Measurement Uncertainty in Microbiological Examination of Foods

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Hilde Skår Norli

Norwegian Veterinary Institute

Veterinærinstituttet
Norwegian Veterinary Institute

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- Sources of uncertainty
- Distribution & expected standard deviations on microbiological examinations
- Estimation of MU in quantitative methods using standard deviation of internal reproducibility
- Checking the estimate

Requirements Accreditation Agency (Norway)

Qualitative analysis

- Identifying and weighting sources of uncertainties (2002)
- Be aware of /estimate the LOD, sensitivity and specificity

Quantitative analysis

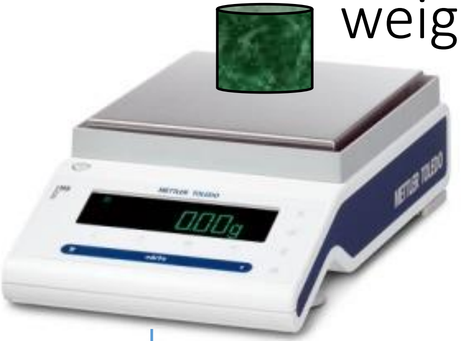
- Identifying and weighting uncertainty sources
- Estimate the measurement uncertainty (2010)

Sources of uncertainty

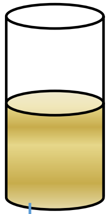
Sampling

Storage

Homogenization

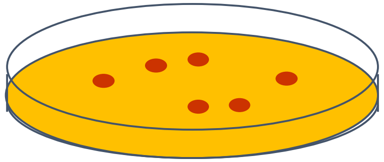


weighing



pipetting

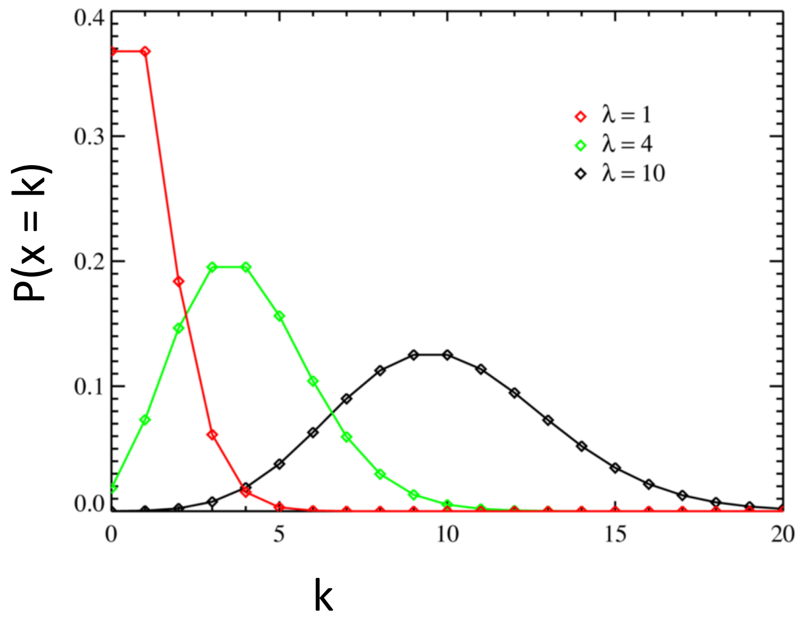
Initial suspension/
primary dilution
and decimal dilutions



