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MEASUREMENT UNCERTAINTY FOR QUANTITATIVE ANALYSES: EN ISO 19036

TECHNICAL UNCERTAINTY

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EN ISO 19036:2019

Microbiology of the food chain - Estimation of measurement uncertainty for quantitative determinations

3 components of measurement uncertainty

- **Technical** Uncertainty
- **Matrix** Uncertainty
- **Distributional** Uncertainty

TECHNICAL UNCERTAINT; U_{tech} (1)

Uncertainty resulting from operational variability associated with the technical steps of the analytical procedures

- associated with the main stages in microbiological method
- considered as a performance characteristic when the method is implemented in a given laboratory
- usually, the largest of the three uncertainty components

TECHNICAL UNCERTAINT; U_{tech} (2)

- Characteristics of the method; technical uncertainty estimated for one method cannot be applied to other methods
- Estimated from the standard deviation of reproducibility on the final result of the measurement process; preferably based on “intralaboratory reproducibility”
- Estimated by performing experiment – Data may be collected in a short period of time as a special exercise

TECHNICAL UNCERTAINT; U_{tech} (3)

Measurements conditions

- the measurement conditions (e.g., A and B) for each test portions should differ in as many ways as possible
- the laboratory sample, where possible, should cover the expected natural variation in contamination level
- the pattern of variation should not be the same for all laboratory (test) samples
- If artificial contamination is needed, spike initial suspension

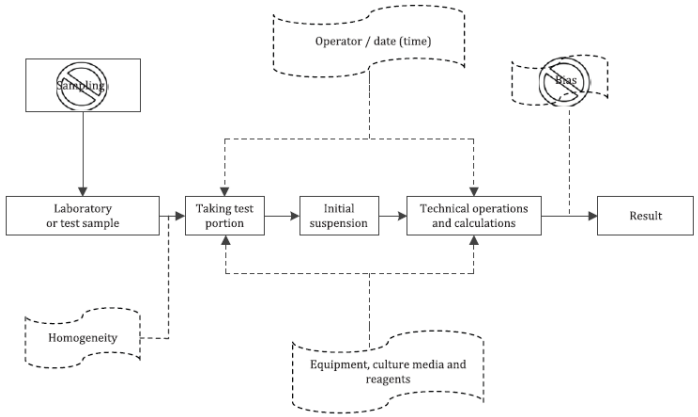
TECHNICAL UNCERTAINT; U_{tech} (3)

Typical sources of uncertainty are:

- stages of the test: e.g., weight of test portion; preparation of initial suspension, serial dilutions, inoculation, incubation, colony counting, confirmation
- batches of culture media, reagents, Equipment: e.g., weighing equipment; vortex/mixers; volumetric measuring/dispensing equipment; pipettes; incubators/baths
- tolerances within method: e.g., temperature range; incubation times
- technicians/operators

TECHNICAL UNCERTAINT; U_{tech} (4)

Technical uncertainty



Reproducibility standard deviation

Intra-laboratory S_{IR}

Inter-laboratory (Method validation) S_R

ILPT S_I

OPTION 1: INTRA-LABORATORY REPRODUCIBILITY

S_{IR} (2)

- ➢ Preferred option for deriving technical uncertainty:
- ✓ Reproductibility conditions are varied in the laboratory
- ✓ Provides uncertainty values that is linked to the laboratory
- ➢ To be determined for each method for at least 2 test portions
- ➢ Samples sujected to artificial contamination (if needed)

OPTION 1: INTRA-LABORATORY REPRODUCIBILITY

S_{IR} (2)

1. Experimental protocol

For each test method, perform the protocol for

- **At least 10 laboratory samples**
- **At least 2 acceptable measurements for each laboratory sample**
- **Repeat design for each laboratory sample**

