Measurement uncertainty in microbiological methods

- Introduction
- Reporting results
- Uncertainty
- Negative uncertainty
- Conclusions

TROLLBOKEN AB

RECENT DEVELOPMENTS IN QUALITY ASSURANCE
Cyprus 12-13 March 2024

Bertil Magnusson, Trollboken AB

Presentation based on

Eurachem
A focus for analytical chemistry in Europe

Accreditation for Microbiological Laboratories

Composition of the ad hoc Working Group

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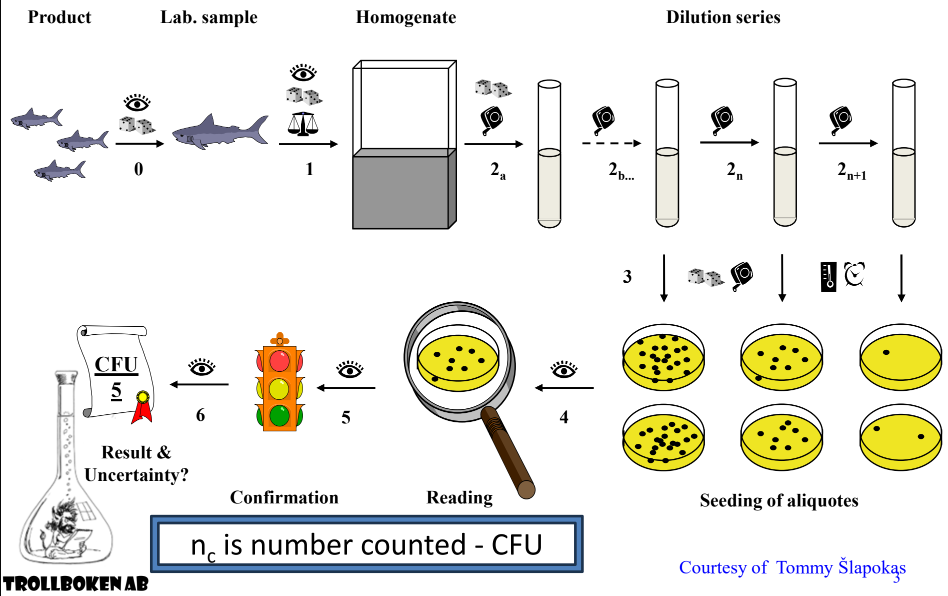
Third Edition 2023

Eurachem guide
&
ISO
7218 Food
8199 Water
19036 Food
29201 Water

Microbiological analytical steps



Uncertainty in measurements

Analysis	Normal Uncertainty	Reporting interval	Guide
Physical	< 10 %	Symmetric	GUM
Chemical	5 – 40 %	Symmetric	Eurachem/ Nordtest
µbiology Water	20 – 60 % 0.1 -0.3 log	Asymmetric	ISO 29201
µbiology Food	0.15 – 0.6 log	Asymmetric	ISO 1903

Uncertainty over the measurement interval/range
For CFU chose 1– 100 CFU

High uncertainty (over 100 %)

If client ask for interval

Report asymmetric interval

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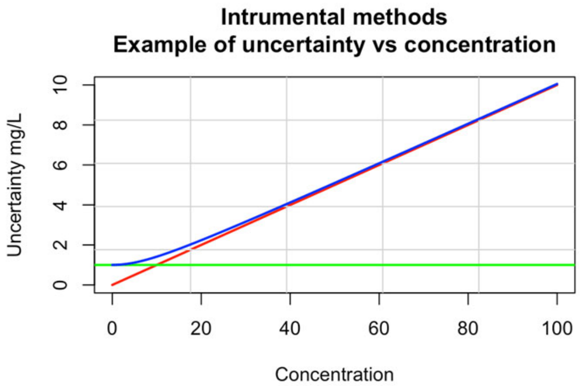
Uncertainty versus concentration



Contributions

1 Constant

2 Proportional

Uncertainty calculated

Low levels (mg/L)

High levels (%)

