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

Trollboken AB

Analytical chemist trying to present UNCERTAINTY in microbiology …

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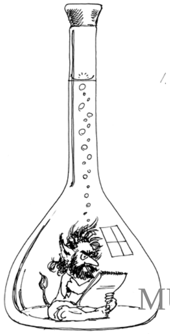
Content

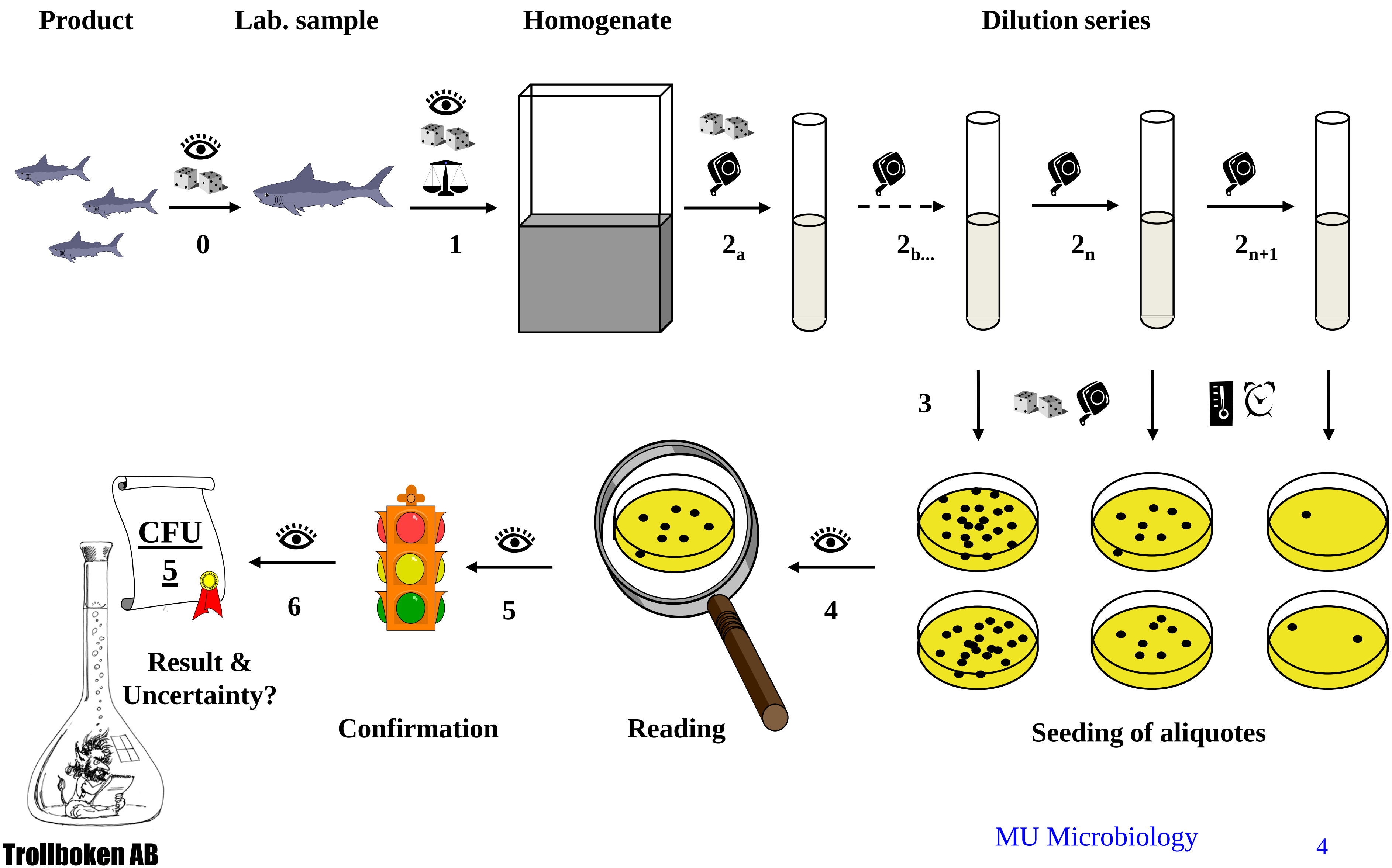
- Microbiology analytical steps
- Reporting results
- Uncertainty
  - Approaches
  - Symbols and units used
  - Distributional (Poisson)
  - Determine operational
  - Expanded
- Reporting uncertainty interval
- Additional components
- Summary reporting
- Eurachem guide

