Comparing Probiotic Plate Count Methods by APLM

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Chair, Measurement & Data Quality Expert Committee and a member of the Probiotic Expert Committee

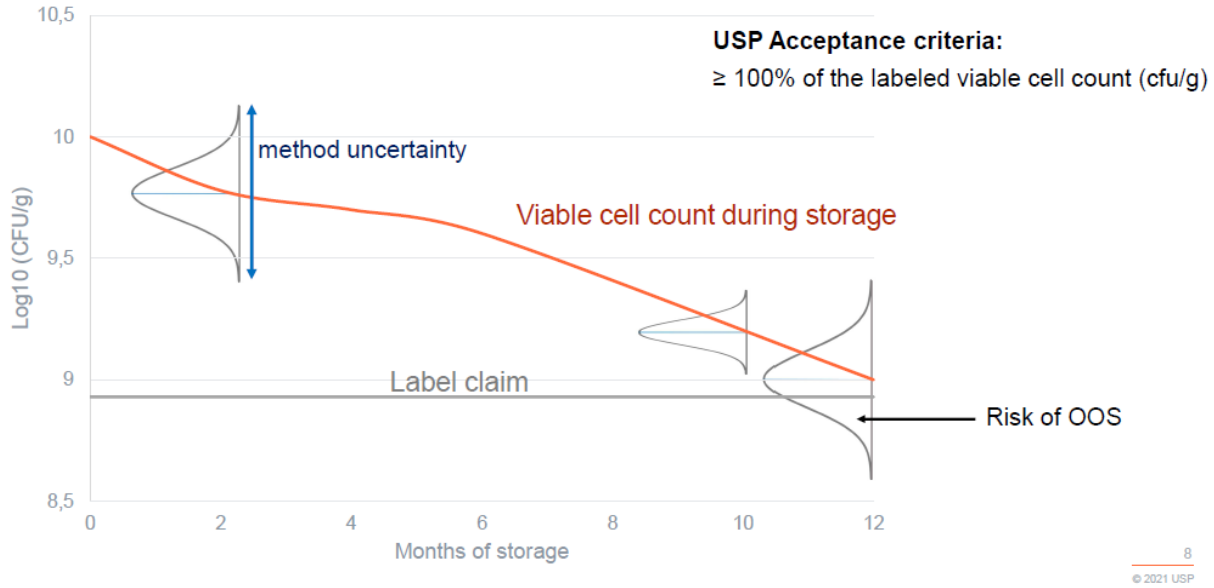
200 usp
The standard of trust

Outline

MS EXCEL Tool

- MS EXCEL and ANOVA Workbook
- Evaluate the MU
- Compare Analytical Procedures
- Your Input
  - Standalone Complete Workbook
  - Workbook as a template for your modification
  - Provide MS EXCEL formulas

Measurement Uncertainty



Variables set at defined levels - conditions

Terminology: conditions, treatment, within groups

How does APLM work?

Risks become ANOVA variables

UNCERTAINTY COMPONENT	CONDITIONS			
	1	2	3	4
Days	A	B	C	D
Analyst	A	B	A	C
Lot of Plating Medium	1	2	1	2
Lot of Suspension / Rehydration Medium	2	2	1	1
Lot of Dilution Buffer	1	2	3	4
Disposable Serological Pipettes	Lot 1	Lot 2	Lot 1	Lot 3
Pipettor with Tips	Set A	Set B	Set A	Set C
pH Meter	A	B	A	B
Analytical Balance	1	2	2	1
Autoclave	1	2	3	2
Agar Tempering Water Bath	2	1	1	2
Incubator	2	3	1	5

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Table Summary

How is it obtained?

How does APLM Work?

ANOVA TABLE

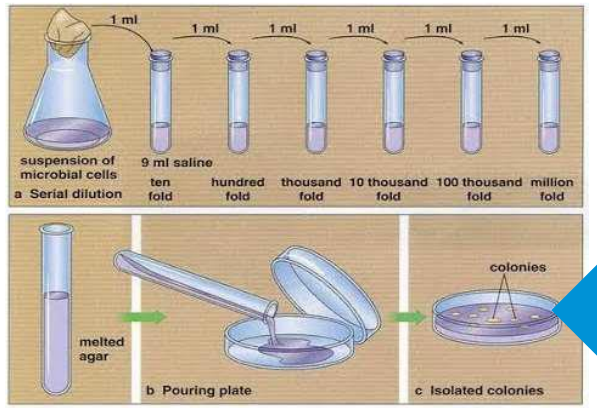
Replicate	Counts Log_{10} CFU/g															
	Condition 1				Condition 2				Condition 3				Condition 4			
	1	2	3	Average	1	2	3	Average	1	2	3	Average	1	2	3	Average
1	11.336	11.478	11.424	11.416	11.236	11.443	11.311	11.330	11.335	11.575	11.234	11.381	11.162	11.326	11.211	11.233
2	11.146	11.312	11.485	11.404	11.442	11.357	11.224	11.341	11.531	11.606	11.584	11.574	10.964	10.996	10.959	10.973
3	11.506	11.688	11.583	11.592	11.466	11.274	11.348	11.356	11.418	11.373	11.386	11.392	11.169	10.946	10.945	11.020
4	11.324	11.363	11.178	11.288	11.167	11.297	11.295	11.253	11.506	11.275	11.322	11.368	10.929	11.018	11.112	11.020
5	11.397	11.519	11.358	11.425	11.424	11.267	11.416	11.369	11.351	11.315	11.282	11.316	11.206	10.986	11.093	11.095
6	11.511	11.639	11.565	11.572	11.416	11.272	11.439	11.376	11.439	11.460	11.695	11.531	10.962	10.815	10.798	10.858
7	11.436	11.510	11.503	11.483	11.511	11.338	11.446	11.432	11.446	11.546	11.441	11.478	11.154	11.290	11.071	11.172
8	11.551	11.700	11.486	11.579	11.193	11.203	11.366	11.254	11.413	11.409	11.389	11.404	11.047	11.191	11.081	11.106
9	11.429	11.607	11.521	11.519	11.283	11.276	11.265	11.275	11.334	11.563	11.018	11.305	10.870	11.005	10.819	10.898
10	11.733	11.712	11.462	11.636	11.258	10.997	11.156	11.137	11.407	11.201	11.486	11.365	10.999	11.074	11.127	11.067
Std. Dev. (S_c)				0.1080				0.0841				0.0888				0.1160
Variance (S_c^2)				0.0117				0.0071				0.0079				0.0134
Average (C)				11.491				11.312				11.411				11.044
Intermediate precision = Pooled Std. Dev (S_{ip})	0.1001															
Std. Dev. for single plate count (S_{PT})	0.1033															
SEM for average of three plate counts (S_{PS})	0.05964															
Std. Dev. for sample preparation (S_{PREP})	0.080393															

