A Statistical Method to Demonstrate Dilutional Linearity for Immunoassays

A.Baclin, C. Bievelet, G. de Lannoy, M.-P. Malice,

Maria Key-Prato, Director

GSK Vaccines, Discovery and Development Biostatistics

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Introduction (1)

Assay validation

- Precision
- Accuracy
- Linearity (calibration? Accuracy ? Dilutional ?)

- EMA guideline on bioanalytical method validation, ICH guidelines on validation of analytical procedures (Q2A and Q2B) delineates methodology but terminology is often vague.

Introduction (2)

- In the case of Ligand binding or immunoassays developed in clinical serology assays only dilutional linearity (also called relative accuracy) can be evaluated.

  - ✓ Absence of external reference material (target is antibodies)
  - ✓ Operate within large analytical ranges
  - ✓ Sample and volume availability
  - ✓ Matrix complexity

The accuracy of an analytical procedure expresses the closeness of agreement between the accepted true value and the test values obtained. When a true value is not available, dilutional linearity/relative accuracy can be established by demonstrating that expected values are obtained with increasing dilution of a known positive sample.

Introduction (3)

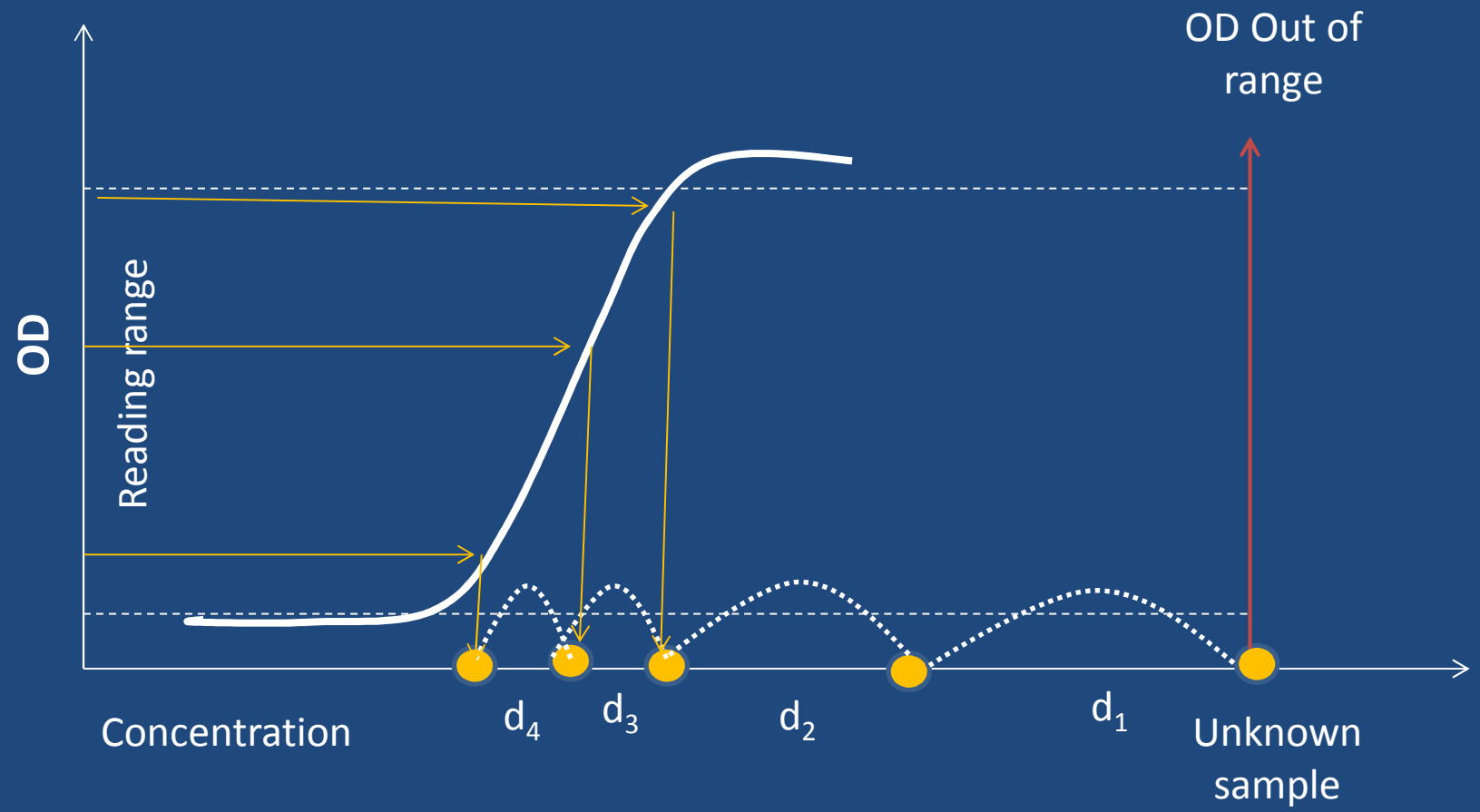
Why do we need to demonstrate dilutional linearity/ relative accuracy ?

