# Measurement Uncertainty
# in
# Microbiology

# Content

elevant guides and documents

Definitions

microbiological analytical steps

SO 29201 Global approach

# Relevant Guides and Documents

he International Vocabulary of Metrology - Basic and General oncepts and Associated Terms (VIM 3): 2011 also published as ISO uide 99

**SO 29201:2012** Water Quality - The variability of test results and the ncertainty of measurement of microbiological enumeration methoc

**SO 11352:2012** Estimation of measurement uncertainty based on alidation and quality control data

SO/IEC Guide 98-3:2008 (en) Uncertainty of measurement - Part 3: uide to the expression of uncertainty in measurement (GUM:1995)

## VIM3

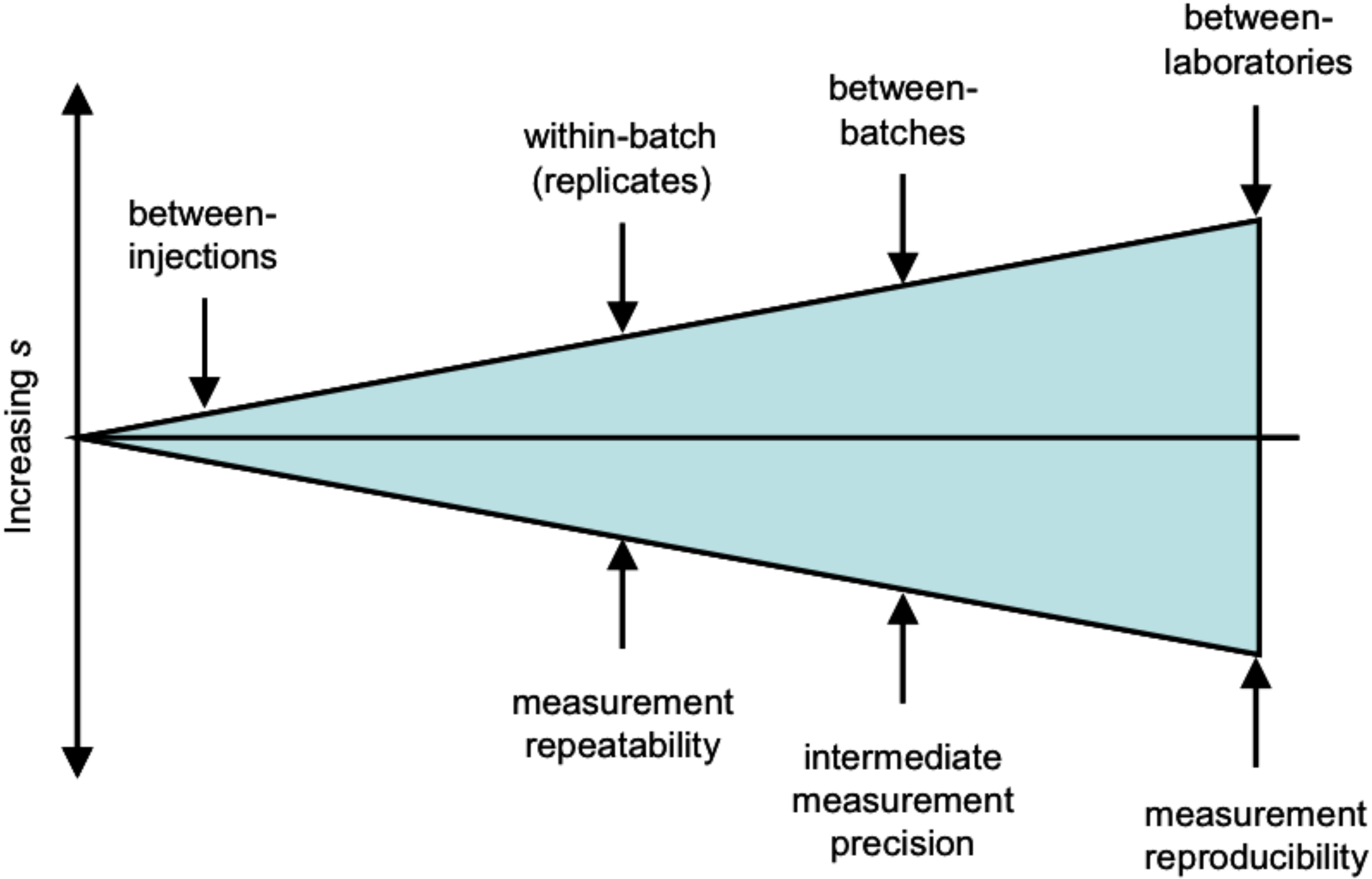
**peatability conditions:** Conditions where independent test results a
btained with the same method on identical test items in the same
aboratory by the same operator using the same equipment within
hort intervals of time.