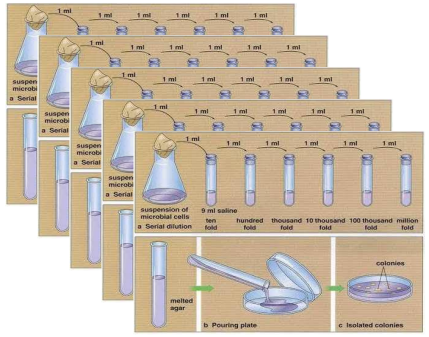
Workbook Example

Design is flexible

- The workbook is designed for
  - up to 4 plates/replicate
  - up to 10 replicates/condition
  - up to 6 conditions
  - For simplicity in this talk, will show only one condition

6 conditions x 10 replicates x 4 plates





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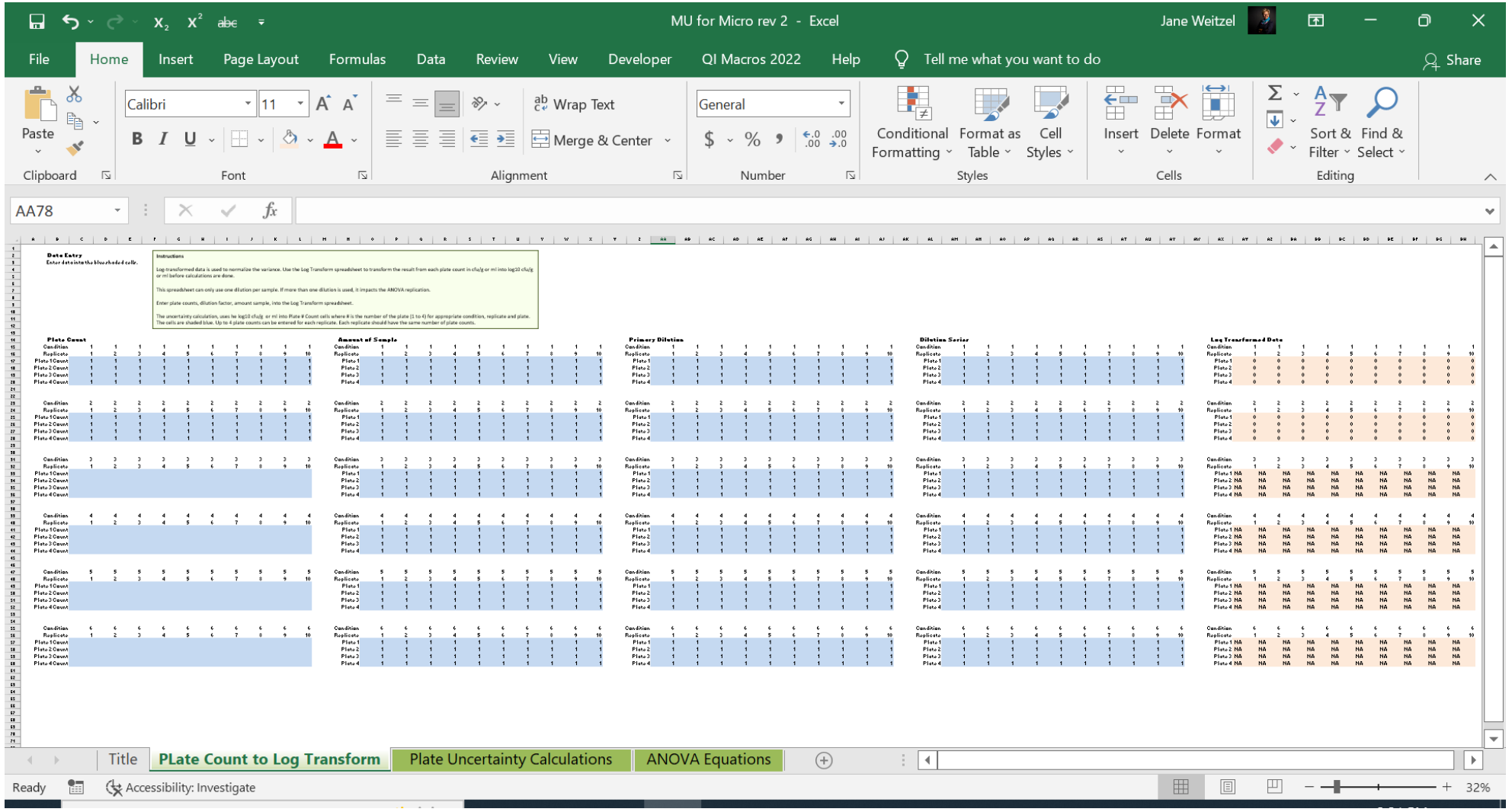
Workbook Overview



Enter Plate Count

Blue cells are for data entry. The value “1” is shown as an example.