EMA Guidance:

For immunoassays, because the narrow range of the calibration standard curve, the analyte of interest, when present in *concentrations exceeding the range of quantification* can be accurately measured by the assay after dilution in blank matrix to bring the analyte concentrations into the validated range for analysis.

Introduction (3)

Example : Elisa Standard 4PL curve



- *Dilute sample until concentration falls in the analysis range*
- *multiply the back-calculated concentration by the dilution factor to get the final sample concentration*

Introduction (4)

- Specific criteria related to accuracy = dilutional linearity (in fact dilution proportionality)

- Question of interest :
  - Test results T_{ij}, i=1,..k; j=1,..,n_i with geometric means t_i
  - Obtained at dilution d_1, d_2,.., d_k (independent dilutions)
  - $H_a : t_1 d_1 = t_2 d_2 = \ldots t_k d_k$

- Equivalence hypothesis question (need to specify 'equivalence bounds')

Dilution proportionality – Reference Known

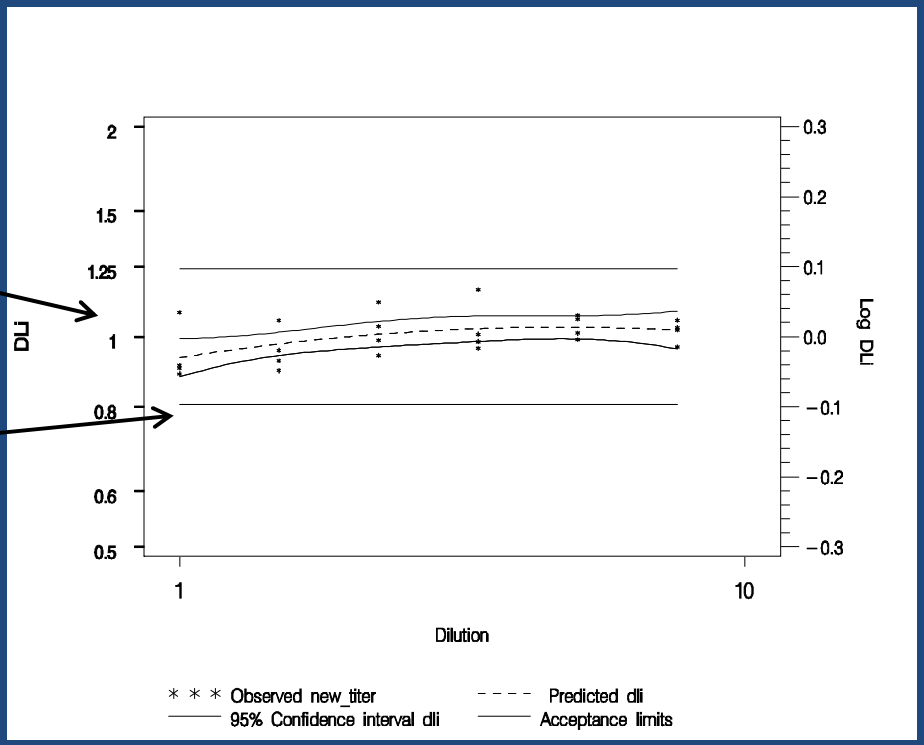
- EMA guideline: « back-calculated titers/concentrations for each dilution should be within 20% of the nominal value after correction for dilution »
  - Estimate or CI within 20% ? Why 20%?
  - Assume nominal concentration or reference value known
- CLSI EP06-A: If reference value is known:
  - Deviations from dilution-proportionality of the predicted titers (linear or higher order) minus reference value computed
  - Confidence intervals for the deviations compared to reference value

