MATRIX UNCERTAINTY-CALCULATIONS IN EXCEL TOOL

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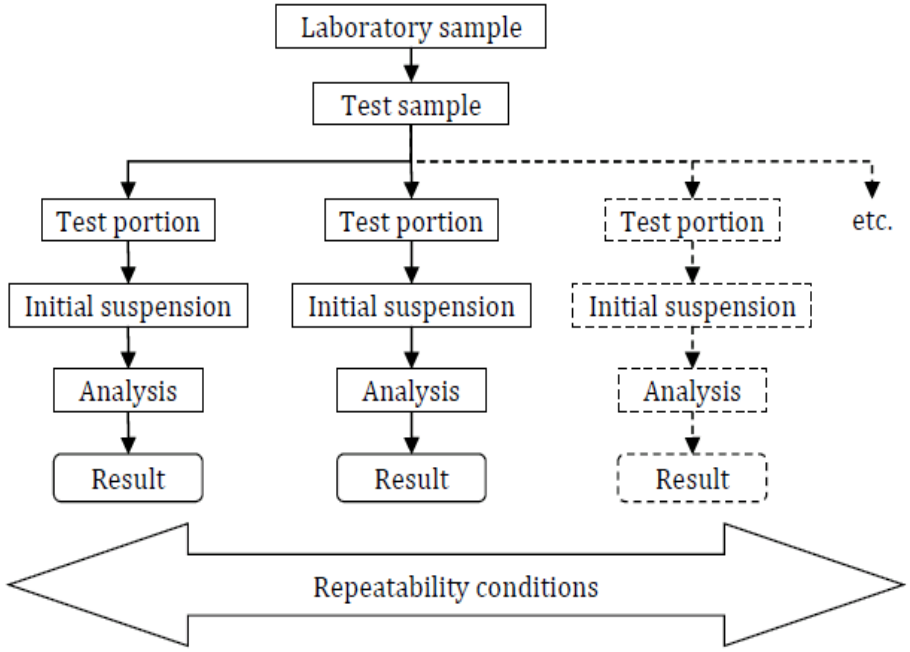
IF MATRIX UNCERTAINTY IS NOT IN DATABASE FOR YOUR MATRIX

- Estimate the *within-laboratory-sample repeatability standard deviation* s_r
- Perform exeriment using specified setup
- Calculate repeatability standard deviation s_r
- Matrix standard uncertainty: $u_{matrix} = s_r$

REPEATABILITY EXPERIMENT

- Repeat design for each laboratory sample
  - do not homogenise
  - do not artificially contaminate
- Matrix uncertainty is regarded as independent of target microorganism and test method used
  - chose target microorganisms for which naturally contaminated samples are likely to be found (e.g. TVC)



From ISO-19036 presenation

REPEATABILITY EXPERIMENT

- take at least two test portions from each laboratory sample
- total number of test portions = at least 10 or more than the number of laboratory samples, i.e.
  - 1 laboratory sample
    - at least 11 results (all in same batch)
  - 10 or more laboratory samples (from same matrix)
    - at least 2 results each (replicates in same batch)

ISO-19036 CALCULATION TOOL

- Excel tool implementing the calculations of ISO 19036 (2019)
  - Developed by Campden BRI (UK)
  - Verified by WG 2 of ISO/TC 34/SC 9
  - Freely available on line at https://committee.iso.org/sites/tc34sc9/home/general-standards/content-left-area/culture-media/iso-19036-estimation-of-measurem.html

EXCEL CALCULATIONS TOOL

- 2nd worksheet “Reproducibility”
  - Calculates the intralaboratory reproducibility standard deviation (s_{IR})
    = 1st option to estimate the technical uncertainty

- 3rd worksheet “Repeatability”
  - Calculates the repeatability standard deviation
    = to estimate the matrix uncertainty

- 4rd worksheet “Combines”
  - Estimate MU
    = to estimate the measurement uncertainty

DATA

1	Laboratory			Contact person		Email address	
2	Country			GDPR agreement	Yes		
3	**Method**			**Repeatability conditions for test portions of the same sample**		**Matrix tested**	
4	Target organism	Total mesophilic aerobic count		Operator repeatability	Yes	Category	Pet food and animal feed
5	Method	ISO 4833-1:2013		Equipment repeatability	Yes	Type	Animal feeds (fish)
6	Accreditation	Yes		Media and reagents repeatability	Yes	Matrix	Flour
7	General comment on the method or matrix			Weight of each test portion 10 g	Yes	Additional description	