	Plate Count									
	Condition	1	1	1	1	1	1	1	1	1	1
	Replicate	1	2	3	4	5	6	7	8	9	10
Plate 1 Count		1	1	1	1	1	1	1	1	1	1
Plate 2 Count		1	1	1	1	1	1	1	1	1	1
Plate 3 Count		1	1	1	1	1	1	1	1	1	1
Plate 4 Count		1	1	1	1	1	1	1	1	1	1

Amount of Sample

Units can be weight or volume and amount is the same for each plate

Amount of Sample										
Condition	1	1	1	1	1	1	1	1	1	1
Replicate	1	2	3	4	5	6	7	8	9	10
Plate 1	1	1	1	1	1	1	1	1	1	1
Plate 2	1	1	1	1	1	1	1	1	1	1
Plate 3	1	1	1	1	1	1	1	1	1	1
Plate 4	1	1	1	1	1	1	1	1	1	1

Primary Dilution

e.g. 10 is 1/10

Primary Dilution										
Condition	1	1	1	1	1	1	1	1	1	1
Replicate	1	2	3	4	5	6	7	8	9	10
Plate 1	1	1	1	1	1	1	1	1	1	1
Plate 2	1	1	1	1	1	1	1	1	1	1
Plate 3	1	1	1	1	1	1	1	1	1	1
Plate 4	1	1	1	1	1	1	1	1	1	1

Dilution Series

Dilution Series										
Condition	1	1	1	1	1	1	1	1	1	1
Replicate	1	2	3	4	5	6	7	8	9	10
Plate 1	1	1	1	1	1	1	1	1	1	1
Plate 2	1	1	1	1	1	1	1	1	1	1
Plate 3	1	1	1	1	1	1	1	1	1	1
Plate 4	1	1	1	1	1	1	1	1	1	1

Calculate the Log Transformed Data

Normally Distributed

Log Transformed Data										
Condition	1	1	1	1	1	1	1	1	1	1
Replicate	1	2	3	4	5	6	7	8	9	10
Plate 1	0	0	0	0	0	0	0	0	0	0
Plate 2	0	0	0	0	0	0	0	0	0	0
Plate 3	0	0	0	0	0	0	0	0	0	0
Plate 4	0	0	0	0	0	0	0	0	0	0

- =IF(ISBLANK(C17),"NA",LOG(C17*AA17*AM17/O17))

u for Log plate count

Cells are orange for formula, automatically update

	ANOVA Data - Average									
		log10 cfu/g	log10 cfu/g	log10 cfu/g	log10 cfu/g	log10 cfu/g	log10 cfu/g			
		Condition 1	Condition 2	Condition 3	Condition 4	Condition 5	Condition 6			
Replicate 1		11.35281	11.49121	NA	NA	NA	NA			
Replicate 2		11.3404	11.48862	NA	NA	NA	NA			
Replicate 3		11.31384	11.54322	NA	NA	NA	NA			
Replicate 4		11.3911	11.49535	NA	NA	NA	NA			
Replicate 5		11.31153	11.53862	NA	NA	NA	NA			
Replicate 6		11.3787	11.55982	NA	NA	NA	NA			
Replicate 7		11.35314	11.50458	NA	NA	NA	NA			
Replicate 8		11.38885	11.51223	NA	NA	NA	NA			
Replicate 9		11.37793	11.50413	NA	NA	NA	NA			
Replicate 10		11.4185	11.5176	NA	NA	NA	NA			
PLATE UNCERTAINTY										
		Σsquares	ΣDEGREES OF Freedom		u for single plate		Number of plates		u for average of plates	
		0.849621	40		0.145741		3		0.084144	

ANOVA Calculations

Count	10						
N	60		ANOVA				
k	6	*Source of Variation*		*df*	*SS*	*MS*	*F*
ybar..	0		Between Groups	5	0	0	#DIV/0!
ybar1.	0		Within Groups	54	0	0	
ybar2.	0		Total	59	0		
ybar3.	0						
ybar4.	0						
ybar5.	0						
ybar6.	0						
SST	0	0	0	0	0	0	
0	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
SS(Tr)	0		***SSE***				
0	0		0				
	0						
	0						
	0						
	0						