Dilution proportionality – Reference UnKnown

- CLSI EP06-A: If reference value is unknown:
  - Tradition to use the median or mean as reference value and proceed as above

Predicted deviation from dilution-proportionality and 95% CI

Acceptance limits



Dilution proportionality – Reference Unknown

Simulations

- 7 dilutions and 5 replications per dilution (CLSI EP06-A guideline)
- Distribution log-normal CV 18%
- Acceptance limits 20% (0.80;1.25)
- 3 different cases of GMR at different dilutions

	GMR Compared With Dil 1					
Case	**Dil 2**	**Dil 3**	**Dil 4**	**Dil 5**	**Dil 6**	**Dil 7**
1	**0.96**	**0.94**	**0.90**	**0.86**	**0.84**	**0.80**
2	**1.03**	**1.08**	**1.13**	**1.16**	**1.22**	**1.25**
3	**1**	**1**	**1**	**1**	**1**	**1**

Type I error

Power

Dilution proportionality – Reference Unknown

Procedure 1: use of overall mean as reference

Procedure 2: use of first dilution mean

Linear model for deviation from reference, 90% CI for deviations compared to reference value

	GMR Compared With Dil 1							
							Error Type	Probability
	Dil 2	Dil 3	Dil 4	Dil 5	Dil 6	Dil 7		
Procedure 1	0.96	0.94	0.90	0.86	0.84	0.80	I	**0.780**
	1.03	1.08	1.13	1.16	1.22	1.25	I	**0.774**
	1	1	1	1	1	1	II	0.012
Procedure 2	0.96	0.94	0.90	0.86	0.84	0.80	I	0.051
	1.03	1.08	1.13	1.16	1.22	1.25	I	0.049
	1	1	1	1	1	1	II	**0.542**

Dilution proportionality – Reference Unknown

- Procedure 1: use of overall mean as reference
  - Leads to unacceptable ‘false non-rejection’ probability
- Procedure 2: use of first dilution mean
  - Appears to have low power with recommended design
  - Uncertainty of reference estimate taken into account when computing 90%CI for deviations

Use of Dose Proportionality Methodology

- Based on paper 'Confidence Interval Criteria for Assessment of Dose Proportionality' Smith, Vandenhende, &al. Pharmaceutical Research, 17,10, 2000

- Assumptions Power model

$$\log T_{ij} = \beta_0 + \beta_1 \log d_i + \varepsilon_{ij} \quad \text{with } \varepsilon_{ij} \sim N(0, \sigma^2)$$

The methodology can be extended if

$$T_{ij} = \beta_0 + \beta_1 d_i + \varepsilon_{ij} \quad \text{with } \varepsilon_{ij} \sim N(0, \sigma^2)$$

Test results T_{ij}, i=1,..k; j=1,..,n_i with geometric means t_i

Use of Dose Proportionality Methodology

- Hypothesis :

$$H_a: \frac{t_1 d_1}{t_k d_k} = 1$$

- Demonstrate 90% CI for β_1 lies entirely in

(-1+ log(0.8)/log(r); -1 + log(1.25)/log(r))

with r=d_k/d_1

Dose Proportionality Methodology: Error rate

Procedure 3: Dose proportionality

Simulations: 7 dilutions and 5 replications per dilution

Distribution log-normal CV 18%

Acceptance limits (0.80;1.25)

						Error Type	Probability
Dil 2	Dil 3	Dil 4	Dil 5	Dil 6	Dil 7		
0.96	0.94	0.90	0.86	0.84	0.80	I	**0.050**
1.03	1.08	1.13	1.16	1.22	1.25	I	**0.049**
1	1	1	1	1	1	II	**0.354**