Focussing on water matrix

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



Microbiological analytical steps



Reporting results

CFU*	Report	Example
< 10	Value and text	5 Result is an estimate
≥ 10	Value & Uncertainty	20 U = 54 %
	Value and asymmetric confidence interval (95%)	20 [12,34]
*CFU = colony forming units		

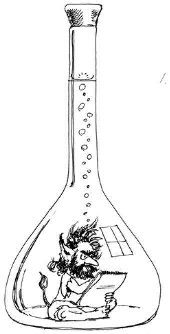
CFU ≥ 10

Inform client

1) **Uncertainty**

or

1) **Give an interval**



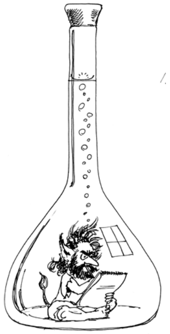
ISO standards say this

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.



Results ≥ 10 CFU
measurement uncertainty
is needed

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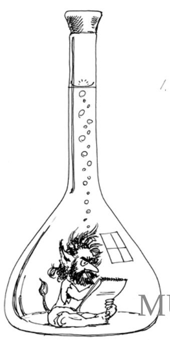
Focussing on water matrix

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 …

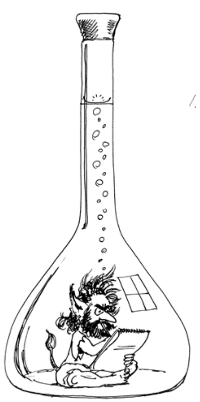
Modelling

Single lab validation/ **Global**

Interlaboratory validation

Proficiency testing

Experimental approaches



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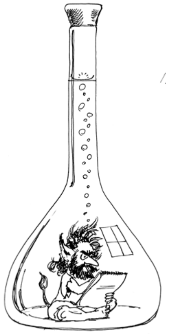
ISO 29201 Water quality

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*



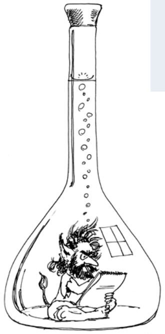
ISO 29201 is a general standard for uncertainty in ubio

Many many other ISO standards

e.g. ISO 19036

Symbols

n_c	Number of counts
u	standard uncertainty
u_{SIR}	Intra (within) laboratory reproduciblity
u_R	Between laboratory reproducibility
$\boldsymbol{u_o}$	**operational or technical uncertainty**
$\boldsymbol{u_d}$	**distributional or Poisson uncertainty**
u_{matrix}	uncertainty arising from imperfect mixing of the laboratory sample
u_c	combined uncertainty
U	Expanded uncertainty



Expanded uncertainty

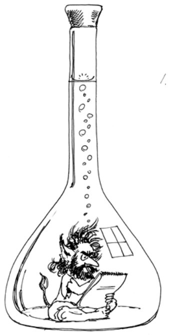
$$U = 2 * u_c$$

Coverage factor k=2

Units for uncertainty

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

Unit	u
CFU	5 CFU
%	30 %
ln	ln 0.30
log_{10}	log_{10} 0.13

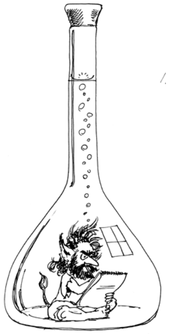


NOTE The uncertainty given can be recalculated to other units

Uncertainty u in %

Uncertainty from in-house validation data

Component	General	ubio
Precision Between days	Intermediate precision	Operational + Distributional
Confirmation	-	Uncertainty of confirmation
Inhomogeneity	Repeatability	Matrix uncertainty