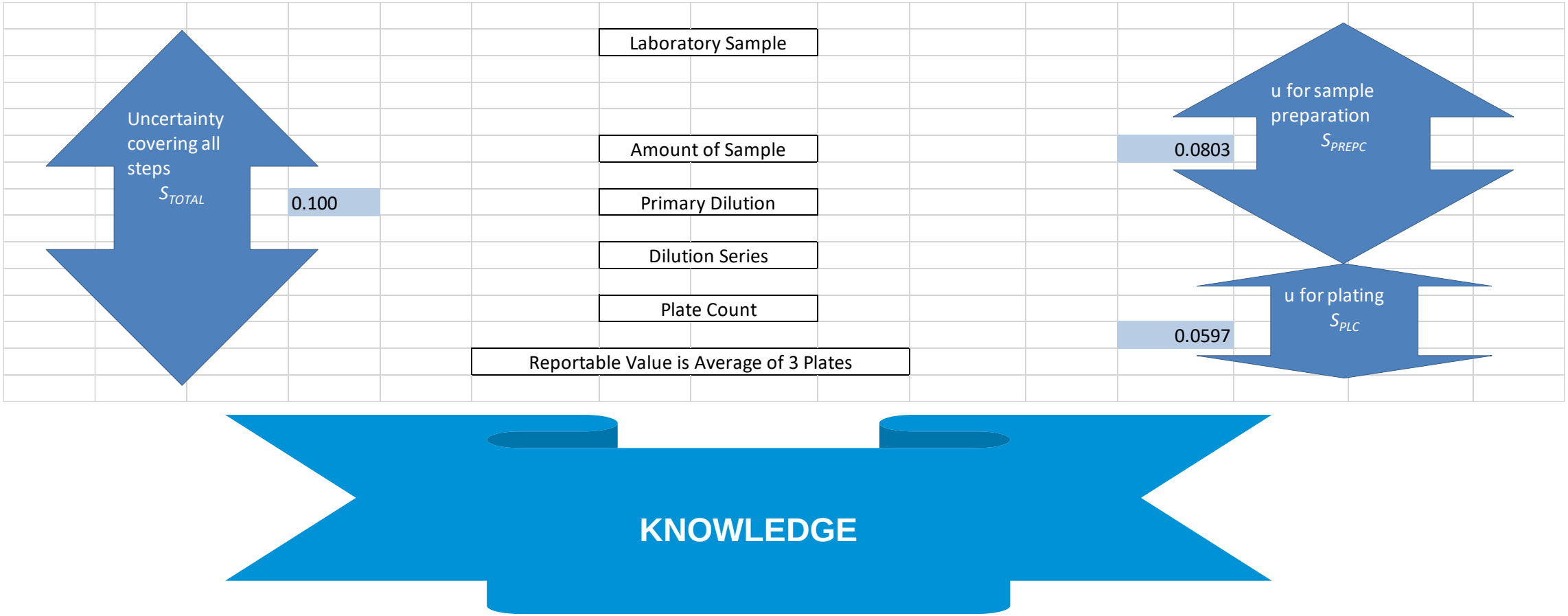
u calculations

repeatability & Intermediate Precision

		Precision Extimates Using ANOVA				**Formula**						
		Repeatability	S_r	0.000		=SQRT(*MS* Within Groups)						
Between Group Standard Deviation			S_{BG}	0.000		=SQRT((*MS* Between Groups - *MS* Within Groups)/Count)						
		Intermediate Precision	S_{IP}	0.000		=SQRT(S_r^2+S_{BG}^2)						
		If Between Group SD is "#NUM", it is the same as repeatability.										

Summary of EXCEL

Formula = $S_{PREPC} = \sqrt{(S_{TOTAL}2 - S_{PLC}^2)}$ =SQRT(D36^2-L41^2)



Comparing Probiotic Plate Count Methods

Use the Knowledge

APLM and Comparisons

Comparing Probiotic Plate Count Methods

Use the Measurand

- Confirm both Analytical Procedures are measuring the same thing.

Use the Risk Analysis in the Comparison

e.g. does the sonication step have the same risk for both procedures?

Risk Assessment

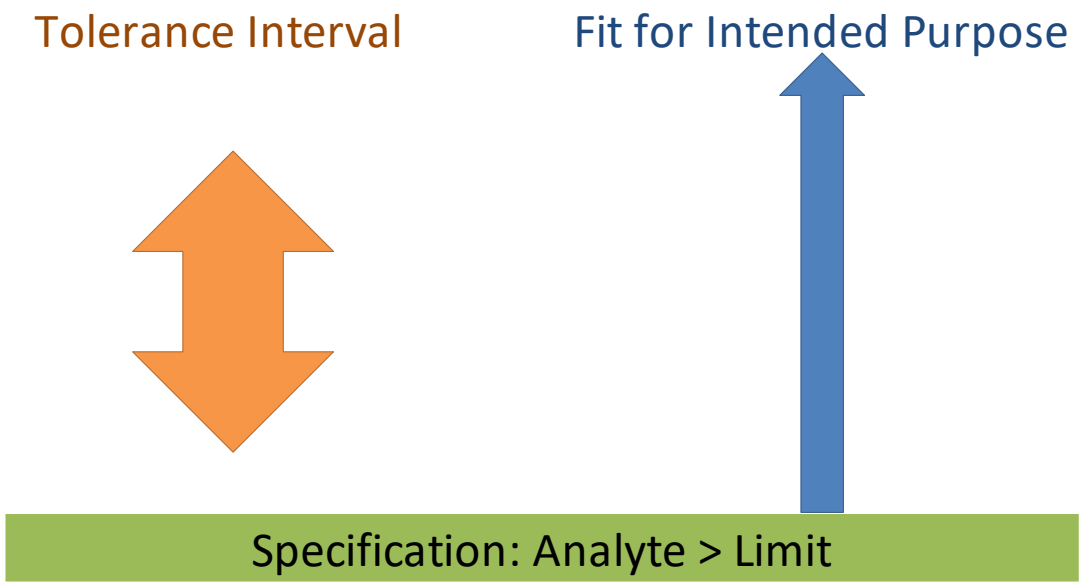
USP GC <1220>

Analytical Unit Operation	Analytical Factor or Variable	Identified Potential Risk	RISK HEAT MAP		Analytical Control Strategy
			Accuracy	Precision	
SAMPLE & REAGENT PREPARATION	Humidity of the laboratory	Moisture absorption by the sample can lead to incorrect weighing or degradation			Monitor environmental controls
	Analyst skill	Incorrect sample preparation; weighing & volumetric dilutions			Training program and records
	Sonication time	Lack of dissolution of the sample or degradation			Establish limit or conditions during development
	Composition of the solvent mixture used in sample preparation	Lack of complete dissolution of the sample			
INSTRUMENT & SYSTEM SET UP	% composition of the solvent in the mobile phase	Column performance, peak shape & retention times			Gravimetric preparation, SSTs
	Column temperature				Establish operation within limits during instrument/system qualification; SSTs to confirm performance
	Batch of column packing material				Establish variability during Stage 1 and design SSTs
	Quality of the solvent	Baseline drift and noise are wavelength dependent and may affect the peak shape			Specify required grade and transmittance characteristics
	Cleaning	Peaks from previous Injections			Establish cleaning protocol, SST

