☛ **NOTE 2** *In those cases where a well-recognized test method specifies limits to the values of the major sources of uncertainty of measurement and specifies the form of presentation of calculated results, the laboratory is considered to have satisfied this clause by following the test method and reporting instructions*

Estimation of Uncertainty of Measurement

- ☛ The estimation of uncertainty of measurement requires the calculation of the degree of uncertainty
- ☛ And now that you are thoroughly confused, we can open this up for discussion.
- ☛ Thanks for bearing with me!

Suggested References

- ☛ Nordic Committee on Food Analysis (NMKL) Procedure NO 8 (1999)
Measurement of Uncertainty in Microbiological Examination of Foods
- ☛ S. Niemelä, Statistical Evaluation of Results from Quantitative Microbiological Examinations, NMKL Report no.1, 2nd ed.