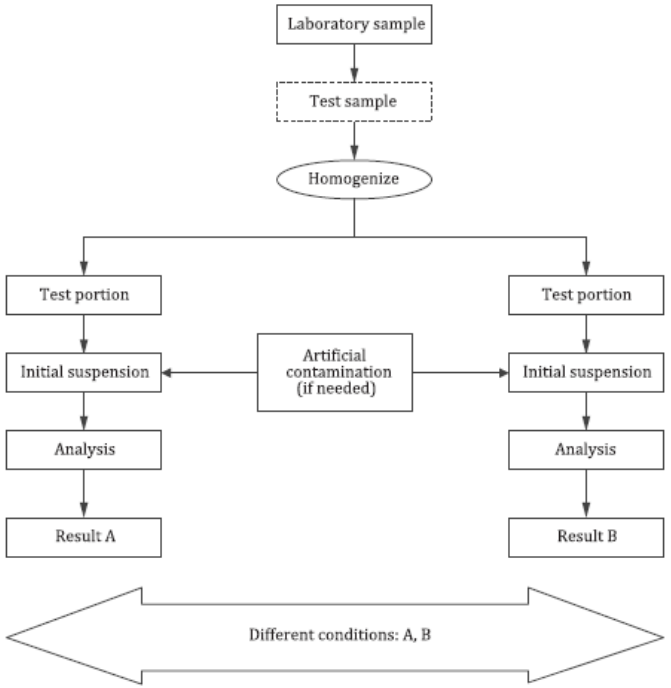


Figure 2 — Experimental protocol for estimation of intralaboratory reproducibility — Two determinations on each laboratory sample

OPTION 1: INTRA-LABORATORY REPRODUCIBILITY

s_{IR} (2)

2. Criteria for excluding and calculation

- The sum of the total colonies on all retained plates

30 ≤ $\sum C$ ≤ 300 cfu/plate or lower as specified in the specific standard

- Transform the result from each test portion into $\log_{10}$ before calculations
- Eg. for 10 lab samples from operators A and B

$$s_{IR} = \sqrt{\frac{1}{2n}\sum_{i=1}^{n}\left(y_{iA} - y_{iB}\right)^2}$$

where

i is the index of the sample, i = 1 to n ($n \geq 10$);

y_{iA}, y_{iB} are the log-transformed data, in $\log_{10}$ cfu/g or ml, from conditions A and B

OPTION 1: INTRA-LABORATORY REPRODUCIBILITY

S_{IR} (2)

2. Acceptable results and culculation

Description of data:		Utech LCSV pour méthode CFA 2009LR28 (données issues du dossier d'ad							
			Optional;						
Standard uncertainty	s_{IR}	0,14823	uncertainty components subtracted from s_{IR} to give $s_{IR:corr}$						
	$s_{IR:corr}$	0,14823	Matrix	Poisson		Confirmation			
Laboratory Sample ID	Result $\log_{10}$		u_{matrix}	ΣC	$u_{Poisson}$	n_p	n_c	u_{conf}	
19Q002517	4,832508913								
19Q002517	4,812913357								
19Q002518	4,913813852								
19Q002518	4,799340549								
19Q002326	3,806179974								
19Q002326	3,672097858								
19Q002327	3,770852012								
19Q002327	3,612783857								
19Q002832	3,342422681								
19Q002832	3,230448921								
19Q002979	2,949390007								
19Q002979	3,230448921								
19Q002566	2,380211242								
19Q002566	2,255272505								

OPTION 2: INTER-LABORATORY S_R (METHOD VALIDATION)

Method used routinely submitted to inter-laboratory validation study

Conditions to meet:
Repetability and reproductibility estimates of precisions with the lab shall not be larger than those obtained in the inter-lab study

- Advantages = use precision data already generated
- Drawbacks

Difficulty to generalise values from the inter-lab sutudy to routine samples.
Unavalaibility of reproducibility parameters from inter-lab method validation study for all methods.
This is why inter-laboratory SR from validation method is only an **OPTION 2**

OPTION 3: INTER-LABORATORY S_R (PROFICIENCY TEST)

Laboratory taken part in ILPT can use S_R to deduce its technical uncertainty

Conditions to meet:

Method used by the lab in PT = Same method that the laboratory uses in routine analyses

- Advantages = avalaible values, large number of PT schemes organized in food microbiology
- Drawbacks

Difficulty to generalise from ILPT to routine analyses performed by the laboratory.

PRACTICAL APPROACH TO ESTIMATE THE STANDARD DEVIATION OF REPRODUCIBILTY (S_R)