Compare Analytical Procedures Performance

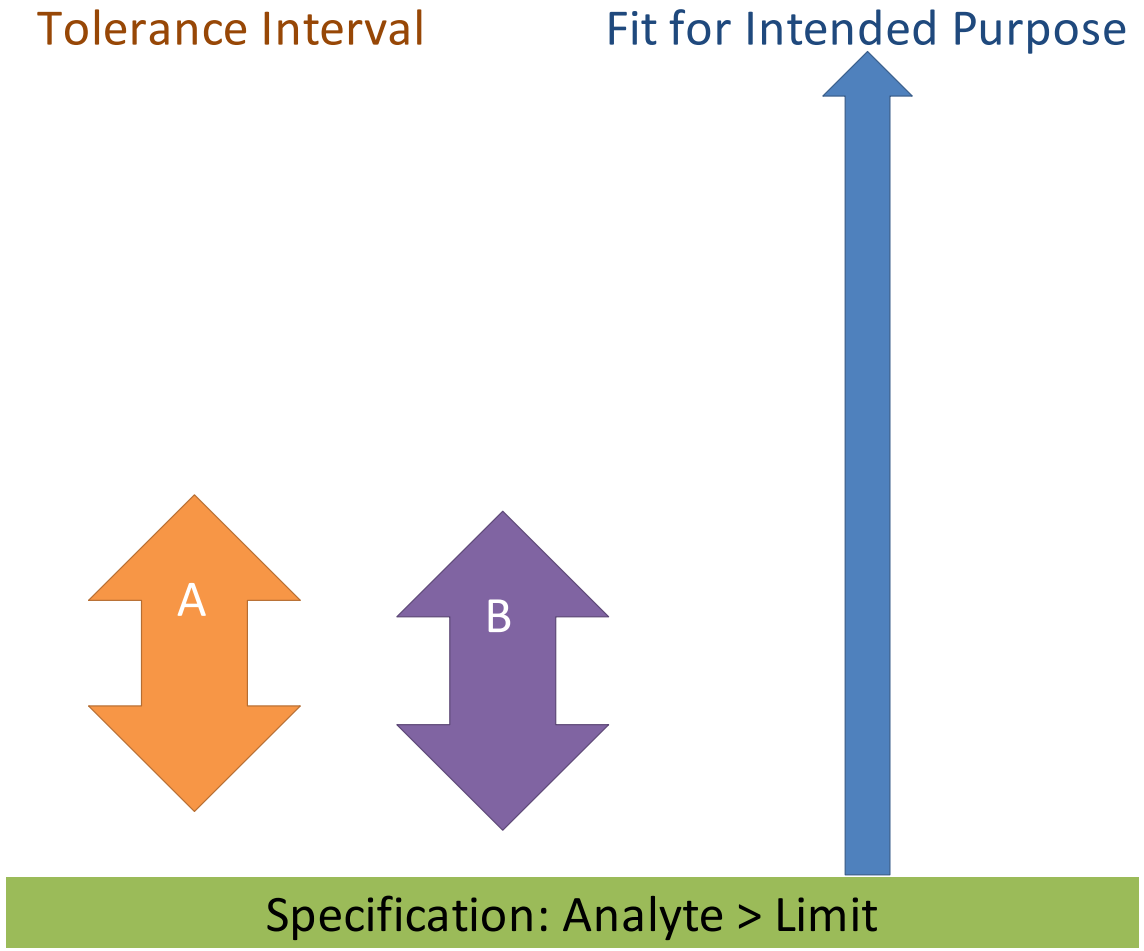
▲<1210> STATISTICAL TOOLS FOR PROCEDURE VALIDATION

- 3.2 When assessing whether an analytical procedure is fit for its intended purpose, it is often useful to consider the combined impact of bias and precision.
- 3.2 2. A tolerance interval (also referred to as a content tolerance interval) is used to demonstrate fit for its intended purpose



