

Method Validation Test Statistical aspects

Goal of the appendix

- Method validation from a statistical perspective
 - Simple
 - More sophisticated
- Obtain objective supporting evidence:
 - Number of steps to be followed

Different steps

- 1. *Ruggedness testing procedure***
2. Statistical support to establish and review
3. Set up of a test
4. Benefits of simulations for a test plan design

Different steps

5. Different types of results and distributions
 - Direct test
 - Indirect test
- 6. *Data check; looking for outliers***
7. Statistical analysis, model and assumptions

Different steps

8. ANOVA

9. GLM

10. Repeatability and reproducibility

1. Normal data: ISO 5725-2

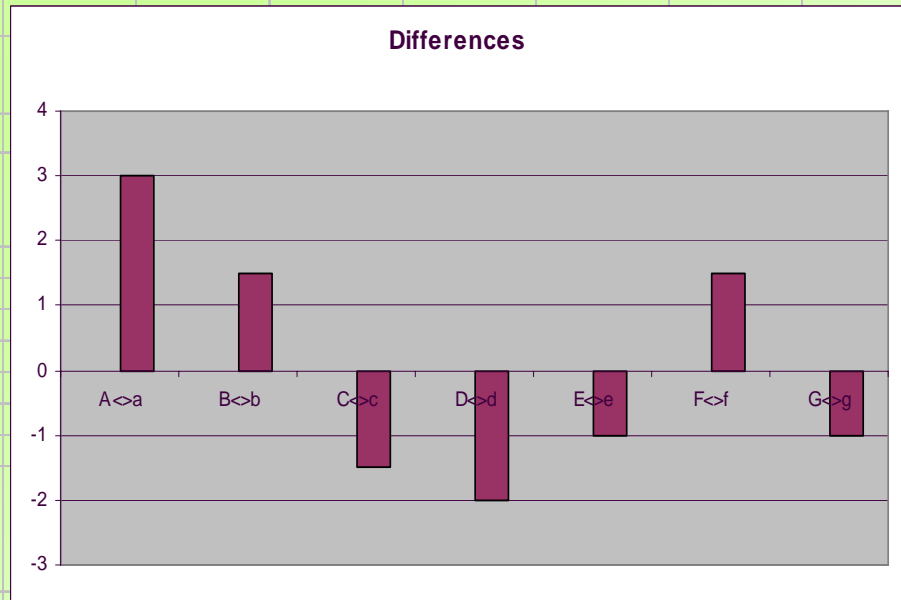
2. Binomial and Poisson data

1. Ruggedness

- Method development in one laboratory
 - No variations tested
- Introduce reasonable variations
 - Several changes at a time
 - Control these steps
- A procedure is rugged if these variations do not give different results