**producibility conditions:** Conditions where test results are obtained
vith the same method on identical test items in different laboratorie
vith different operators using different equipment.

**cision** closeness of agreement between measured quantity values
btained by replicate measurements on the same or similar objects
nder specified conditions

# Precision estimates as observed imprecision

**VIM3**



# VIM3

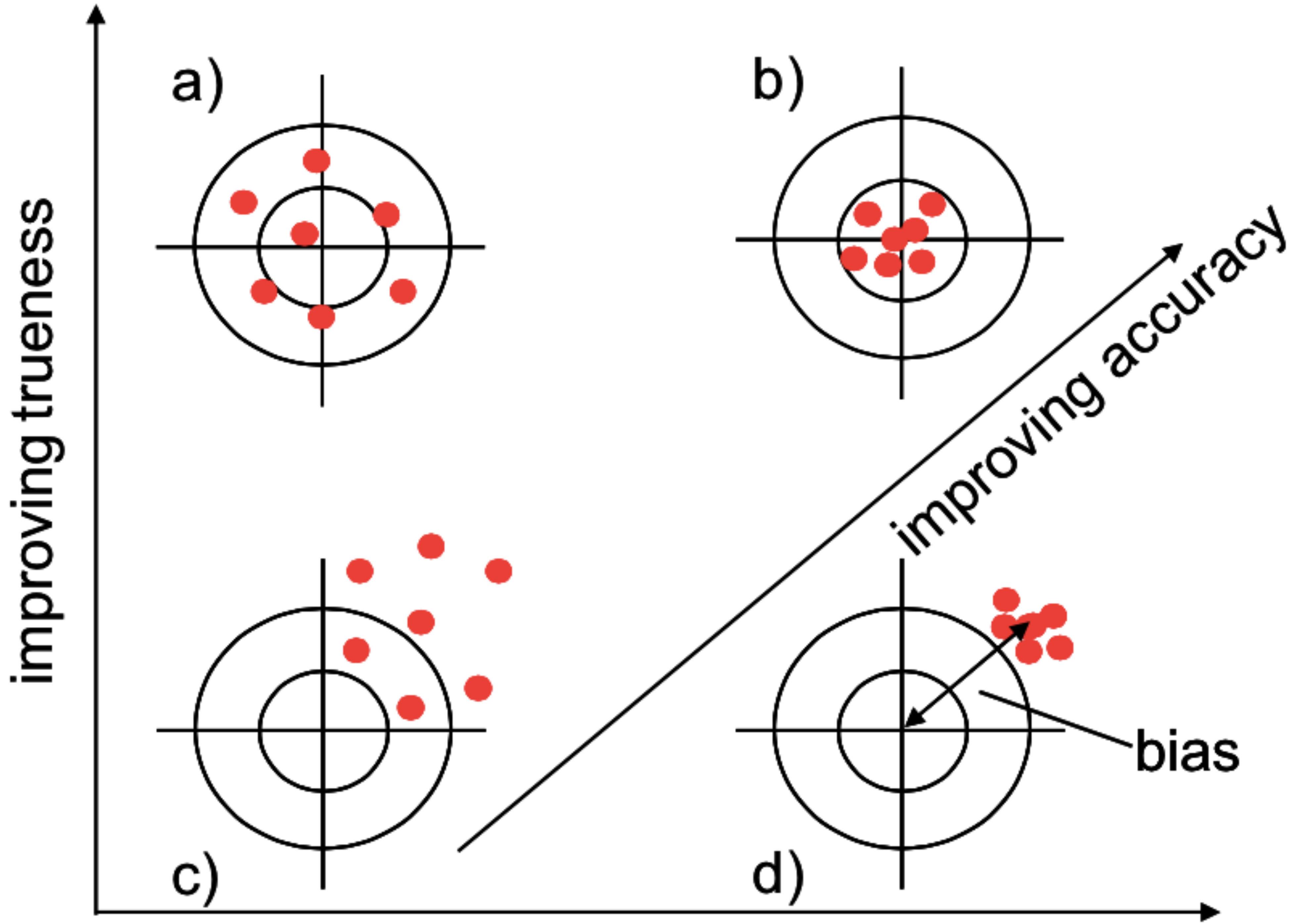
**eness** closeness of agreement between the average of an infinite umber of replicate measured quantity values and a reference quantit alue (not a quantity value!)