		Sample and conditions				Results								
10	*ref_n*	*sample_ID*	*test_portion_ID*	*start_date*	*start_time*	*dilution_1*	*volume_1*	*colony_count_dil1_plate1*	*colony_count_dil1_plate2*	*dilution_2*	*volume_2*	*colony*	*colony*	*confirme*
11	1	1	1a	2021-10-08	09:30	−2	1.0	109		−3	1.0	14		
12	2	1	1b	2021-10-08	09:30	−2	1.0	87		−3	1.0	8		
13	3	1	1c	2021-10-08	09:30	−2	1.0	143		−3	1.0	19		
14	4	1	1d	2021-10-08	09:30	−2	1.0	99		−3	1.0	12		
15	5	1	1e	2021-10-08	09:30	−2	1.0	130		−3	1.0	12		
16	6	1	1f	2021-10-08	09:30	−2	1.0	86		−3	1.0	8		
17	7	1	1g	2021-10-08	09:30	−2	1.0	99		−3	1.0	8		
18	8	1	1h	2021-10-08	09:30	−2	1.0	102		−3	1.0	9		
19	9	1	1i	2021-10-08	09:30	−2	1.0	111		−3	1.0	10		
20	10	1	1j	2021-10-08	09:30	−2	1.0	138		−3	1.0	18		
21	11	1	1k	2021-10-08	09:30	−2	1.0	112		−3	1.0	14		
22	12													
23	13													
24	14													
25	15													
26	16													
27	17													
28	18													
29	19													
30	20													

CALCULATE CONC AND LOG10CONC

Make new columns with appropriate formulae

	Operator repeatability	Yes	**Category**	Raw meat and ready-to-cook meat products (except poultry)
	Equipment repeatability	Yes	**Type**	Fresh meats (unprocessed)
	Media and reagents repeatability	Yes	**Matrix**	Pork meat cuts
	Weight of each test portion 10 g	Yes	**Additional description**	Laboratory samples - portions cut of pork meat (300-500g), refrigerated, collected from retail

Results

dilution_1	volume_1	colony_count_dil1_plate1	colony_count_dil1_plate2	dilution_2	volume_2	colony_	colony_	confirmed	comment	konc	log10conc	r
−1	1.0	76		−2	1.0	6						
−1	1.0	42		−2	1.0	6						
−1	1.0	114		−2	1.0	9						
−1	1.0	66		−2	1.0	6						
−2	1.0	180		−3	1.0	15						
−2	1.0	259		−3	1.0	23						
−1		156		−2	1.0	14						
−1		113		−2	1.0	10						
−3		65		−4	1.0	9						
−3		41		−4	1.0	6						
−3		99		−4	1.0	8						
−3		143		−4	1.0	17						
−3	1.0	30		−4	1.0	4						
−3	1.0	43		−4	1.0	5						
−2	1.0	154		−3	1.0	12						
−2	1.0	118		−3	1.0	8						
−2	1.0	103		−3	1.0	11						
−2	1.0	142		−3	1.0	19						
−3	1.0	99		−4	1.0	11						
−3	1.0	127		−4	1.0	12						