Colony counting

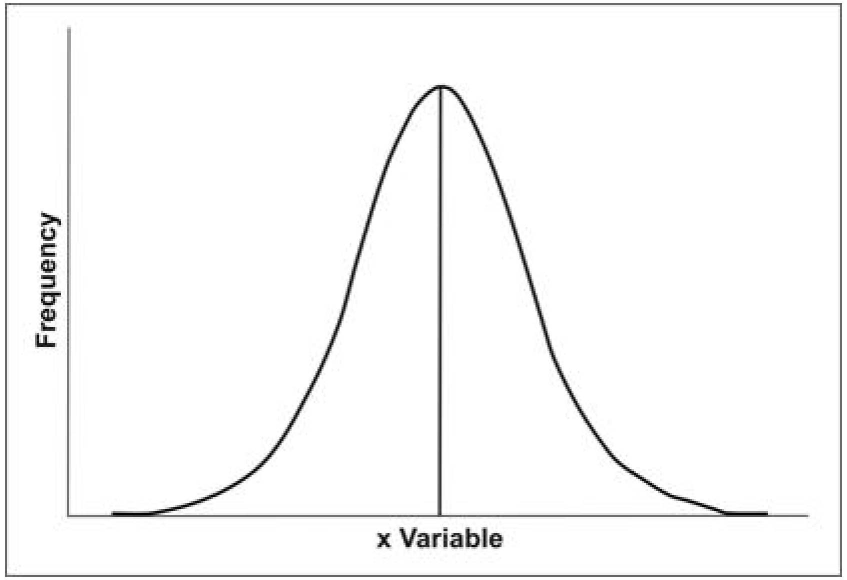
growth and death

Distribution - Poisson



- Dividing the population in two parts; whereof one of the two happenings occurs with a probability p and the other with a probability of q, and $p + q = 1$.
  When one of the happenings, p, is small we have Poisson distribution.
- The probability of finding the target bacteria might be small -> Poisson distribution

Distribution - Normal



In order to obtain normal distribution the results need to be transformed into $\log_{10}$ before statistical calculations are carried out.