Microbiology in water matrix

Main components

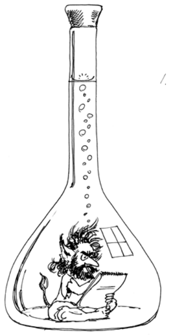
$$u_c = \sqrt{u_o^2 + u_d^2}$$

Main components for uncertainty

Operational u_o (technical)

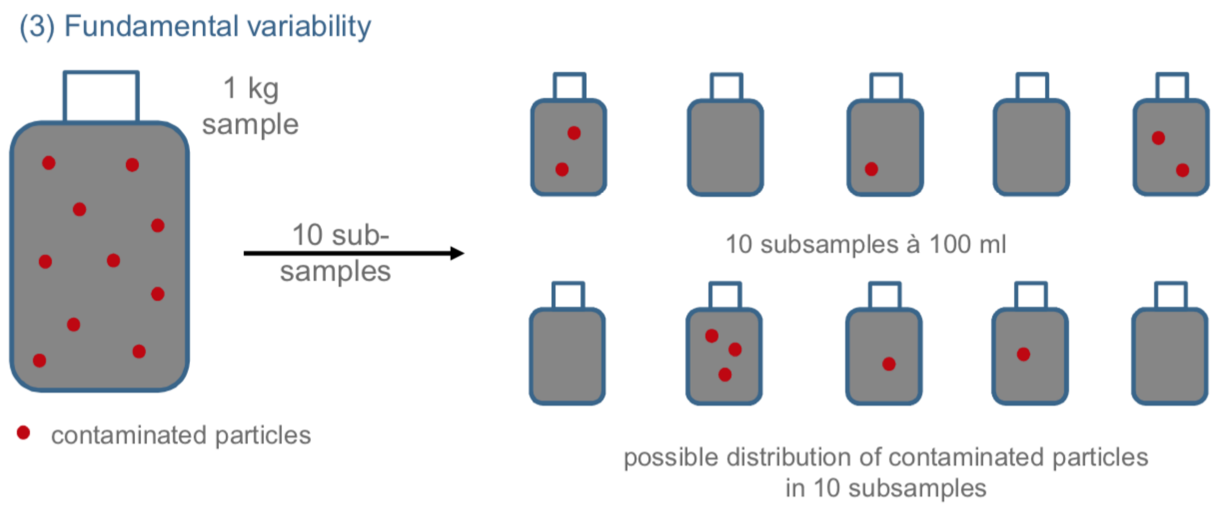
Distributional u_d (Poisson)

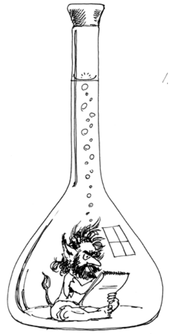
$$u_c = \sqrt{u_o^2 + u_d^2}$$



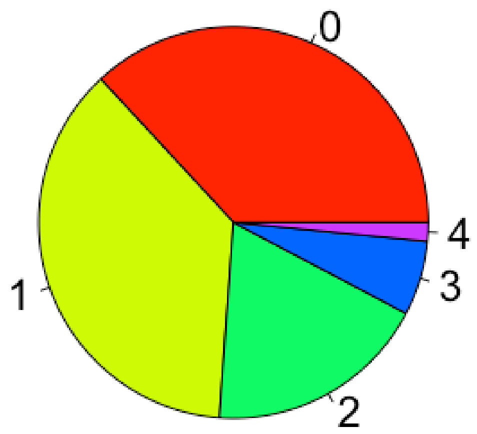
Only distributional uncertainty is needed for CFU < 10