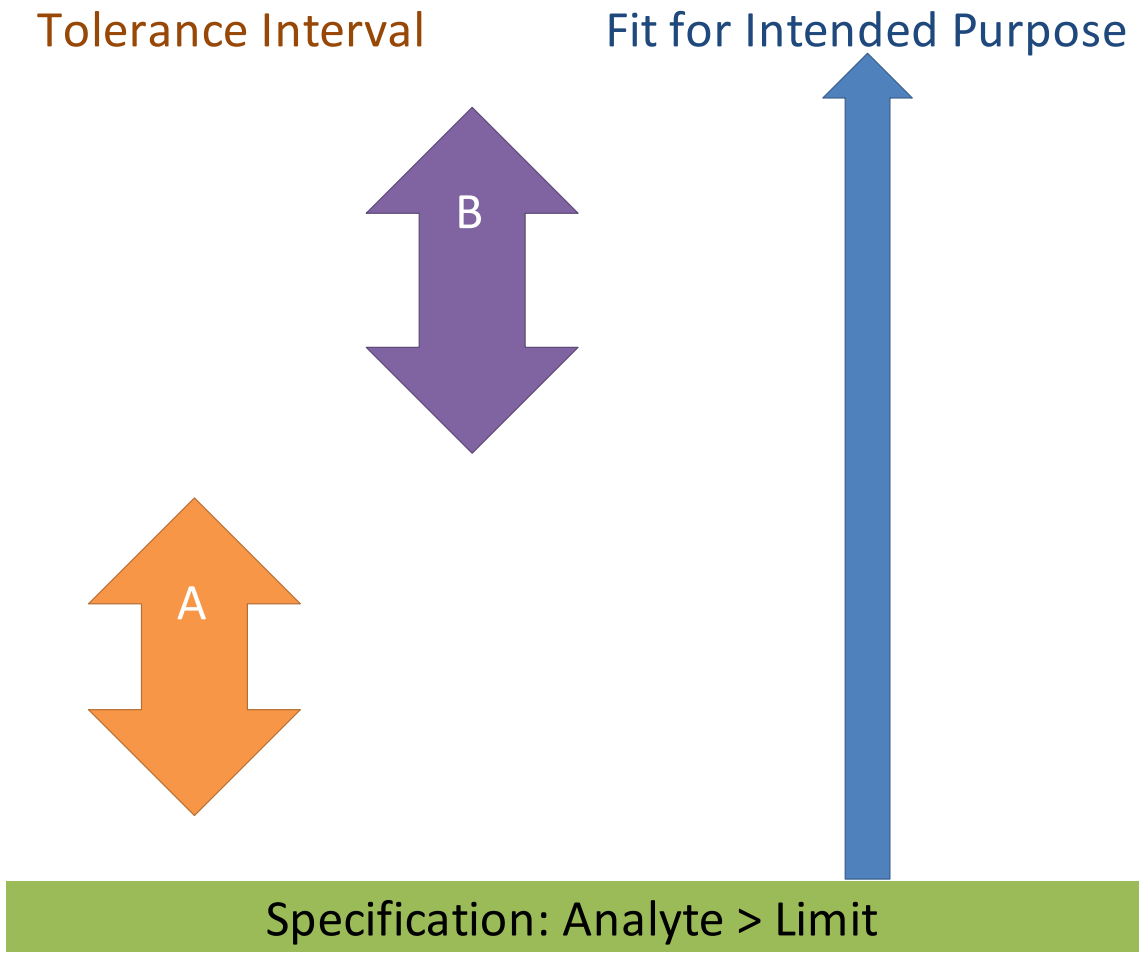
Compare Procedure A to Procedure B

Scenario 1



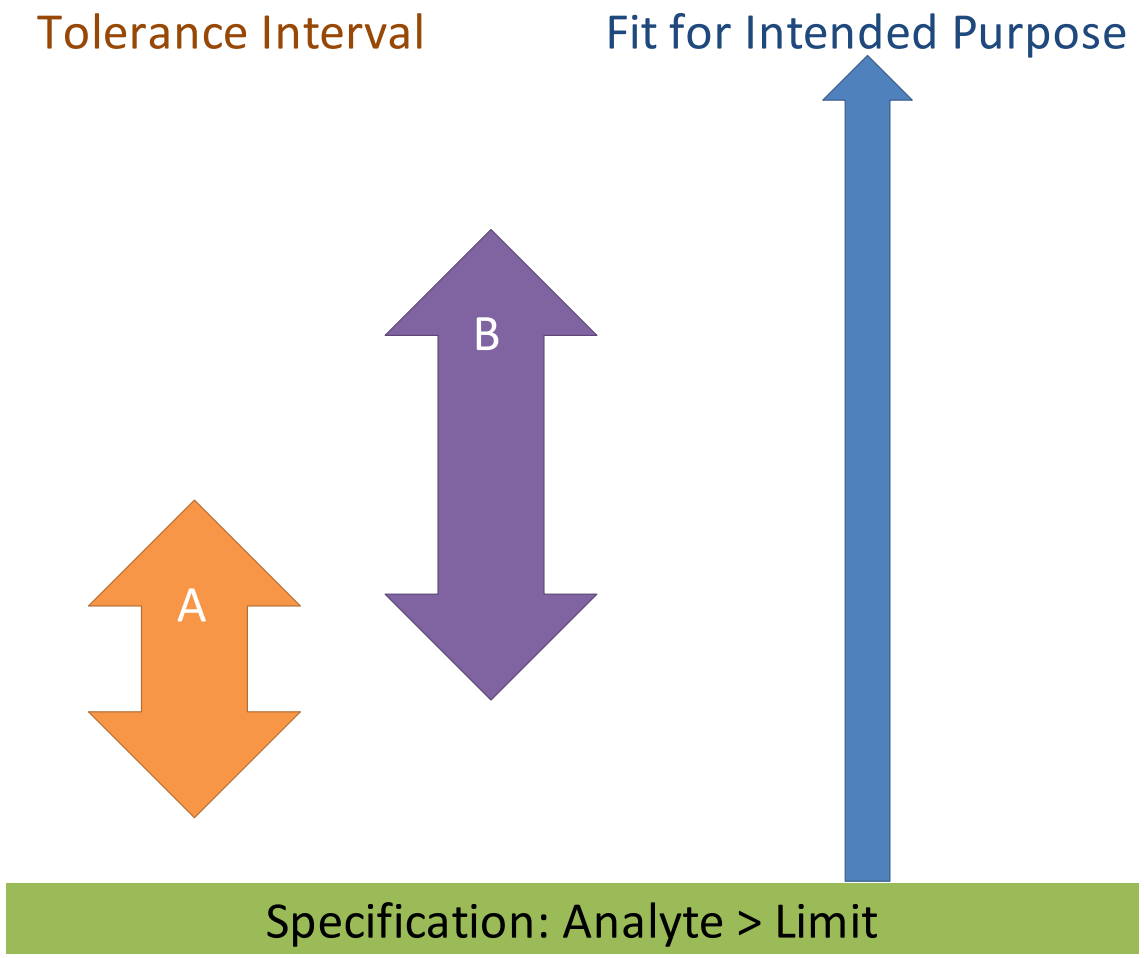
Compare Procedure A to Procedure B

Scenario 2



Compare Procedure A to Procedure B

Scenario 3



TI Equation 11 in <1220>

Symbols are explained on next slide.