Use of RSD

RSD = relative standard deviation

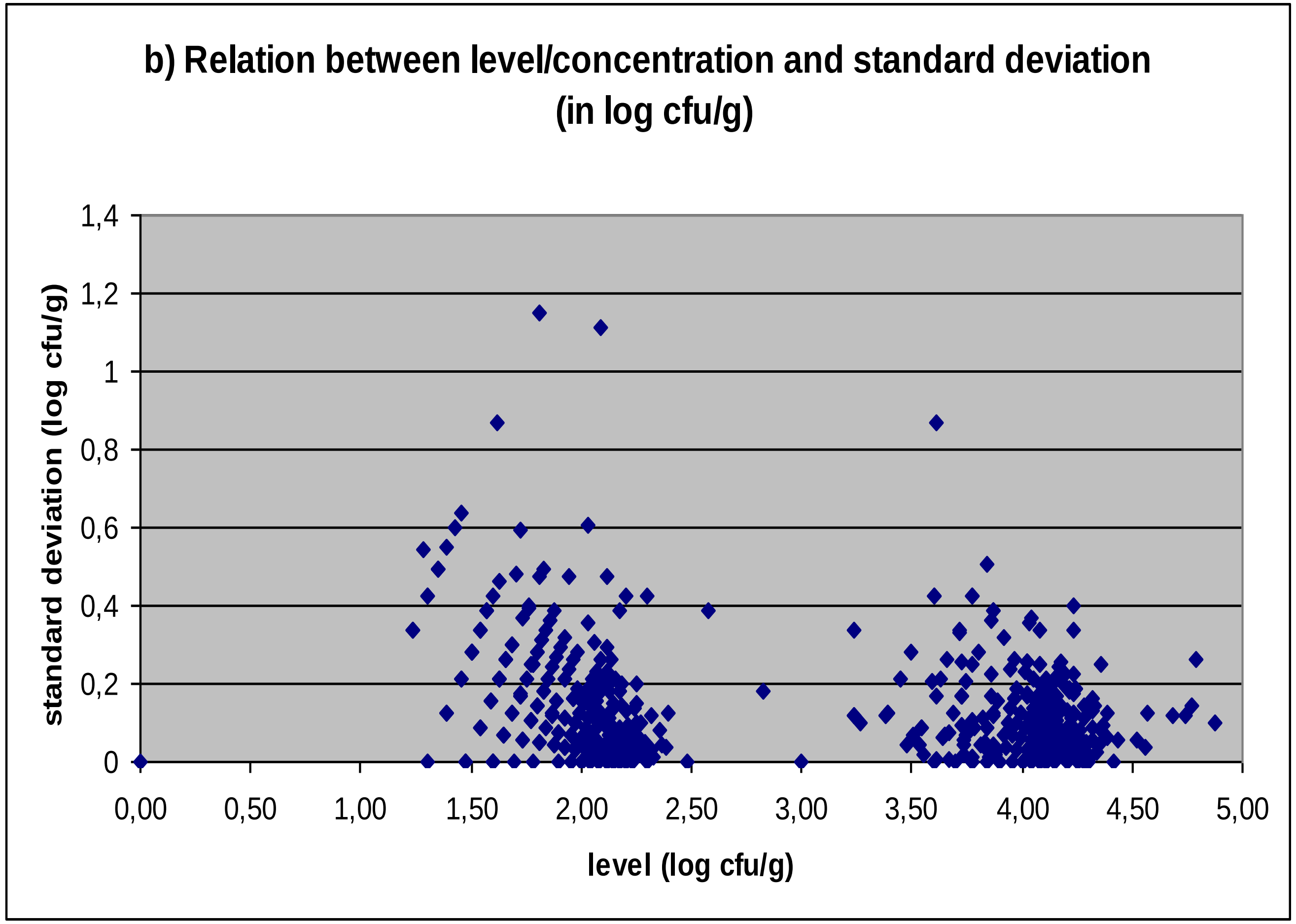
RSD = SD/ X · 100

Chemistry: RSD is used in MU

Microbiology: RSD should not be used

- SD is constant for different levels

Relation between level and SD



- 681 parallels (1362 analyses) of different microbes
- no increase in SD by increasing level, the relation is almost constant. No RSD!
- 98% of the results are below 0.5 $\log_{10}$ cfu/g,
- 96% of the results are below 0.4 $\log_{10}$ cfu/g,
- 94% of the results are below 0.35 $\log_{10}$ cfu/g,
- SD < 0.4 $\log_{10}$ cfu/g (at 95% confidence)

Estimation of Measurement Uncertainty

NMKL Proc 8, 2004 and ISO/NP 19036:2016 (current 2006)

- Use of $\log_{10}$ data
- Global approach (not step by step)
- Standard deviation of the internal reproducibility
  - Reproducible conditions: different time, analysts, reagents
- Standard deviation of reproducibility of the method derived from an interlaboratory study
- Standard deviation of reproducibility derived from an PT-scheme.

Design NMKL & ISO

NMKL:
Relevant matrices
Approved analyst

Laboratory sample

Homogenize

Test sample

Test Portion A

Test Portion B

Initial suspension

Initial Suspension

ISO: 10 lab samples

NMKL:
10 results/ plates
From A

ISO:
Result A

ISO:
Result B

NMKL:
10 results/ plates
from B

Reproducible conditions

MU Study Plan

- Homogenize before and after microbial inoculate is added
  - ISO (EURL Listeria): For a homogeneous matrix ; s_{matrix}= 0.1 log cfu/g
- Use of stressed organisms
- Not too low contamination level (Poisson distribution)
- Otherwise no need for several levels as s_r (in $\log_{10}$) is constant

MU Design

NMKL:
10 results from A +
10 results from B

= 20 results

NMKL:

- Similar to collaborative study

$S_{iR} = \sqrt{(S_r^2 + S_L^2)}$

S_{iR} = internal reproducibility,
S_r = repeatability
S_L = standard deviation between-series

ISO: 10 lab samples

1 result from A	1 result from B

ISO:

Standard deviation of the mean of the difference.

$D = A_1 - B_1$

$S_{iR} = S_D$

Example: NMKL

No	A	B
1	3.67	3.50
2	3.66	3.66
3	3.72	3.50
4	3.85	3.70
5	3.70	3.40
6	4.02	3.80
7	3.87	3.65
8	3.90	3.50
9	3.74	3.48
10	3.45	3.50

Mean for each, A & B	3.76	3.57
SD for each, A & B	0.16	0.13
Combined SD of A+B	$\sqrt{}(0.16^2 + 013^2)/2$=0.14	
Mean of A+B	(3.76+3.57)/2 = 3.66	
SD of A+B, s_x	$\sqrt{\frac{(3.76-3.66)^2+(3.57-3.66^2)}{1}}$ =0.13	
$s_L{}^2$ – variance between A&B	$0.13^2+0.14^2/10$= 0.015	
Reproducibility, s_{iR}	$\sqrt{}(0.14^2+0.015)$= 0.19	
MU = 2·u = 2· s_{iR}	**0.38**	