Uncertainty due to subsampling
Poisson – distributional





CFU = 1



Distributional

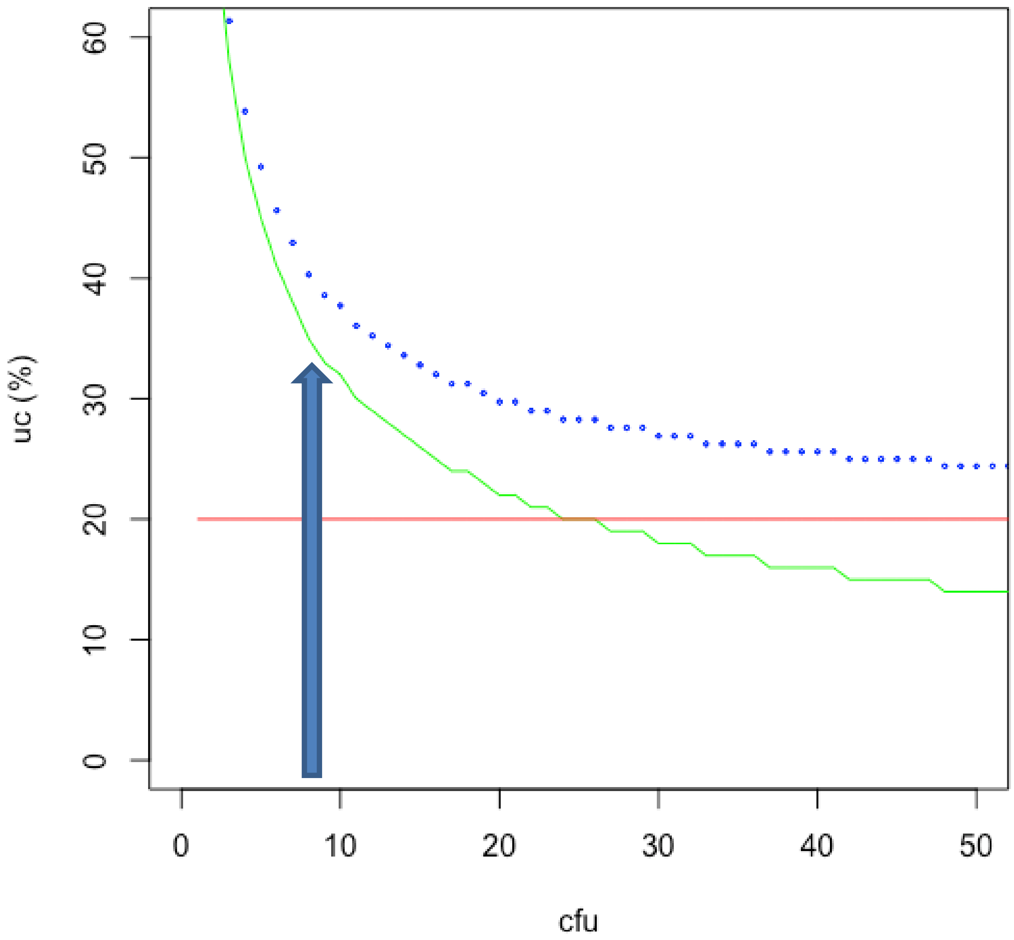
$$u_d = \sqrt{1/n_c} * 100$$

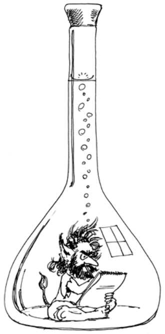
CFU = 1

$$u_d = 100\ \%$$

Standard uncertainty

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



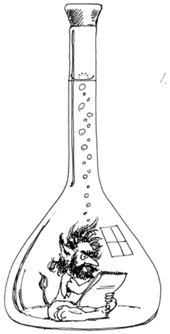


Distributional uncertainty can be calculated

Main components for uncertainty

Operational u_o
(technical)