**s** Difference between mean measured value from a large series of tes esults and an accepted reference value (a certified or nominal value). he measure of trueness is normally expressed in term of bias

**uracy** closeness of agreement between a measured quantity value *single measurement!*) and a true quantity value of a measurand *precision and trueness*)

# Trueness and Precision

**VIM3**



**easurement uncertainty (MU)**

M3: non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the nformation used

/IEC 98-3 a parameter associated with the result of measurement hat characterizes the dispersion of the values that could reasonably be attributed to the measurand (measure of imprecision)

# ISO 29201:2012

/IEC 98-3:2008 component approach or bottom-up or step-by-step

ck box approach or top-down or global approach

U can be applied to results obtained by the same method in the same aboratory

blem: „variation without a cause“ that accompanies counts is not taken in ccount

cision in microbiological analysis depends on two important parameters:

ncertainty of technical measuring procedure (operational variability) and

ariation due to distribution of particles (distribution uncertainty)

# ISO 29201:2012

**ra-laboratory reproducibility**

**intermediate reproducibility**

**= intermediate precision**

**ative standard uncertainty** $u_{rel}$ in percent = coefficient of variation
CV)

# Steps in microbiological analysis

**product (food) / water**

***sampling***

**sample (laboratory)**

***mixing***

**homogenate - initial suspension**

***diluting***

**dilution series - final suspension**

***volumetric handling***

**inoculation (seeding) of plates**

***incubation***

**reading**

***picking of presumptives***

**confirmation**

***calculation***

# Principles of ISO 29201

press MU of a microbiological result *(y)* as a combination of
o major components

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

$u_c$ = the combined relative standard uncertainty

$u_o^2$ = operational variability (technical, experimental)

$u_d^2$ = ”intrinsic” variability (distributional)