Example: ISO

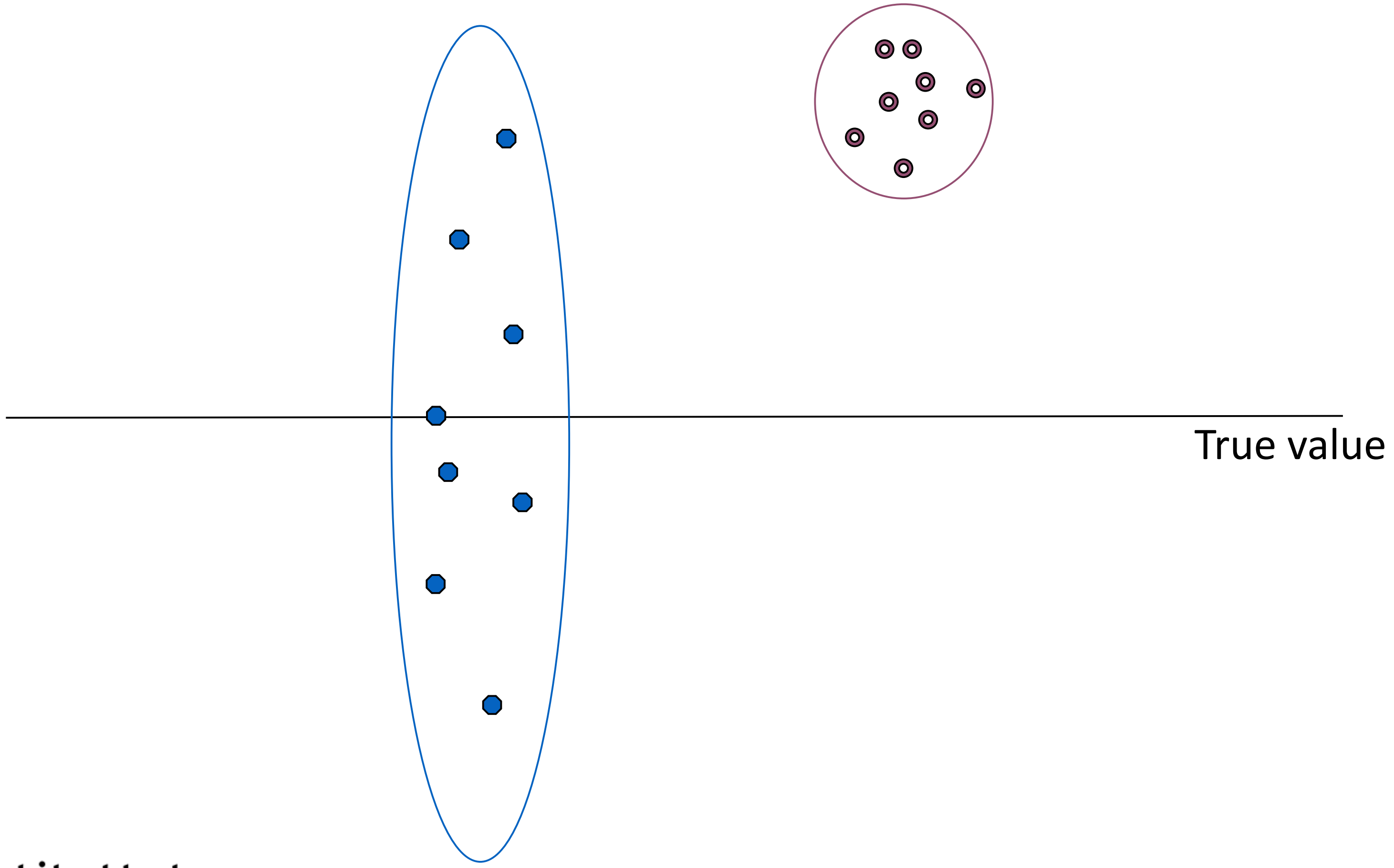
No	A	B	(a-b)^2
1	3.67	3.50	0.029
2	3.66	3.66	0.000
3	3.72	3.50	0.048
4	3.85	3.70	0.023
5	3.70	3.40	0.090
6	4.02	3.80	0.048
7	3.87	3.65	0.048
8	3.90	3.50	0.160
9	3.74	3.48	0.068
10	3.45	3.50	0.002
Sum			0.52

$$s_{iR} = \sqrt{\frac{\sum_{i=1}^{n}(a_i - b_i)^2}{2n}} = \sqrt{\frac{0.52}{2 \cdot 10}} = 0.16$$