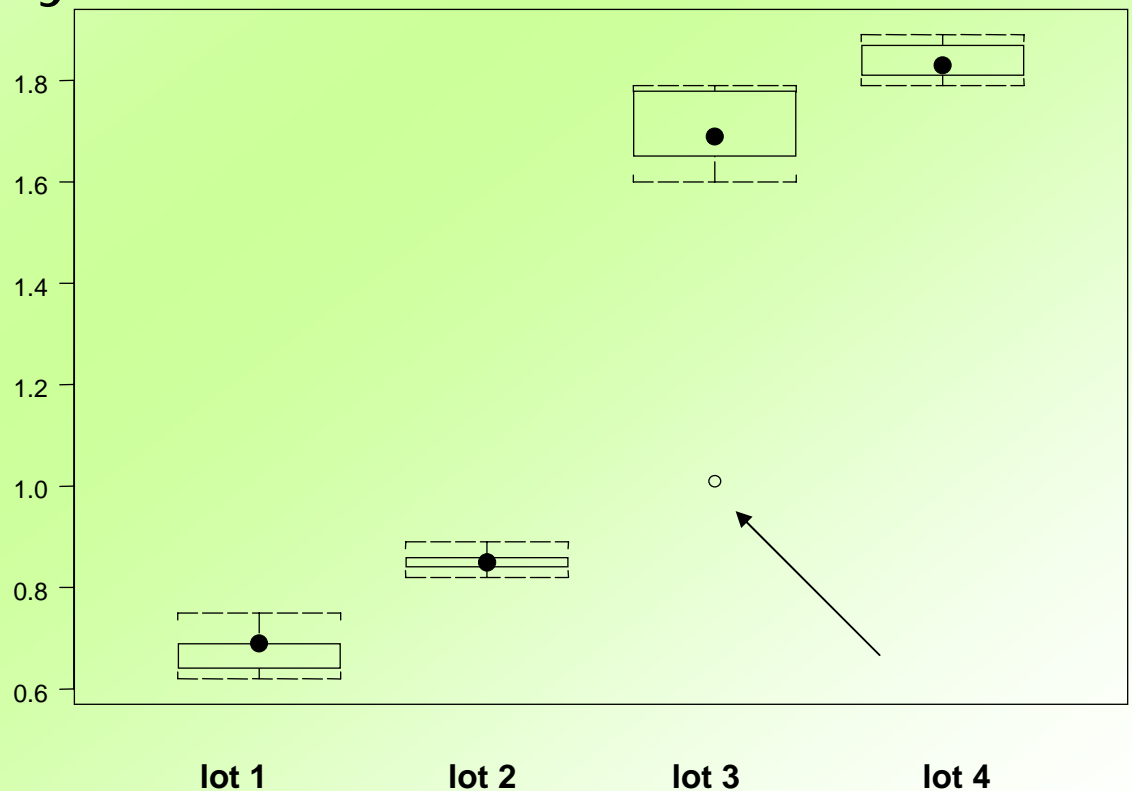
Example ruggedness

Eight combinations for 2 to 7 factors used to test the ruggedness of an analytical method								
<i>enter results in green cells</i>								
Factor Value								
A or a	A	A	A	A	a	a	a	a
B or b	B	B	b	b	B	B	b	b
C or c	C	c	C	c	C	c	C	c
D or d	D	D	d	d	d	d	D	D
E or e	E	e	E	e	e	E	e	E
F or f	F	f	f	F	F	f	f	F
G or g	G	g	g	G	g	G	G	g
Observed result	12	14	12	15	13	11	7	10
<i>mean</i>	11,75							
<i>standard deviation</i>	2,49284691							
		<i>difference</i>	<i>difference /mean</i>	<i>absolute value of difference</i>				
	A<>a	3	25,53%	3				
	B<>b	1,5	12,77%	1,5				
	C<>c	-1,5	-12,77%	1,5				
	D<>d	-2	-17,02%	2				
	E<>e	-1	-8,51%	1				
	F<>f	1,5	12,77%	1,5				
	G<>g	-1	-8,51%	1				
		<i>mean of absolute value of differences</i>		1,64285714				