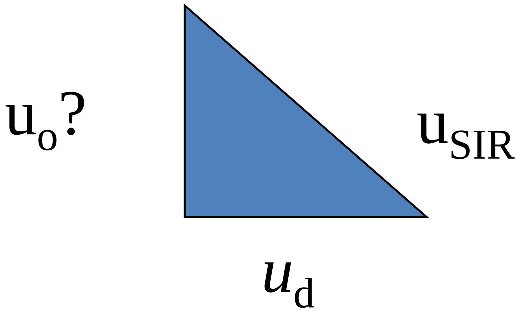
Distributional u_d



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

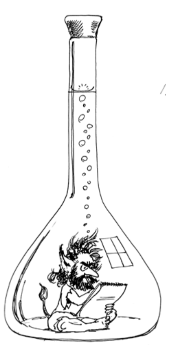
Operational is needed for CFU ≥ 10

Estimate u_o in %



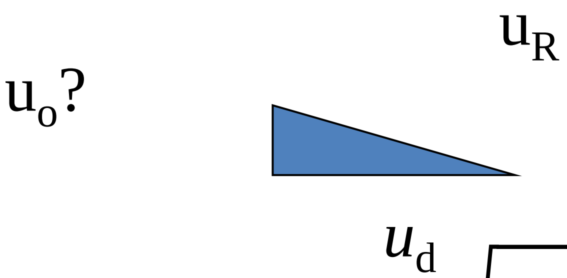
$$u_o = \sqrt{u_{SIR}^2 - u_d^2}$$

u_{SIR} Intra (within) laboratory reproducibility

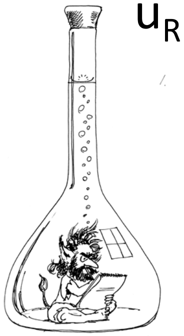


Operational uncertainty is estimated as a difference

Estimate u_o in %



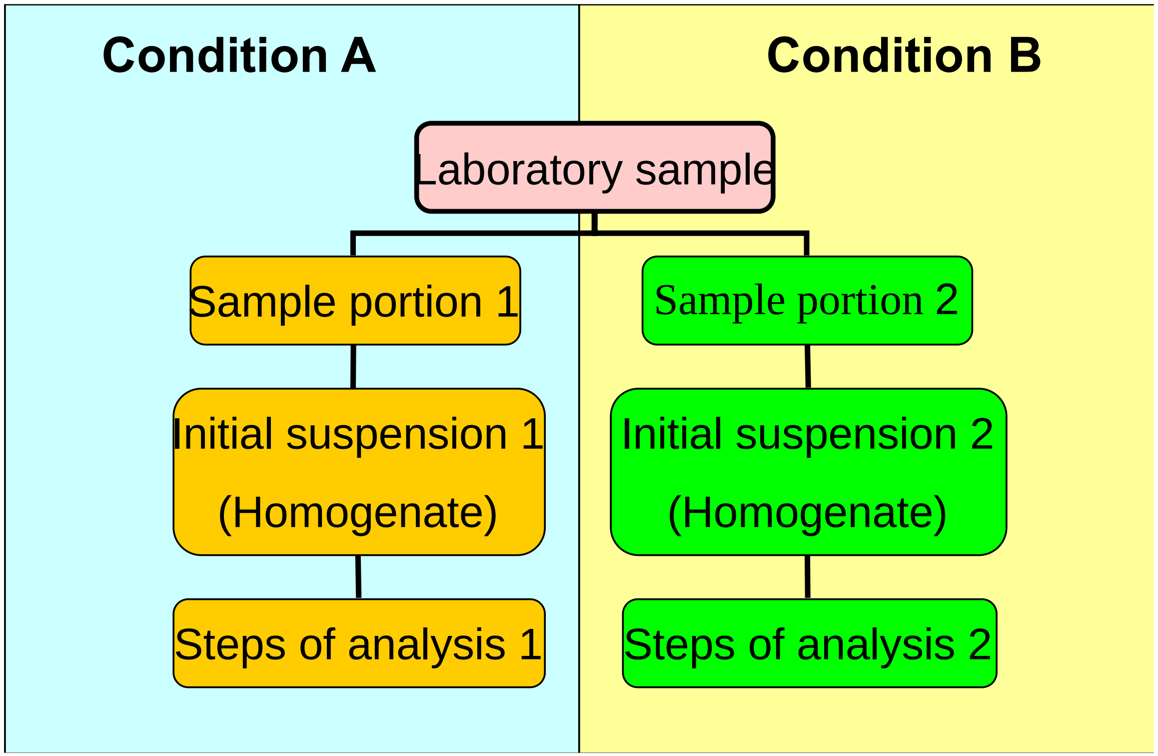
$$u_o = \sqrt{u_R^2 - u_d^2}$$



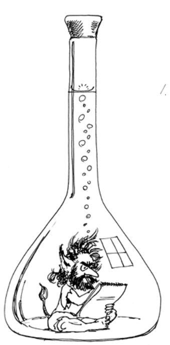
u_R Intra (within) laboratory reproducibility