EXAMPLE OF INTRALABORATORY S_R

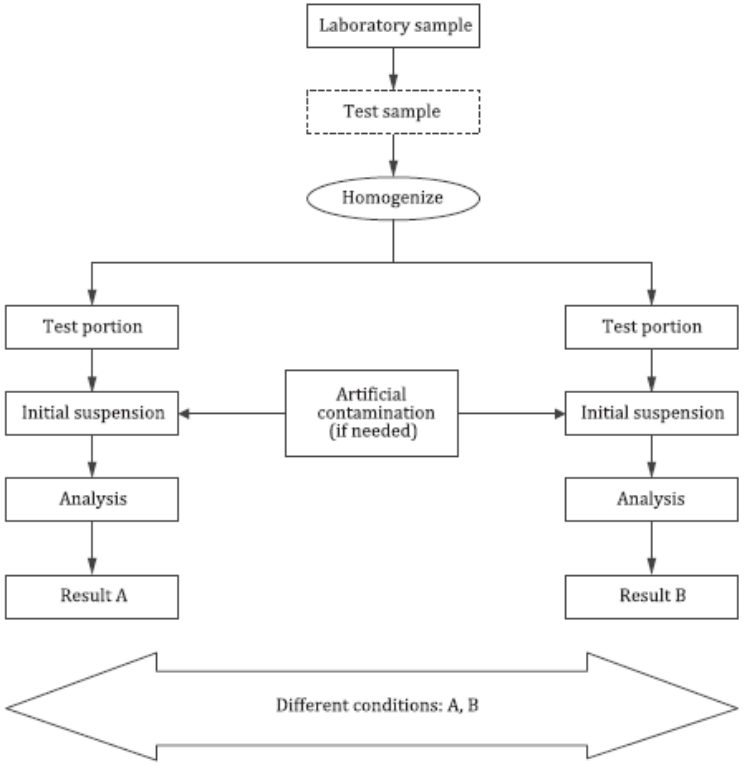


Figure 2 — Experimental protocol for estimation of intralaboratory reproducibility — Two determinations on each laboratory sample

Take 10 test portions from the same sample

Artificial or naturally contaminated sample Artificial contamination should be performed in initial suspension and should cover natural variation (

Homogenize before and after contamination

Implement the method on all the suspensions by inoculating the appropriate dilutiions

CALCULATION OF INTRALABORATORY S_R

anses

Example of CPS using EN ISO 6888-1 in cheese (Mozzarella)

Lab sample	Test portion	Inoculated volume	Dilution factor and copunted colonies					Total colonies counted	Result (EN ISO 7218	log10 CFU/g	Criteria compliance
			Nombre de	d1	C1	d2	C2				
1	1A	0,1	1	-1	57	-2	4	61	5,5E+03	3,74	conform
2	2A	0,1	1	-2	56	-3	2	58	5,3E+04	4,72	conform
3	3A	0,1	1	-2	54	-3	4	58	5,3E+04	4,72	conform
4	4A	0,1	1	-2	71	-3	5	76	6,9E+04	4,84	conform
5	5A	0,1	1	-3	65	-4	6	71	6,5E+05	5,81	conform
6	6A	0,1	1	-3	35	-4	7	42	3,8E+05	5,58	conform
7	7A	0,1	1	-3	48	-4	8	56	5,1E+05	5,71	conform
8	8A,	0,1	1	-4	63	-5	5	68	6,2E+06	6,79	conform
9	9A	0,1	1	-4	48	-5	4	52	4,7E+06	6,67	conform
10	10A	0,1	1	-4	57	-5	8	65	5,9E+06	6,77	conform
11	11A	0,1	1	-5	64	-6	11	75	6,8E+07	7,83	conform
12	12A	0,1	1	-5	53	-6	4	57	5,2E+07	7,71	conform

*: Dilution "-1" corresponds to intial solution

Criteria for the acceptability of the result

1- For each test portion, the sum of colonies retained for counting must be ≥ 30.

2- Methods including partial confirmation: at least half of the tested colonies confirmed

CALCULATION OF INTRALABORATORY S_R

anses

Example of CPS using EN ISO 6888-1 in cheese (Mozzarella)

Lab sample	Test portion	Inoculated volume	Dilution factor and copunted colonies					Total colonies counted	Result (EN ISO 7218	log10 CFU/g	Criteria compliance
			Nombre de	d1	C1	d2	C2				
1	1B	0,1	1	-1	84	-2	19	103	9,4E+03	3,97	conform
2	2B	0,1	1	-2	84	-3	8	92	8,4E+04	4,92	conform
3	3B	0,1	1	-2	86	-3	9	95	8,6E+04	4,94	conform
4	4B	0,1	1	-2	108	-3	9	117	1,1E+05	5,03	conform
5	5B	0,1	1	-3	104	-4	3	107	9,7E+05	5,99	conform
6	6B	0,1	1	-3	86	-4	6	92	8,4E+05	5,92	conform
7	7B	0,1	1	-3	122	-4	15	137	1,2E+06	6,10	conform
8	8B	0,1	1	-4	135	-5	11	146	1,3E+07	7,12	conform
9	9B	0,1	1	-4	123	-5	11	134	1,2E+07	7,09	conform
10	10B	0,1	1	-4	96	-5	18	114	1,0E+07	7,02	conform
11	11B	0,1	1	-5	110	-6	6	116	1,1E+08	8,02	conform
12	12B	0,1	1	-5	109	-6	20	129	1,2E+08	8,07	conform

*: Dilution "-1" corresponds to intial solution

Criteria for the acceptability of the result

1- For each test portion, the sum of colonies retained for counting must be ≥ 30.