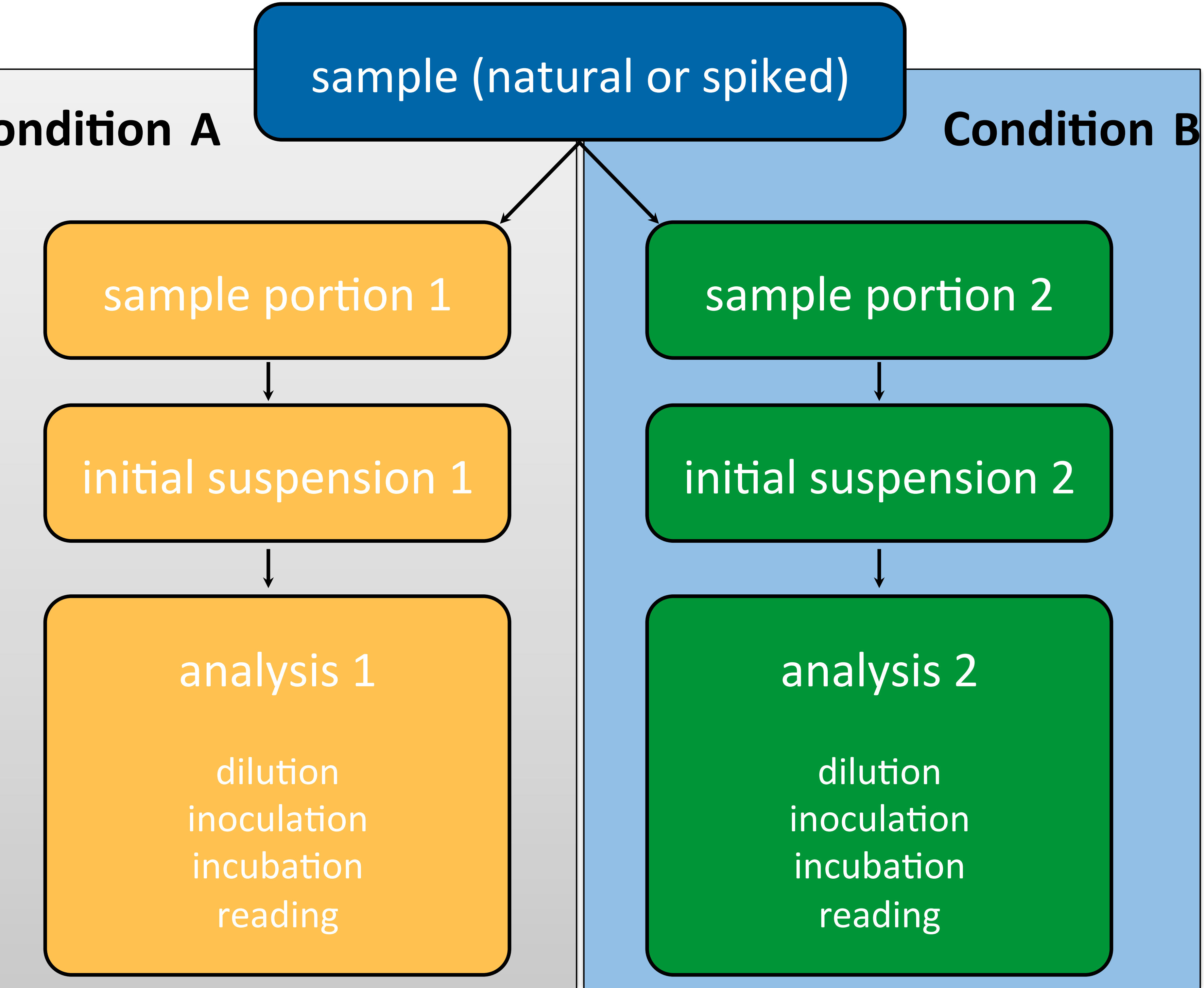
expanded uncertainty with coverage factor k=2

$$U = 2 * u_c = 2 * \sqrt{(u_o^2 + u_d^2)}$$

# uncertainty structure

| structure | final suspension | uncertainty component | depends on |
|---|---|---|---|
| subsampling and mixing | before | $u_o$ | matrix/material |
| dilution | before | $u_o$ | matrix/material |
| inoculation | within final suspension (random distribution of particles) | $\mathbf{u_d}$ | matrix/material |
| incubation | after | $u_o$ | method |
| reading: counting and possibly confirming (presumptive) | after | $u_o$ | operator/ equipment for detection |

# Experimental design




- at least 30 sa or more!
- separately fo every proced matrix and ta organism
- intralaborato reproducibilit conditions

# ISO 29201 global approach (Annex F)

nalyse 30 samples in duplicate with high variability
operator+ time+ equipment)

alculate reproducibility standard uncertainty $u^2_R$ from the 2 counts
each sample; *use log scales to ensure that the value of the paramete*
*not sensitive to contamination level (dilution)*

$$u^2_R = (\log n_{c1} - \log n_{c2})^2 \; / \; 2$$

alculate the intrinsic relative distribution uncertainty for each samp
$u^2_d$

$$u^2_d = 2 * 0{,}1886 \; / \; ( n_{c1} + n_{c2})$$

alculate **mean** relative operational uncertainty $u^2_o$

$$u^2_o = u^2_R - u^2_d$$

# ISO 29201 global approach (Annex F)

or new results (single counts) calculate combined uncertainty of the est result as:

$$u_c(x) = \sqrt{(u^2_o - u^2_d)} \text{ with } u^2_d = 0{,}1886/n$$

$u_o$ *is the mean relative operational uncertainty for the given sample e and parameter*

$u_d$ *is the relative distribution uncertainty (intrinsic variation assumin sson distribution) of the count*

expanded uncertainty $U = 2 * u_c = 2 * \sqrt{(u^2_o - u^2_d)}$

***te:*** for water analysis uncertainty values can be converted to the elative scale by multiplication with the conversion factor of 5,3