When operational uncertainty is small we can often not estimate it

Operational uncertainty - experiment



n_{c1} n_{c2}



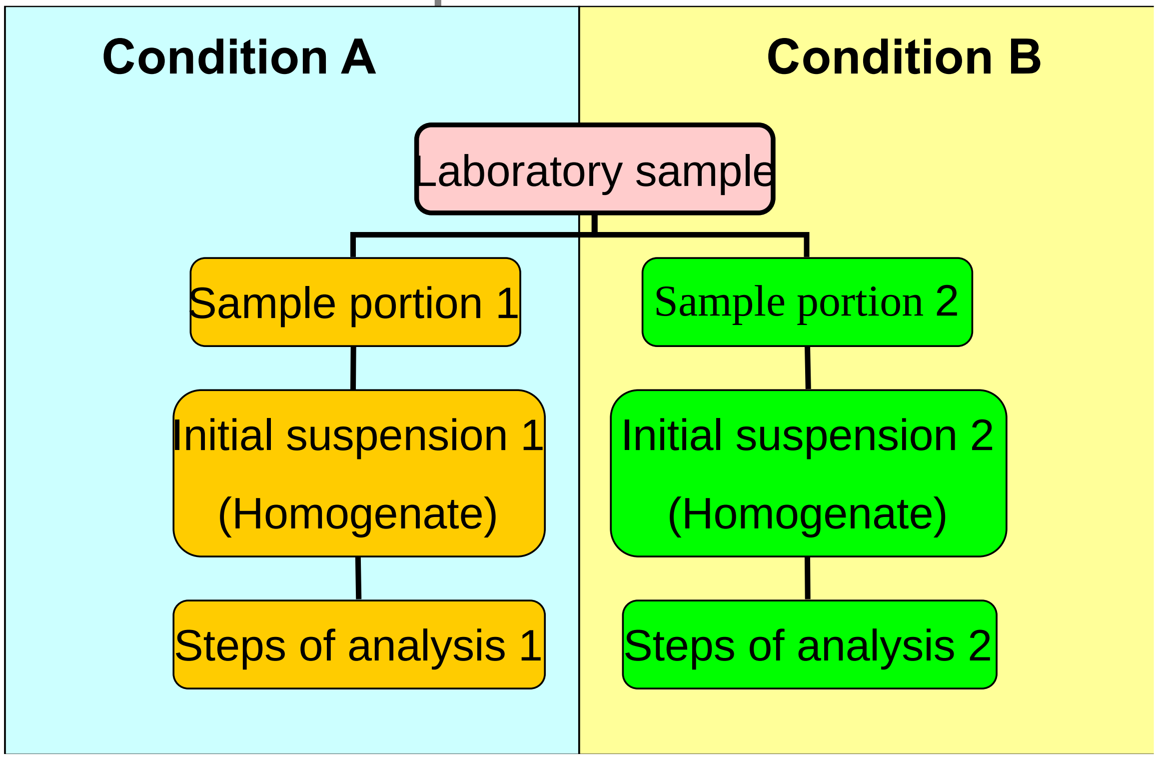
Calculate u_R (sd) for n_{c1} and n_{c2}

"Global approach"