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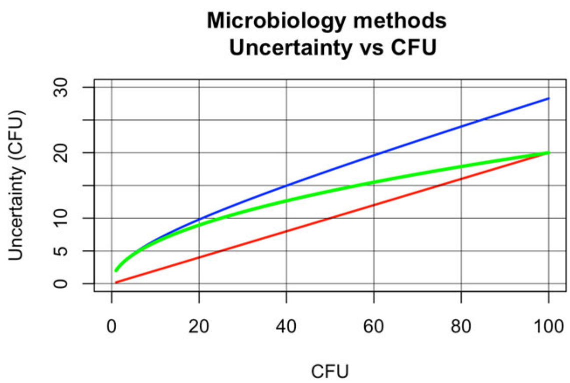
Uncertainty versus concentration



Contributions

1 Poisson

2 Technical/Operational

Uncertainty calculated

High levels (%) or (Log10)

Technical/operational

Low levels - known Poisson

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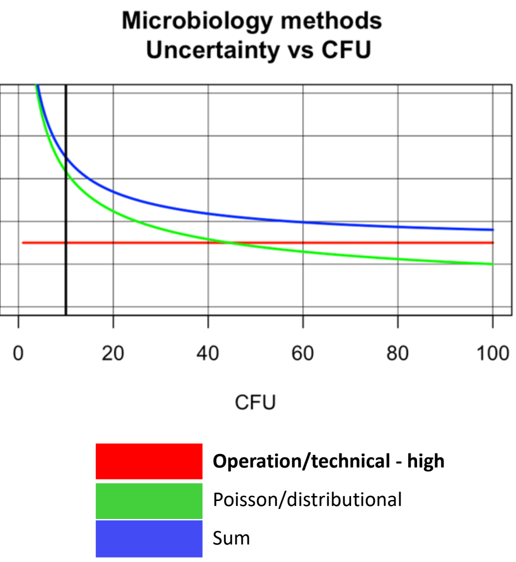
Relative uncertainty



Poisson dominates at lower counts

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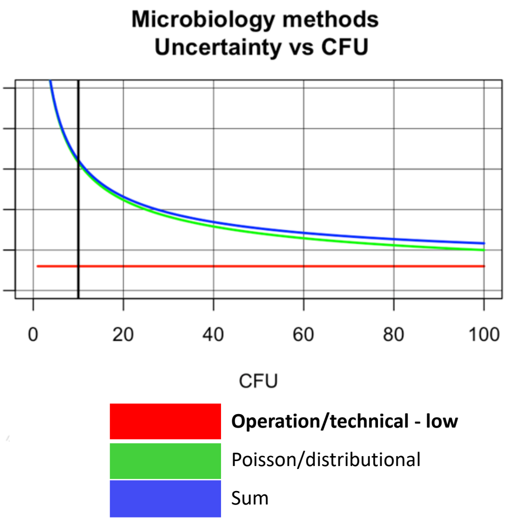
Relative uncertainty



Poisson dominates

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ISO standards say this

Table 1 - Expression of results in CFU/ml or per analytical test portion

Counted colonies	Reporting of results	
	ISO 8199 Water matrix	**ISO 7218 Food matrix**
0	Not detected or < 1	< 1
1-2	Microorganisms are present	Microorganisms present but < 4
3	Report results as an estimate	Microorganisms present but < 4
4 - 9	Report results as an estimate	Report results as an estimate
≥ 10	Report results	Report results

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.