$u = \sqrt{(s^2_{iR} + s^2_{Poisson} + s^2_{matrix})}$	$= \sqrt{(0.16^2 + 0.1^2)}$ $= 0.19$
MU = 2·u Unit	= 2· 0.19 = **0.38** log cfu/g

NMKL: MU = 2 x s_R	**0.38 log cfu/g**

Trueness & precision



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Trueness / Bias

- Use of CRM
- Participation in PT-schemes

Participate in PT to check if the estimate of MU is OK

$$Y \in X \pm MU$$

Y = “true” value
∈ = belongs to
X = our obtained value

Trueness (bias) + Precision (S_R)
= Accuracy(MU)

Checking the estimate of MU

$$\text{Zeta-score, } \zeta = \left(\frac{(X - Y)}{\sqrt{u_{lab}^2 + \left(\frac{s_{PT}}{\sqrt{n}} \right)^2}} \right)$$

Z-score

X = our result
Y = “true result” (mean value)
u = standard uncertainty of our method
s_{PT} = SD of true result (participants)

Checking the estimate of MU

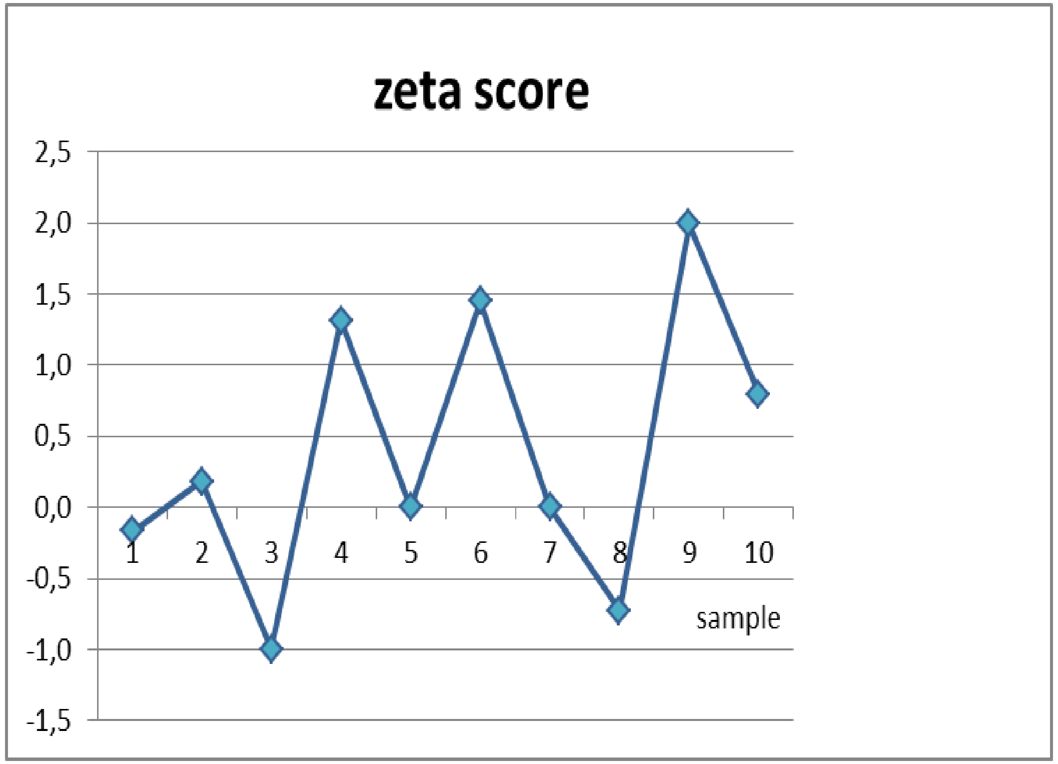
-2 ≤ zeta /z -score ≥ 2

Yes: the MU is OK,

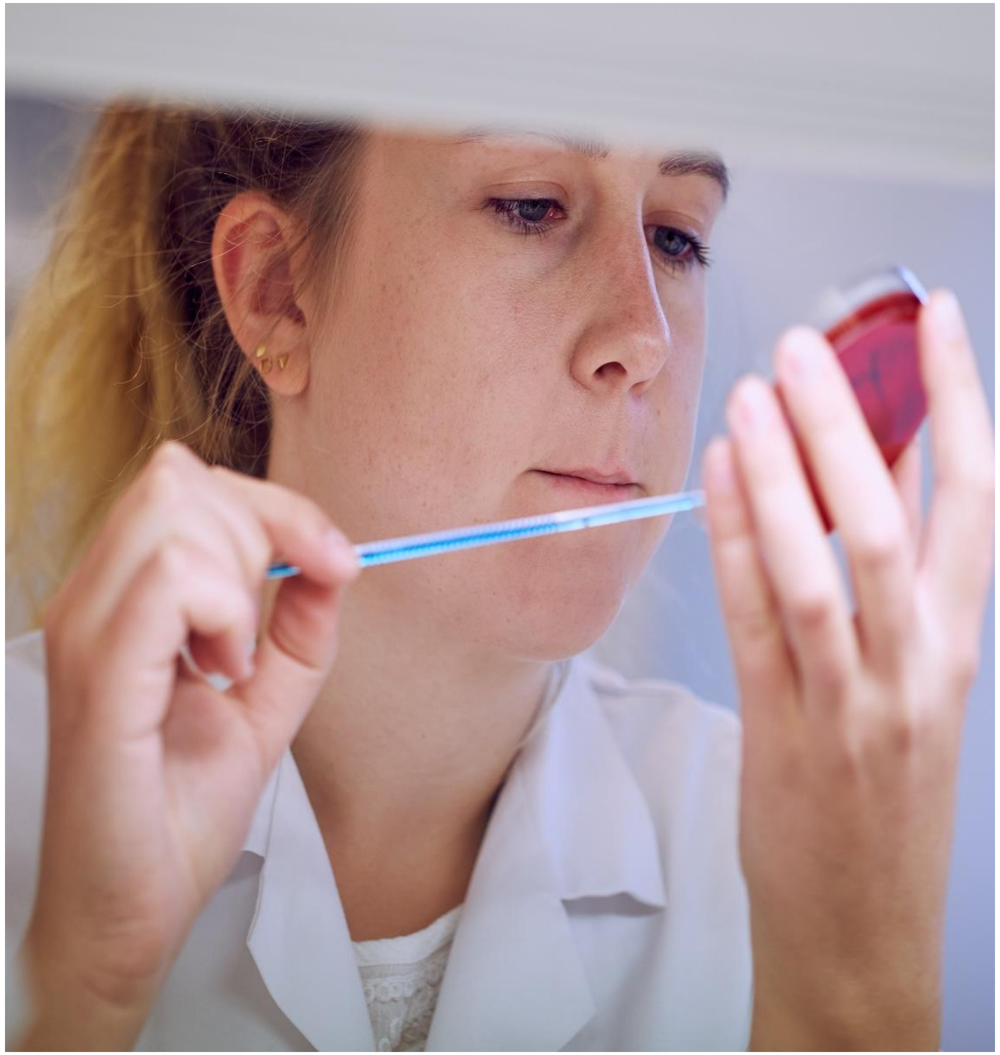
i.e. if S_R is OK (≤0.4 log cfu/g)

1: MU might be too narrow and needs to be expanded.

2: MU might be too wide



Most importantly, Competence!!



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References

- NMKL Procedure No. 8, 4th Ed., 2004: Measurement of uncertainty in quantitative microbiological examination of foods

- NMKL Procedure No. 32, 2017 Verification of microbiological methods

- ISO/NP 19036:2016. Microbiology of the food chain – Guidelines for the estimations of measurement uncertainty for quantitative determinations