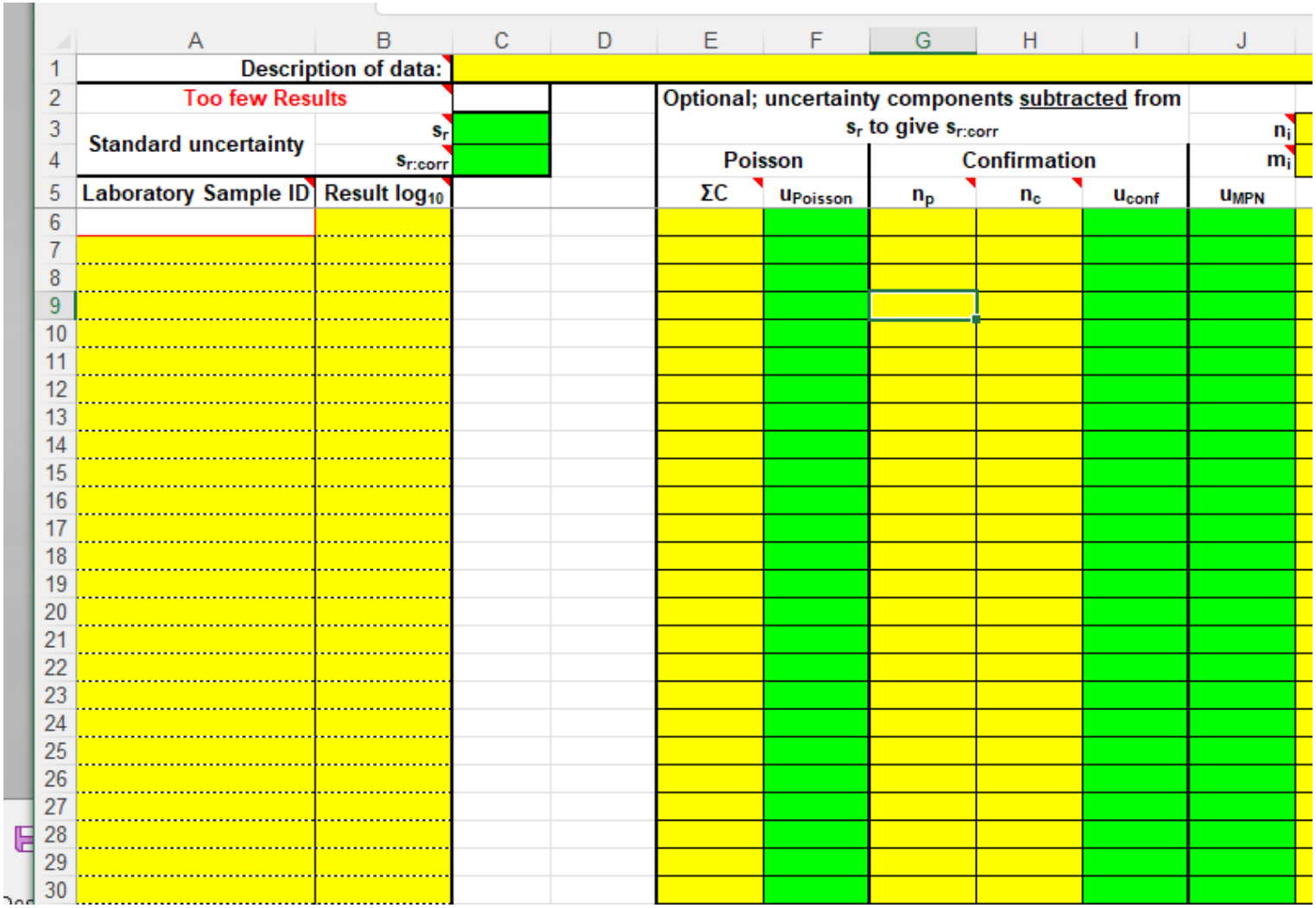
Indicate the volume inoculated (in ml) of the first dilution to use in calculation, e.g. 0.1, 1.0 (or 1,0, 0,1 etc, depending on format in Excel).

- How would you make the formula?

ENTER DATA IN TOOL ”REPEATABILITY”

Enter data – check README for format *etc.*

Ignore the optional columns (poisson etc)