Reporting results
CFU < 10

Reporting results < 10

CFU	Report	Example
0-3	< Value & text	< 1 Not detected
4-9	Value % text	5 *Result is an estimate*

Inform client

Value and text

Reporting results ≥ 10

CFU	Issue	Report
≥ 10	Client request	Report as client requests
	Close to a limit	Propose value and uncertainty interval
	No request	Value

According to ISO/IEC 17025

you may report just the value if no request or need

Reporting results with uncertainty

CFU	Alt 1	Example
Water ISO 29201		
≥ 10 Water	Value & Uncertainty	20 cfu/100 ml U = 54 %
	Value [interval]	20 [12, 34]* cfu/100 ml
Food ISO 19036		
≥ 10 Food	Value & Uncertainty	2,18 log cfu/g U = 0.67 log cfu/g
	Value [interval] CFU units	2,18[1,51,2.85] 150 [32, 701]* cfu

*Note asymmetric interval in cfu

[-8, +12]

Inform client

1) Uncertainty or

2) Give an interval

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Reporting in microbiology

- Units
  - %
  - log10
- Reporting
  - < 10 CFU - Reported with text
  - ≥ 10 CFU – Report:
    - uncertainty when needed
    - asymmetric interval
- At low and medium levels uncertainty is known – Poisson

U < 10 %

GUM

Microbiology higher uncertainty

ISO 29201

ISO 19036

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ISO 19036 Food

*Microbiology of the food chain – Estimation of measurement uncertainty for quantitative determinations*Covers:

- *All variants of methods of*
  - ***colony counts***
  - ***most probable number (MPN)***
  - *Some instrumental and molecular methods such as flow cytometry and PCR (polymer chain reactions)*

ISO 19031 is a general standard for uncertainty in ubio

Similar approaches for colony counts and MPN as in ISO 29201

ISO 29201 Water quality

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

Covers:

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

ISO 29201 is a general standard for uncertainty in ubio

Here global approach with colony counts

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Symbols

s	Standard deviation
u	standard uncertainty ≈ *s*
u_R	Intra (within) laboratory reproducibility standard uncertainty
U	Expanded uncertainty

Expanded uncertainty

$$U = 2 * u_c$$

Coverage factor k=2

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Symbols - ubiology

n_c	**Number of counts counted**
u_d	Distributional or Poisson
u_o	Operational or tecnical uncertainty
u_{matrix}	Matrix
u_{conf}	Confirmation

$$\boldsymbol{u_c^2 = u_d^2 + u_o^2} + u_{matrix}^2 + u_{conf}^2$$

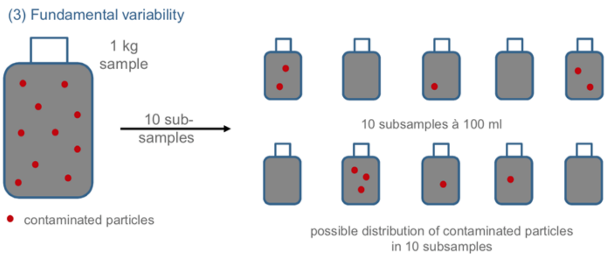
$$u_d$$

Microbiology

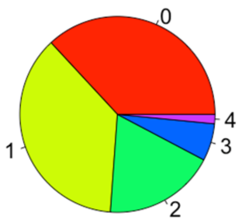
Four component to combine

We will talk about the first two

Uncertainty due to subsampling Poisson – distributional



CFU = 1



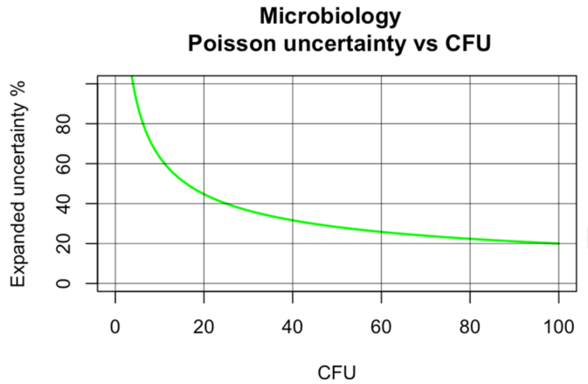
Microbiology

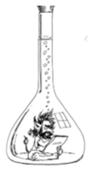
$$u_d = \sqrt{1/n_c} * 100$$

CFU = 1

$$u_d = 100\ \%$$

Poisson





$$u_d =$$
$$= \sqrt{1/n_c} * 100\ \%$$

Vid 100 CFU

u_d = 10 %

U_d = 20 %

Symbols - ubiology

n_c	**Number of counts counted**
u_d	Distributional or Poisson
u_o	Operational or tecnical uncertainty
u_{matrix}	Matrix
u_{conf}	Confirmation