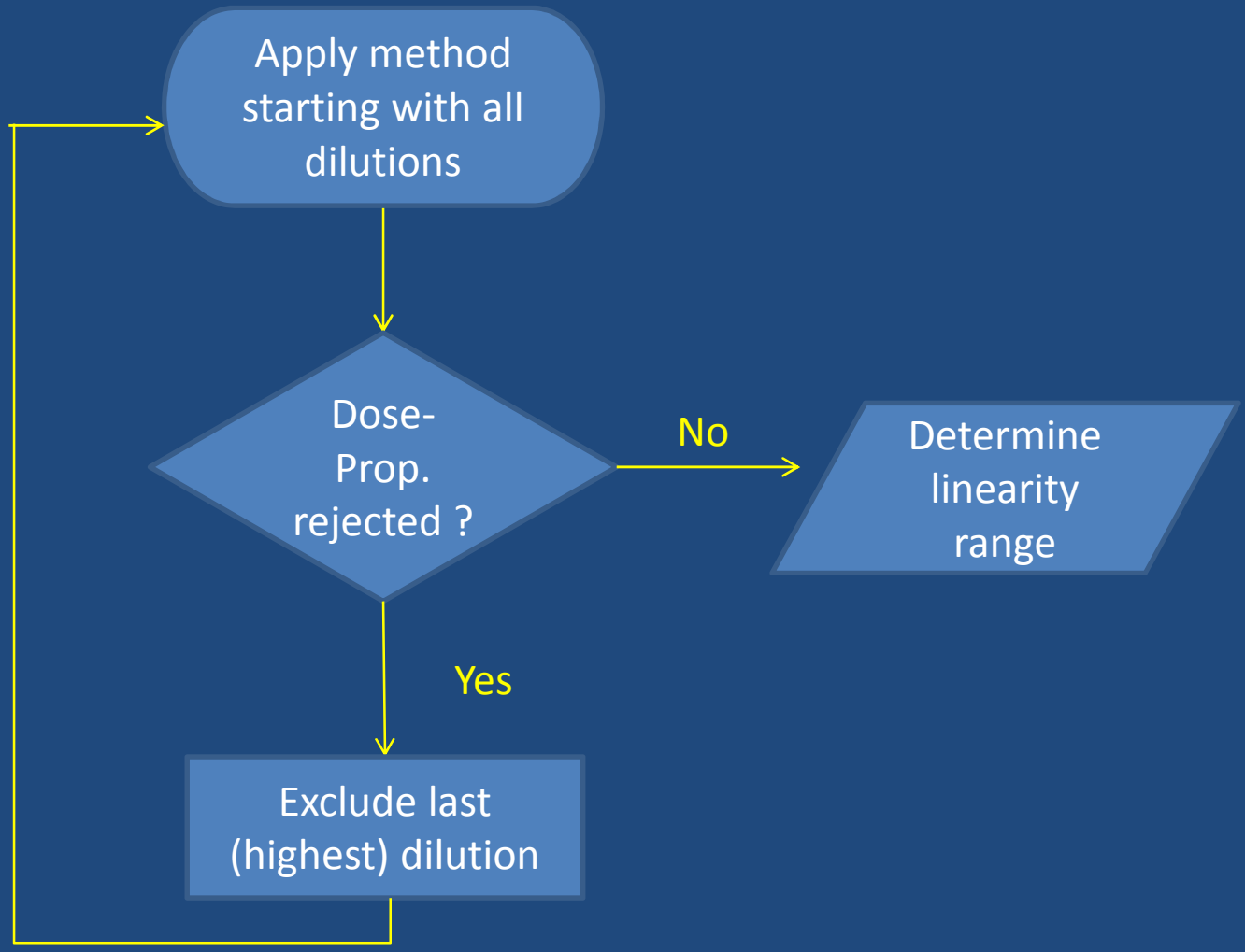
Increasing Power

First dilution plays a specific role since reference value
More specifically for procedure 2 and 3 (confidence interval calculations)