# Example

| $n_{c1}$ | $n_{c2}$ | $log(n_{c1})$ | $log(n_{c2})$ | $u^2_R$ <br> $(\log n_{c1} - \log n_{c2})^2/2$ | $u^2_d$ <br> 2*0,1886 / (c1 +c2) | $u^2$… <br> (log… <br> $u^2_R$ - … |
|---|---|---|---|---|---|---|
| 5 | 8 | 0,6990 | 0,9031 | 0,0208 | 0,0290 | -0,00… |
| 15 | 11 | 1,1761 | 1,0414 | 0,0091 | 0,0145 | -0,00… |
| 11 | 19 | 1,0414 | 1,2788 | 0,0282 | 0,0126 | 0,01… |
| 21 | 39 | 1,3222 | 1,5911 | 0,0361 | 0,0063 | 0,02… |
| 68 | 45 | 1,8325 | 1,6532 | 0,0161 | 0,0033 | 0,01… |
| 151 | 203 | 2,1790 | 2,3075 | 0,0083 | 0,0011 | 0,00… |
| | | | | relative reproducibility standard variance | intrinsic variability (distribution) | relative op… unce… |

… = 0,0086 $u^2_{o,rel}$ = 5,3 * 0,0086 = 0,0457

# Exercise

| $n_{c1}$ | $n_{c2}$ | *log($n_{c1}$)* | *log($n_{c2}$)* | $u^2_R$ | $u^2_d$ | $u^2_o$ *(log10)* |
|---|---|---|---|---|---|---|
| | | | | (log $n_{c1}$ -log $n_{c2}$ )$^2$/2 | 2*0,1886 / (c1 +c2) | $u^2_R$ - $u^2_d$ |
| 27 | 22 | | | | | |
| 34 | 22 | | | | | |
| 36 | 26 | | | | | |
| … | … | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | mean | | | |

## C. perfingens

| mean | $u^2_R$ | $u^2_d$ | $u^2_o$ | conversion $u^2_o * 5,3$ | $u_o$ | % |
|---|---|---|---|---|---|---|
| 10 | 0,0187 | 0,0028 | 0,0160 | 0,0846 | 0,2908 | 29 |
| 20 | 0,0198 | 0,0163 | -0,0025 | -0,0134 | | |
| 30 | 0,0198 | 0,0092 | 0,0105 | 0,0558 | 0,2362 | 24 |

## E. coli

| | $u^2_R$ | $u^2_d$ | $u^2_o$ | conversion $u^2_o * 5,3$ | $u_o$ | % |
|---|---|---|---|---|---|---|
| 10 | 0,0151 | 0,0098 | 0,0053 | 0,0282 | 0,1679 | 17 |
| 20 | 0,0193 | 0,0101 | 0,0092 | 0,0487 | 0,2207 | 22 |
| 30 | 0,0157 | 0,0103 | 0,0053 | 0,0283 | 0,1682 | 17 |

for new results (single counts) calculate combined uncertainty of th
test result as:

$$u_c(x) = \sqrt{(u^2_{o} + u^2_{d})} \text{ with } u^2_d = 0{,}1886/n$$

.2.2 Combined uncertainty of colony counts

he scale of measurement is chosen according to the intended use of the combined uncertainty:

terval scale:

$$u_c = \sqrt{n_z + u^2_{o,rel} n^2_z}$$

here

$u_{o,rel}$ is the relative operational uncertainty component;

$n_z$ is the number of colonies observed.

# Measurement uncertainty - final remarks

- methods available that are suitable for microbiology
- not very much used
- not very much liked
- gives an order of magnitude to the uncertainty of your results
- helps to compare and evaluate methods

- did not go into details of other methods (RM, PT samples…)

Terminology in Analytical Measurement: Introduction to VIM 3 (2011)

Traceability in Chemical Measurement (2003)

The Selection and use of Reference Materials (2002)

Guide to Quality in Analytical Chemistry: An Aid to Accreditation (2002)

Accreditation for Microbiological Laboratories (2013)

Selection, Use and Interpretation of Proficiency Testing (PT) Schemes by Laboratories (2011)

Quantifying Uncertainty in Analytical Measurement, 3rd Edition (2012)

Measurement uncertainty arising from sampling (2007)

Use of uncertainty information in compliance assessment (2007)

The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics (201

Harmonised Guidelines for the Use of Recovery Information in Analytical Measurements (1998)

Quality Assurance for Research and Development and Non-routine Analysis (1998) | translations available