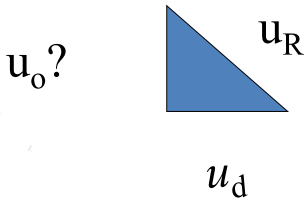
$$u_c^2 = u_d^2 + u_o^2 + u_{matrix}^2 + u_{conf}^2$$

$$u_o$$

Microbiology
Four component
to combine

Calculate u_o

u_o	Operational or technical uncertainty
u_R	Intra (within) laboratory reproducibility standard uncertainty i %
u_d	Distributional or Poisson



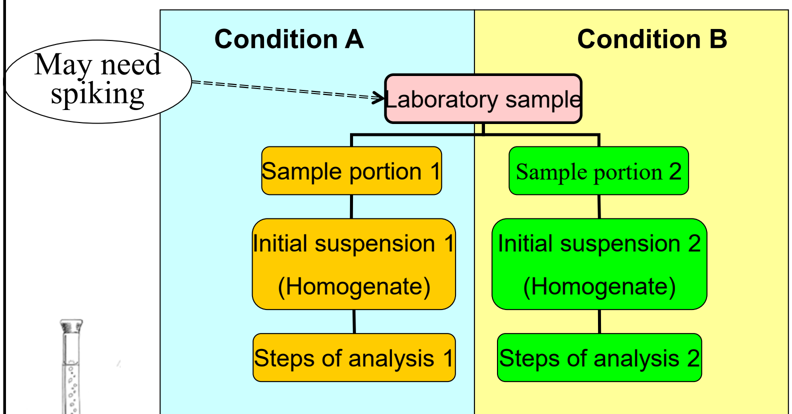
U_o

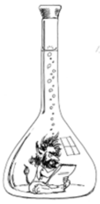
is calculated from

the difference

$$\sqrt{u_R^2 - u_d^2}$$

Global design, ISO 19036 and ISO 29201





This should be repeated with at least 10 samples, usually on different days. ISO 29201 recommends more than 30 samples

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Global design

For each analytic parameter

- Choose relevant matrices to include

For each matrix: 10 samples usually on different days

- For each sample: Perform a complete analysis of 2 test portions, 1 and 2, under conditions A and B
  - Vary the conditions as much as possible: DIFFERENT
    - » technicians (and if stable sample even days)
    - » batches of media, diluents etc.,
    - » equipment (mixers, pH-meters, pipettes etc.)
    - » incubators, different times within interval

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Global design – Example ISO 29201

Table F.1 — Calculation with common logarithms

Sample No	Dilution[a]	n_{c_1}	n_{c_2}	lg n_{c_1}	lg n_{c_2}	$u_{R'}^2$	u_d^2	u_o^2
1	−4	5	8	0,699 0	0,903 1	0,020 8	0,029 0	−0,008 2
2	−3	15	11	1,176 1	1,041 4	0,009 1	0,014 5	−0,005 4
3	−4	11	19	1,041 4	1,278 8	0,028 2	0,012 6	0,015 6
4	−6	21	39	1,322 2	1,591 1	0,036 1	0,006 3	0,029 8
5	−5	68	45	1,832 5	1,653 2	0,016 1	0,003 3	0,012 8
6	−4	151	203	2,179 0	2,307 5	0,008 3	0,001 1	0,007 2
					Mean	0,019 8	0,011 1	0,008 6

[a] Dilution needs not to be considered when working with logarithms.

u_R is reproducibility (correct Rw) - log
u_d poisson, distributional uncertainty - log
u_o operation uncertainty (at high counts) - log

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Global design – Example ISO 29201