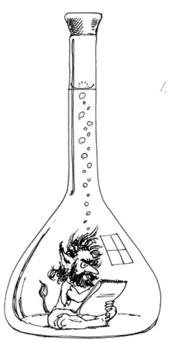
Large differences condition A and B

30 duplicates are suitable

Operational uncertainty - experiment



n_{c1} n_{c2}

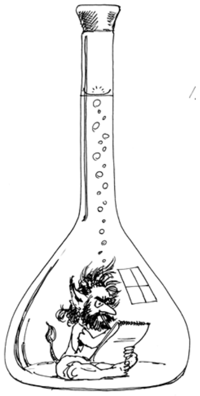


n_{C1}	n_{C2}	Mean	u_R (%)	u_{d_rel} (%)
5	8	6,5	33	39

When operational uncertainty is small we can often not estimate it

”Global”, ISO 29201 (Example in $\log_{10}$ units)

Sample	Dilution	C_1	C_2	$\log(C_1)$	$\log(C_2)$	s^2_R	u^2_d	u^2_o
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.
This can be converted to %, in this case u_o= 21 %.

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Reporting results

Operational u_o is 21 %

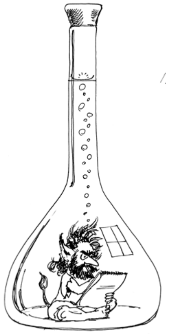
Distributional u_d depends on CFU

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

Confidence interval

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

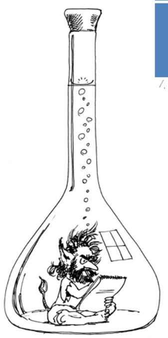
$$U_{max} = n \times \exp\left(\frac{2u_c}{100}\right)$$



Operational is needed for CFU ≥ 10

Calculation of interval
Example with operation. 15 %

Count CFU	u_o %	u_d %	u_c %	U %	U_{min} CFU	U_{max} CFU
10	15	32	35	70	5	20
15	15	26	30	60	8	27
20	15	22	27	54	12	34
30	15	18	23	46	19	48
40	15	16	22	44	26	62
50	15	14	21	42	33	76
75	15	12	19	38	51	110
100	15	10	18	36	70	143



Here is calculated an asymmetric interval (95 %)

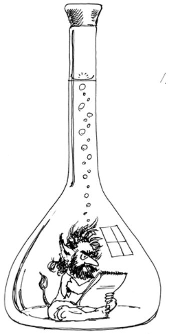
Similar for MPN

Additional uncertainty components

$$u_c = \sqrt{u_{\mathrm{d}}^2 + u_o^2 + u_{\mathrm{conf}}^2 + u_{\mathrm{matrix}}^2}$$

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

based on …

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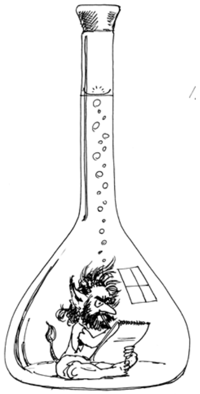
Single lab validation/ **Global**