	A	B	C	D	E	F	G	H	I	J
1	Description of data:									
2	Too few Results				Optional; uncertainty components subtracted from s_r to give $s_{r:corr}$					
3	Standard uncertainty	s_r								n_i
4		$s_{r:corr}$			Poisson		Confirmation			m_i
5	Laboratory Sample ID	Result $\log_{10}$			ΣC	$u_{Poisson}$	n_p	n_c	u_{conf}	u_{MPN}
6										
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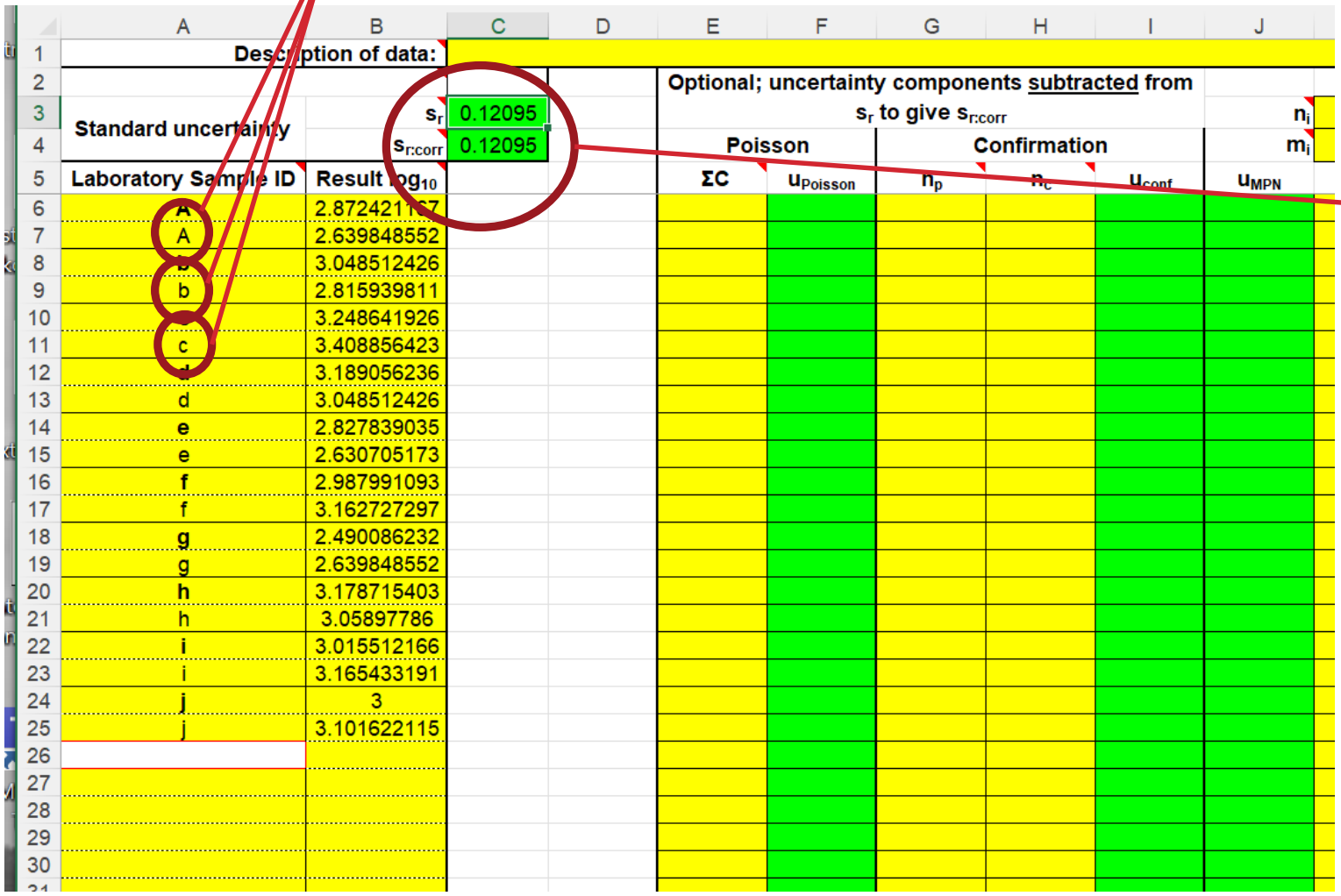
ENTER DATA IN TOOL ”REPEATABILITY”

OBS: Repeated test portions from same sample should have the same Sample ID (make sure to document)

Could also be 10+ test portions from one sample!



	A	B	C	D	E	F	G	H	I	J
1	Description of data:									
2					Optional; uncertainty components subtracted from					
3	Standard uncertainty	s_r	0.12095		s_r to give $s_{r:corr}$					n_i
4		$s_{r:corr}$	0.12095		Poisson		Confirmation			m_i
5	Laboratory Sample ID	Result $\log_{10}$			ΣC	$u_{Poisson}$	n_p	n_c	u_{conf}	u_{MPN}
6	A	2.872421167								
7	A	2.639848552								
8	b	3.048512426								
9	b	2.815939811								
10	c	3.248641926								
11	c	3.408856423								
12	d	3.189056236								
13	d	3.048512426								
14	e	2.827839035								
15	e	2.630705173								
16	f	2.987991093								
17	f	3.162727297								
18	g	2.490086232								
19	g	2.639848552								
20	h	3.178715403								
21	h	3.05897786								
22	i	3.015512166								
23	i	3.165433191								
24	j	3								
25	j	3.101622115								
26										
27										
28										
29										
30										

s_r is our estimate of matrix uncertainty

s_{rcorr} is obtained if we enter values in the distribution uncertainty columns. NOT used in standard.