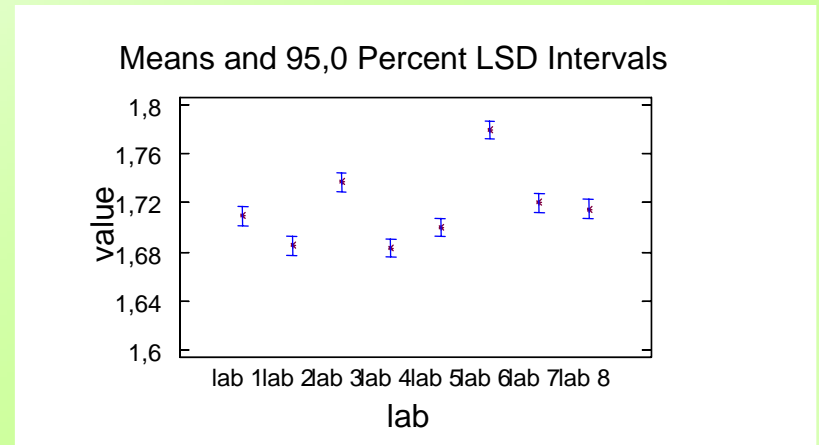
6. Data check for outliers

- Check the quality of the data by visualising
 - Box plot

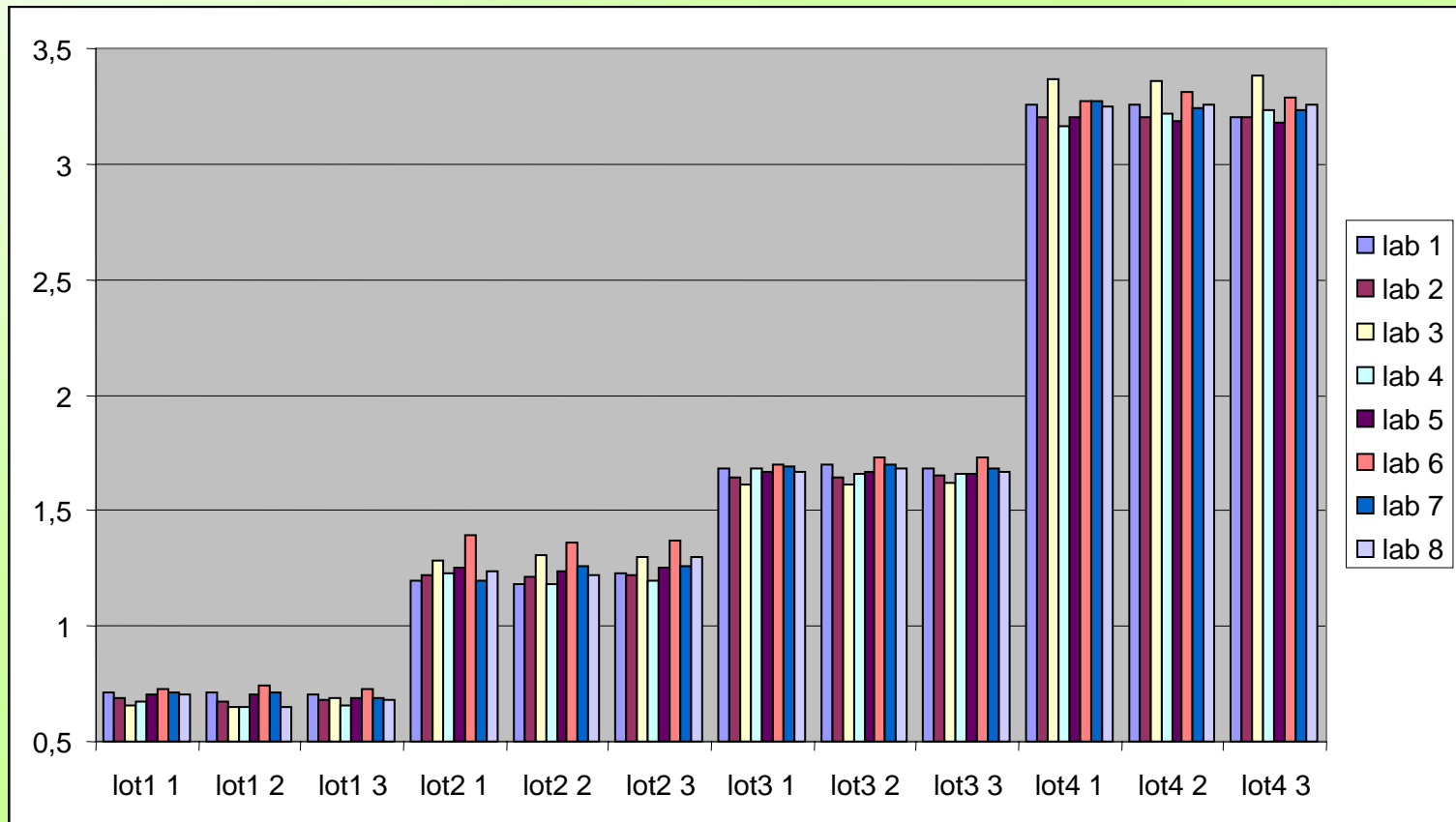


8. ANOVA

- ANalysis Of VAriance
- Assumptions
 - *Independent observations*
 - *Same variability of comparable sub-data sets*
 - Normal distribution
- Fit for quantitative values
- For method validation 2 factors is recommended



Example ANOVA (1)



Example ANOVA (2)

Examples of Analysis of Variance table

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN FACTORS					
A:lab	0,0811791	7	0,011597	34,57	0,0000
B:sample	86,8585	3	28,9528	86319,08	0,0000
INTERACTION					
A*B	0,0943291	21	0,00449186	13,39	0,0000
RESIDUAL	0,0214667	64	0,000335416		
TOTAL (CORRECTED)					
	87,0555	95			

9. GLM

- Generalized Linear Models
- Assumptions
 - *Independent observations*
 - Results are linearly modeled

******* Regression Analysis *******

Response variate: cposcount

Distribution: Poisson

Link function: Log

Fitted terms: Constant + lot + lab + lot.lab

Example GLM (1)

lab!	media!	dil!	lotrep!	rep!	xap	other
1	1	0	1	1	300	0
1	1	0	1	2	300	0
1	1	1	1	1	300	2
1	1	1	1	2	300	2
1	1	2	1	1	54	0
1	1	2	1	2	48	1
1	1	3	1	1	4	0
1	1	3	1	2	0	0
1	1	0	2	1	300	0
1	1	0	2	2	300	0
1	1	1	2	1	300	0
1	1	1	2	2	300	0
1	1	2	2	1	108	0
1	1	2	2	2	99	0
1	1	3	2	1	11	0
1	1	3	2	2	2	0

Example GLM (2)

*** Accumulated analysis of deviance ***

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ lot	3	17042375.	5680792.	657.59	<.001
+ lab	6	24052884.	4008814.	464.05	<.001
+ lot.lab	18	2475610.	137534.	15.92	<.001
+ lsf	113	19695339.	174295.	20.18	<.001
Residual	419	3619646.	8639.		
Total	559	66885854.	119653.		

10. Repeatability and reproducibility

- ISO5725-2 for quantitative data
 - Free tool from ISTA website
- Repeatability
 - Average variability of results within a laboratory on a given level
- Reproducibility
 - Average variability within laboratories + average variability between laboratories on a given level

Example ISO5725-2

h values shows if a lab has a tendency to under or over estimate



This work has been done on request from the Method Validation Program.

It has not been distributed out of the MVP group.

We consider it as a draft that can be improved.

If ISTA confirms the need, we will contact the Technical Committees which will be the users.

Thank you very much
for your attention!!

Next STA workshop

- From 25 to 29 August 2008 at Naktuinbouw, the Netherlands