Interlaboratory validation

Proficiency testing

Experimental approaches



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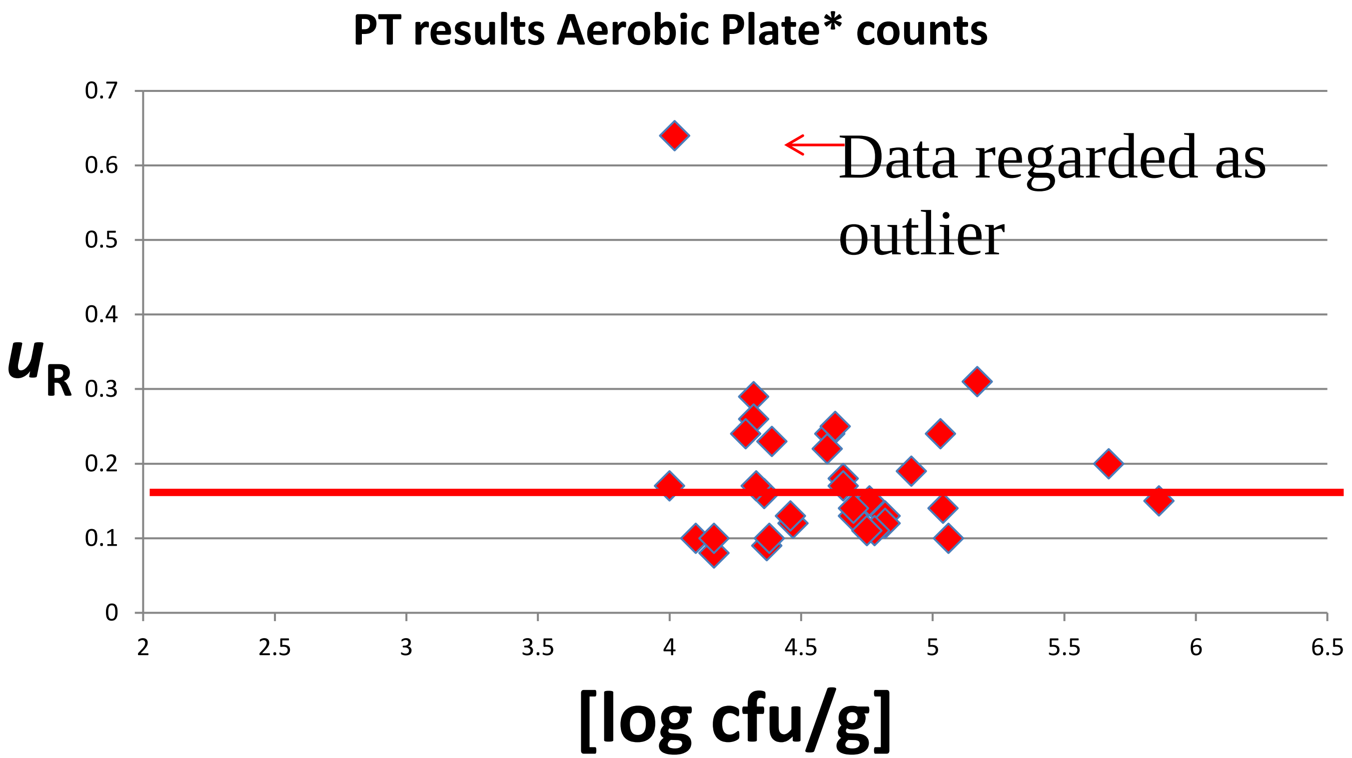
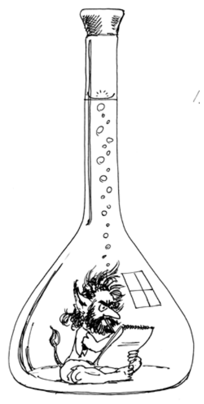
Estimating u_R (s_R from proficiency testing (NMKL 86)





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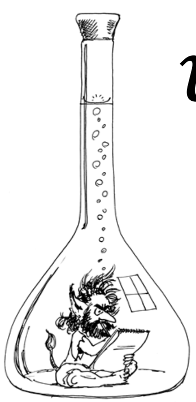
The aerobic plate count* (APC) is intended to indicate the level of microorganism in a product.

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)
  - $\boldsymbol{u_d} = \sqrt{1/n_c}*100 = \sqrt{1/20}*100 = 22\ \%$

$$u_c = \sqrt{u_d^2 + u_R^2} = \sqrt{22^2 + 41^2} = 47\ \%$$



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Reporting result with confidence interval

180 cfu/g [82,395]

Where 82 – 395 is the asymmetric confidence interval

Summary - reporting results

CFU*	Report	Example
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*CFU = colony forming units

CFU ≥ 10

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or

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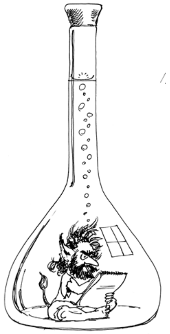


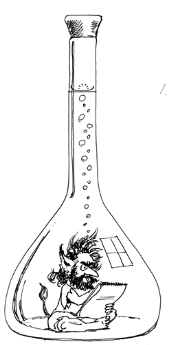

Eurachem

A focus for analytical chemistry in Europe

Accreditation for Microbiological Laboratories

Thanks for listening

Third Edition 2022



New version of the Guide late 2022