WHAT ABOUT THE REST OF THE COLUMNS?

	A	B	C	D	E	F	G	H	I	J
1	Description of data:									
2					Optional; uncertainty components subtracted from s_r to give $s_{r:corr}$					n_i
3	Standard uncertainty	s_r	0.12095							
4		$s_{r:corr}$	0.12095		Poisson		Confirmation			m_i
5	Laboratory Sample ID	Result $\log_{10}$			ΣC	$u_{Poisson}$	n_p	n_c	u_{conf}	u_{MPN}
6	A	2.872421167								
7	A	2.639848552								
8	b	3.048512426								
9	b	2.815939811								
10	c	3.248641926								
11	c	3.408856423								
12	d	3.189056236								
13	d	3.048512426								
14	e	2.827839035								
15	e	2.630705173								
16	f	2.987991093								
17	f	3.162727297								
18	g	2.490086232								
19	g	2.639848552								
20	h	3.178715403								
21	h	3.05897786								
22	i	3.015512166								
23	i	3.165433191								
24	j	3								
25	j	3.101622115								
26										
27										
28										
29										
30										

Leave empty!

Possible to calculate but we don´t

When >30 colonies counted $U_{poisson}$ is ínsignificant

When 5/5 colonies are confirmed $U_{confirm}$ is ínsignificant

Matrix uncertainty is calculated using organisms that does not need confirmation

CAN YOU CALCULATE MANUALLY?

konc	log10conc	räknade	n = 10	SIR
745.45455	2.8724212	82	0.05409	0.12095
436.36364	2.6398486	48		
1118.1818	3.0485124	123	0.05409	
654.54545	2.8159398	72		
1772.7273	3.2486419	195	0.02567	
2563.6364	3.4088564	282		
1545.4545	3.1890562	170	0.01975	
1118.1818	3.0485124	123		
672.72727	2.827839	74	0.03886	
427.27273	2.6307052	47		
972.72727	2.9879911	107	0.03053	
1454.5455	3.1627273	160		
309.09091	2.4900862	34	0.02243	
436.36364	2.6398486	48		
1509.0909	3.1787154	166	0.01434	
1145.4545	3.0589779	126		
1036.3636	3.0155122	114	0.02248	
1463.6364	3.1654332	161		

Root(sum of squares)

Diff^2

THANK YOU FOR YOUR ATTENTION

Do not forget to report new estimtes of matrix uncertainty to NRL or EURL!

CALCULATE MU OF A NEW RESULT (SHEET ”COMBINED”)

Matrix U from database or own study

	A	B	C	D	E	F	G	H	I	J	K	L
1	Sample ID	Result $\log_{10}$	Uncertainty			Optional components added to technical uncertainty to give combined uncertainty						
2			Combined									
3			Standard	Expanded	Technical	Matrix	Poisson		Confirmation			r
4			$u_c(y)$	U	u_{tech}	u_{matrix}	ΣC	$u_{Poisson}$	n_p	n_c	u_{conf}	u_{MPN}
5	1a	2.872421167	0.23372687	0.4674537	0.2	0.12095						
6	1b	2.859648552	0.23372687	0.4674537	0.2	0.12095						
7	2a	3.048512426	0.23372687	0.4674537	0.2	0.12095						
8	2b	2.815939811	0.23372687	0.4674537	0.2	0.12095						
9	3a	3.248641926	0.23372687	0.4674537	0.2	0.12095						
10	3b	3.408856423	0.23372687	0.4674537	0.2	0.12095						
11	4a	3.189056236	0.23372687	0.4674537	0.2	0.12095						
12	4b	3.048512426	0.23372687	0.4674537	0.2	0.12095						
13	5a	2.827839035	0.23372687	0.4674537	0.2	0.12095						
14	5b	2.630705173	0.23372687	0.4674537	0.2	0.12095						

Sample ID and log-concentration from

Beware that matrix uncertainty may be copied automatically from sheet ”repeatability”
(but not in my hands…)