Sample	Dilution	C_1	C_2	Log C_1	Log C_2	u_R^2	u_d^2	u_o^2
1	-4	5	8	0.6990	0.9031	0.0208	0.0290	-0.0082
2	-3	15	11	1.1761	1.0414	0.0091	0.0145	-0.0054
3	-4	11	19	1.0414	1.2788	0.0282	0.0126	0.0156
4	-6	21	39	1.3222	1.5911	0.0361	0.0063	0.0299
5	-5	68	45	1.8325	1.6532	0.0161	0.0033	0.0127
6	-4	151	203	2.1790	2.3075	0.0083	0.0011	0.0072
					Mean:	**0.0198**	**0.0111**	**0.0086**

$u_o^2(log_{10})$ = 0.0086 – operation/technical variability = 21 %

Necessary to add more samples, preferably

≥30 samples usually on different days

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CourtesyTommy Šlapokas

Summary
Steps to determine operational u_o

1. Reproducibility (within-lab) (u_R) is determined for each duplicate
2. Operational/technical variability u_d is calculated for mean CFU(Poisson)
3. u_o^2 operational variability is calculated for each sample as:
4. Mean of u_o^2 from all samples is calculated
   – positive - square root $u_o = \sqrt{u_o^2}$
   – negative go to Eurachem Guide …

$$u_o^2 = \sqrt{u_R^2 - u_d^2}$$

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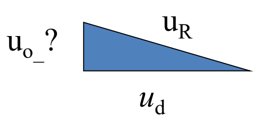
Calculating u_o operational/technical uncertainty

$$u_{o\_} = \sqrt{u_R^2 - u_{d\_rel}^2}$$



Eurachem Guide:

… the issue of obtaining negative results when estimating operational uncertainty from duplicates is treated in Annex C.

u_o is negative?

30 duplicates

Median CFU = 50

Go to Eurachem Annex C

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Eurachem Guide Annex C – Table 7

Number of duplicates	Median (CFU)	u_d (%)	$\hat{u}_o$* (%)
10	30	18	15
20	30	18	11
30	30	18	9
10	50	14	11
20	50	14	8
30	50	14	6

*one-sided upper confidence limit (95 %) for the estimate of u_o when the variance is negative.

u_o

negative?

Use Table C7

$u_o \leq x$

Simulations using

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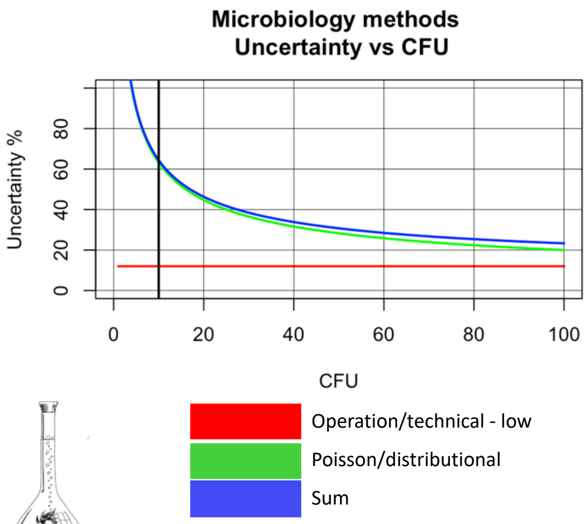
Expanded relative uncertainty



Example with an operational uncertainty of u_o = 6 %

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Measurement uncertainty in microbiological methods

Introduction

Reporting results

Uncertainty

Negative sampling uncertainty

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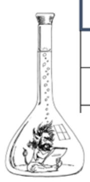
Negative sampling uncertainty

Eurachem Guide Annex D

introduce the estimation of sampling uncertainty from duplicates following the guidance in the Eurachem Guide Measurement uncertainty arising from sampling and Nordtest TR 604

Sample C 1		Sample C 2	
CFU $C_{1.1}$	CFU $C_{1.2}$	CFU $C_{2.1}$	CFU $C_{2.2}$
63	45	41	45
37	26	28	30



Using an EXCEL add-in RANOVA to evaluate results from the "duplicate method"

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Negative sampling uncertainty