2- Methods including partial confirmation: at least half of the tested colonies confirmed

CALCULATION OF INTRALABORATORY S_R

Description of data:																
Too few Results			Optional;							MPN						
Standard uncertainty	s_{IR}	0	uncertainty components subtracted from s_{IR} to give $s_{IR:corr}$							n_i						
	$s_{IR:corr}$		Matrix	Poisson		Confirmation				m_i						
Laboratory Sample ID	Result $\log_{10}$		u_{matrix}	ΣC	$u_{Poisson}$	n_p	n_c	u_{conf}		u_{MPN}	x_i					
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CALCULATION OF INTRALABORATORY S_R

Example of total microflora using EN ISO 4833-1 (Amd1 2022) in cheese (Mozzarella)

Lab sample	Test portion	Inoculated volume	Dilution factor and copunted colonies					Total colonies counted	Result (EN ISO 7218	log10 CFU/g	Criteria compliance
			Nombre de	d1	C1	d2	C2				
1	1A	1	1	-5	11	-6	0	11	1,0E+06	6,00	not conform
2	2A	1	1	-5	5	-6	0	5	4,5E+05	5,66	not conform
3	3A	1	1	-5	16	-6	1	17	1,5E+06	6,19	not conform
4	4A	1	1	-5	10	-6	0	10	9,1E+05	5,96	not conform
5	5A	1	1	-3	155	-4	12	167	1,5E+05	5,18	conform
6	6A	1	1	-3	98	-4	7	105	9,5E+04	4,98	conform
7	7A	1	1	-4	58	-5	10	68	6,2E+05	5,79	conform
8	8A	1	1	-3	229	-4	21	250	2,3E+05	5,36	conform
9	9A	1	1	-4	198	-5	28	226	2,1E+05	5,31	conform
10	10A	1	1	-4	69	-5	7	76	6,9E+05	5,84	conform
11	11A	1	1	-3	102	-4	14	116	1,1E+05	5,02	conform
12	12A	1	1	-3	183	-4	17	200	1,8E+05	5,26	conform

*: Dilution "-1" corresponds to intial solution

Criteria for the acceptability of the result

1- For each test portion, the sum of colonies retained for counting must be ≥ 30.

2- Methods including partial confirmation: at least half of the tested colonies confirmed

CALCULATION OF INTRALABORATORY S_R

Example of total microflora using EN ISO 4833-1 (Amd1 2022) in naturally contaminated frozen melon

Lab sample	Test portion	Inoculated volume	Dilution factor and copunted colonies					Total colonies counted	Result (EN ISO 7218	log10 CFU/g	Criteria compliance
			Nombre de	d1	C1	d2	C2				
1	1B	1	1	-5	6	-6	1	7	6,4E+05	5,80	not conform
2	2B	1	1	-5	10	-6	0	10	9,1E+05	5,96	not conform
3	3B	1	1	-5	15	-6	0	15	1,4E+06	6,13	not conform
4	4B	1	1	-5	10	-6	0	10	9,1E+05	5,96	not conform
5	5B	1	1	-3	158	-4	25	183	1,7E+05	5,22	conform
6	6B	1	1	-3	149	-4	14	163	1,5E+05	5,17	conform
7	7B	1	1	-4	35	-5	5	40	3,6E+05	5,56	conform
8	8B	1	1	-3	222	-4	21	243	2,2E+05	5,34	conform
9	9B	1	1	-3	31	-4	2	33	3,0E+04	4,48	conform
10	10B	1	1	-4	43	-5	1	44	4,0E+05	5,60	conform
11	11B	1	1	-3	177	-4	14	191	1,7E+05	5,24	conform
12	12B	1	1	-3	197	-4	25	222	2,0E+05	5,30	conform

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CALCULATION OF INTRALABORATORY S_R

Description of data:																
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	$s_{IR:corr}$		Matrix	Poisson		Confirmation				m_i						
Laboratory Sample ID	Result $\log_{10}$		u_{matrix}	ΣC	$u_{Poisson}$	n_p	n_c	u_{conf}		u_{MPN}	x_i					
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