$$\bar{Y} \pm K \times S$$

$$K = \sqrt{\frac{Z^2_{(1+P)/2} \times (n-1)}{\chi^2_{\alpha:n-1}} \times \left(1+\frac{1}{n}\right)} \qquad (11)$$

EXCEL to Calculate Tolerance Interval

Create an EXCEL spreadsheet to calculate the TI

⟨ 1210 ⟩ STATISTICAL TOOLS FOR PROCEDURE VALIDATION

3.2 Combined Validation of Accuracy and Precision

$\Pr(-\lambda + \tau < Y < \lambda + \tau) \geq P$

Pr = reference probability

λ = acceptable limit

Y = a reportable value

τ = true or accepted reference value

P = desired probability value

2. A tolerance interval (also referred to as a content tolerance interval) is used to demonstrate …

The 100(1 − α)% tolerance interval used to validate Equation 9 is

$$\bar{Y} \pm K \times S$$

$$K = \sqrt{\frac{Z^2_{(1+P)/2} \times (n-1)}{\chi^2_{\alpha:n-1}} \times \left(1+\frac{1}{n}\right)} \qquad (11)$$

$\bar{Y}$ = sample mean

K = result found from Equation 11

S = result found from Equation 4 in <1210>

Z = the square of the standard normal percentile with area (1 + P)/2 to the left

n = number of reportable values

χ = a chi-squared percentile with area α to the left and (n − 1) degrees of freedom

An Example

Details

- strain Lactobacillus acidophilus
- analysis release > 150 billion\g
  - (261 billion at the time of analysis)

Enter Plate Count

	Plate Count									
	Condition	1	1	1	1	1	1	1	1	1	1
	Replicate	1	2	3	4	5	6	7	8	9	10
Plate 1 Count		20	20	23	23	23	19	21	22	27	23
Plate 2 Count		26	25	19	27	22	30	26	23	24	29
Plate 3 Count		22	21	20	24	17	24	21	29	21	27
Plate 4 Count											
	Condition	2	2	2	2	2	2	2	2	2	2
	Replicate	1	2	3	4	5	6	7	8	9	10
Plate 1 Count		30	36	32	32	31	34	30	37	29	31
Plate 2 Count		32	28	36	29	36	38	32	31	34	36
Plate 3 Count		31	29	37	33	37	37	34	30	33	32
Plate 4 Count											

Enter Dilution

Diluton Series										
Condition	1	1	1	1	1	1	1	1	1	1
Replicate	1	2	3	4	5	6	7	8	9	10
Plate 1	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10
Plate 2	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10
Plate 3	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10
Plate 4										
Condition	2	2	2	2	2	2	2	2	2	2
Replicate	1	2	3	4	5	6	7	8	9	10
Plate 1	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10
Plate 2	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10
Plate 3	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10	1.00E+10
Plate 4										

Workbook calculates Log Transformed Data

Log Transformed Data										
Condition	1	1	1	1	1	1	1	1	1	1
Replicate	1	2	3	4	5	6	7	8	9	10
Plate 1	11.30103	11.30103	11.36173	11.36173	11.36173	11.27875	11.32222	11.34242	11.43136	11.36173
Plate 2	11.41497	11.39794	11.27875	11.43136	11.34242	11.47712	11.41497	11.36173	11.38021	11.4624
Plate 3	11.34242	11.32222	11.30103	11.38021	11.23045	11.38021	11.32222	11.4624	11.32222	11.43136
Plate 4										
Condition	2	2	2	2	2	2	2	2	2	2
Replicate	1	2	3	4	5	6	7	8	9	10
Plate 1	11.47712	11.5563	11.50515	11.50515	11.49136	11.53148	11.47712	11.5682	11.4624	11.49136
Plate 2	11.50515	11.44716	11.5563	11.4624	11.5563	11.57978	11.50515	11.49136	11.53148	11.5563
Plate 3	11.49136	11.4624	11.5682	11.51851	11.5682	11.5682	11.53148	11.47712	11.51851	11.50515
Plate 4										

Workbook Calculates the u of Plate Count

	ANOVA Data - Average									
		log10 cfu/g	log10 cfu/g	log10 cfu/g	log10 cfu/g	log10 cfu/g	log10 cfu/g			
		Condition 1	Condition 2	Condition 3	Condition 4	Condition 5	Condition 6			
	Replicate 1	11.35281	11.49121	NA	NA	NA	NA			
	Replicate 2	11.3404	11.48862	NA	NA	NA	NA			
	Replicate 3	11.31384	11.54322	NA	NA	NA	NA			
	Replicate 4	11.3911	11.49535	NA	NA	NA	NA			
	Replicate 5	11.31153	11.53862	NA	NA	NA	NA			
	Replicate 6	11.3787	11.55982	NA	NA	NA	NA			
	Replicate 7	11.35314	11.50458	NA	NA	NA	NA			
	Replicate 8	11.38885	11.51223	NA	NA	NA	NA			
	Replicate 9	11.37793	11.50413	NA	NA	NA	NA			
	Replicate 10	11.4185	11.5176	NA	NA	NA	NA			
PLATE UNCERTAINTY										
		Σsquares	ΣDEGREES OF Freedom		u for single plate		Number of plates		u for average of plates	
		0.849621	40		0.145741		3		0.084144	