Output from Excel add-in RANOVA

Classical ANOVA No. Targets 10

Mean	35.3	Confidence limits		Confidence limits
Total Sdev	16.473	(11.945, 27.498)		
	Btn Target		Sampling	
Standard deviation	14.299	(9.2055, 26.537)	0	(0, 8.5372)
% of total variance	75.35		0.00	
Expanded relative uncertainty (95%)			0.00	(0, 48.37)
Uncertainty Factor (95%)			1	(0, 1.4932)

No. Targets 10

	Confidence limits
Sampling	
0	(0, 8.5372)
0.00	
0.00	(0, 48.37)
1	(0, 1.4932)

From upper confidence limit of uncertainty factor

1.49

$U_{samp} \leq 20$ %

See Eurachem guide for details

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Water samples – low counts

- ISO 29201 7.5

When one component dominates the combined uncertainty, it is possible to omit the smaller component (2.4.2). This is particularly advantageous for situations in which the operational variability is the insignificant component. Then the intrinsic variability can be considered representative of the combined uncertainty.

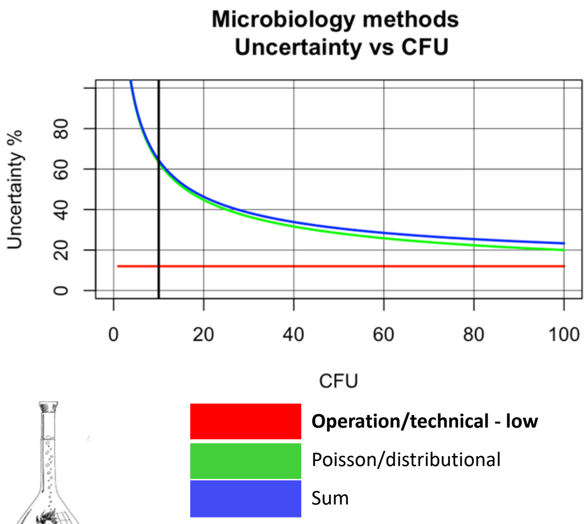
No experimental work is required

Low n_c

$$u_{c\_rel} \approx \sqrt{1/n_c} * 100$$

”The uncertainty is obtained <u>only</u> from the test result assuming Poisson”

Water samples – low counts



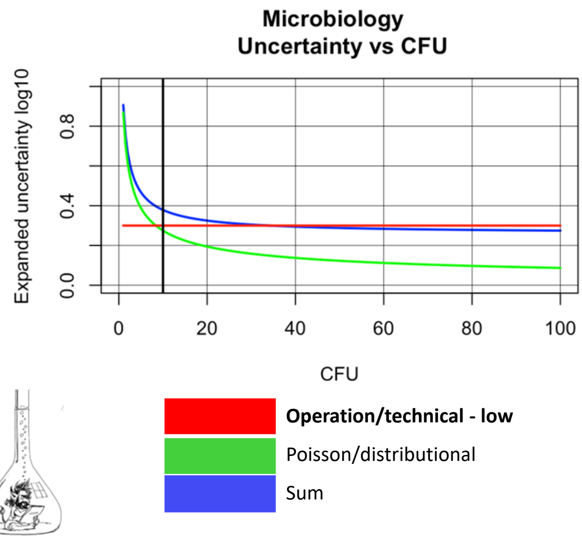
Poisson dominates

From n_c*

$$U \approx 2\sqrt{1/n_c} * 100\ \%$$

*Number of counts counted

Food samples – high operational/technical uncertainty



Operational/ technical dominates

U≈ 0.3 log10

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Reporting in microbiology

- Units
  - %
  - log10
- Reporting
  - < 10 CFU - Reported with text
  - ≥ 10 CFU – Report:
    - uncertainty when needed
    - asymmetric interval
- Uncertainty
  - low operational use Poisson
  - high operational use operational
  - otherwise combine

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

U < 10 %

GUM

Microbiology higher uncertainty

ISO 29201

ISO 19036

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Measurement uncertainty in microbiological methods

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Uncertainty

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🙏🙏🙏 & ?

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