Workbook Calculates ANOVA

Count	10						
N	20		ANOVA				
k	2	*Source of Variation*		*df*	*SS*	*MS*	*F*
ybar..	11.43911	Between Groups		1	0.116832	0.116832	131.1644
ybar1.	11.36268	Within Groups		18	0.016033	0.000891	
ybar2.	11.51554		Total	19	0.132865		
ybar3.							
ybar4.							
ybar5.							
ybar6.							
SST	0.007448	0.002715					
0.132865	0.009744	0.002451					
	0.015693	0.010839					
	0.002305	0.003164					
	0.016276	0.009903					
	0.00365	0.014571					
	0.007391	0.004287					
	0.002526	0.005346					
	0.003743	0.004228					
	0.000425	0.006162					
SS(Tr)	0.058416		***SSE***				
0.116832	0.058416		0.016033				

Workbook Calculates the Precisions

Repeatability & Intermediate Precision (for the 2 conditions)

		Precision Extimates Using ANOVA				**Formula**							
		Repeatability	S_r	0.030		=SQRT(*MS* Within Groups)							
Between Group Standard Deviation			S_{BG}	0.108		=SQRT((*MS* Between Groups - *MS* Within Groups)/Count)							
		Intermediate Precision	S_{IP}	0.112		=SQRT(S_r^2+S_{BG}^2)							

Compare the 2 Conditions

Calculate the Tolerance Intervals

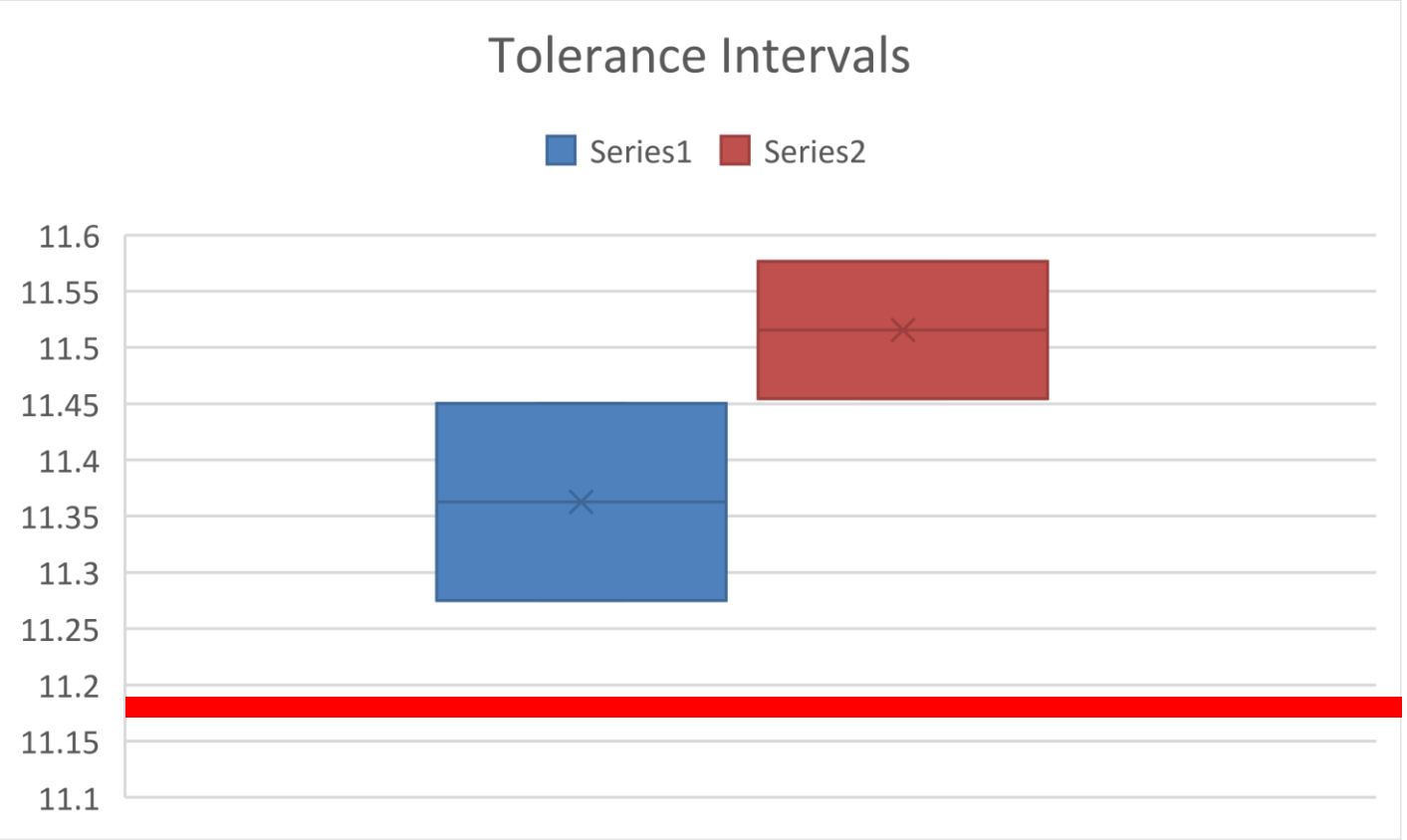
Tolerance intervals

Condition	Mean	S	Tolerance Interval			
1	11.36	0.0346	11.275	to	11.450	$\log_{10}$ cfu/g powder
2	11.52	0.0242	11.454	to	11.577	$\log_{10}$ cfu/g powder

Confidence Level			0.9
Percent Coverage			0.9
n			10
K			2.535

TI Comparison

Condition is “Series”, The specification is > 11.17



Your Feedback

Workbook

- Would a workbook be useful?
- Would the EXCEL formulas be useful?

Thank You

The standard of trust

Stay Connected

Jane Weitzel | E-mail: mljweitzel@msn.com

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