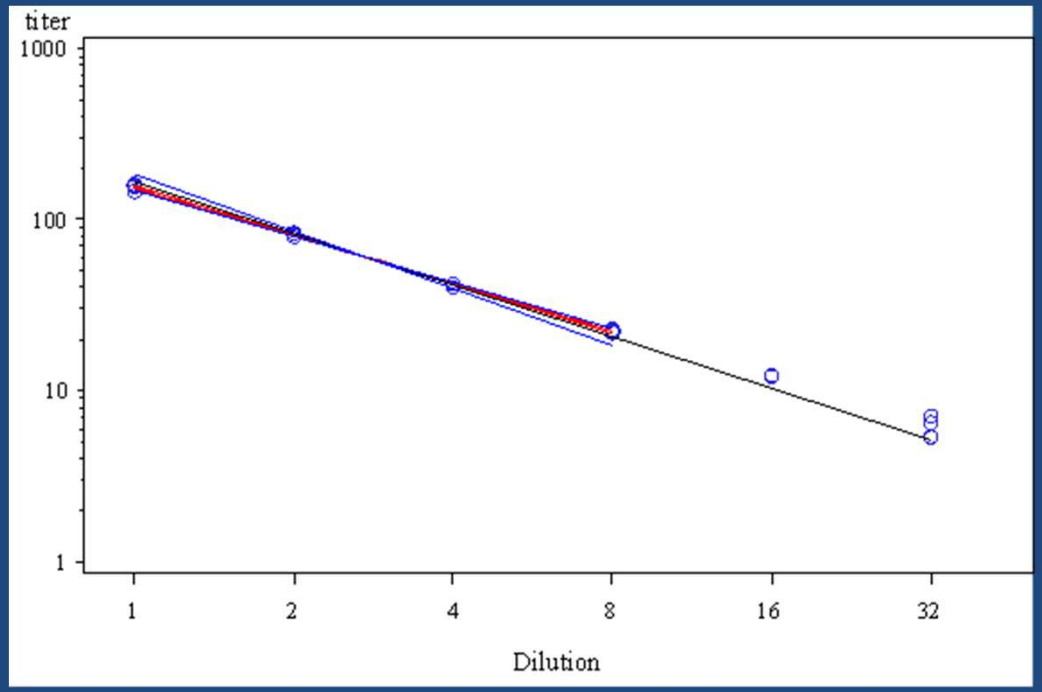
Increase sample size at first dilution to n=10

	Error Type	Probability
Procedure 2	**II**	**0.242**
Procedure 3	**II**	**0.134**

Dose Proportionality Methodology: Recursive



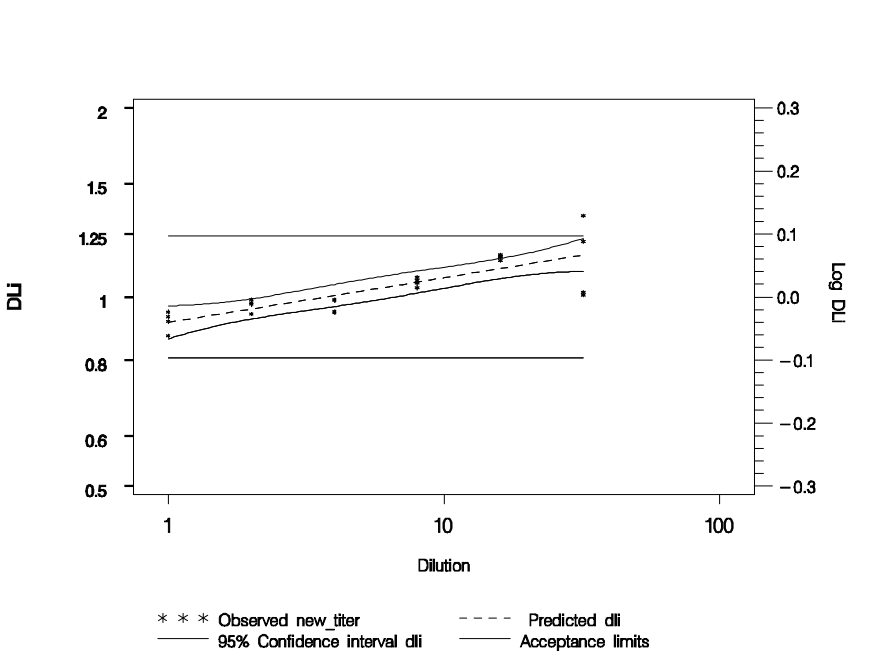
Use of Dose Proportionality Methodology: Presentation of results



Dilution		Titer		Slope			Target		
From	To	From	To	Lower	Value	Upper	Lower	Upper	ok
1	32	6.2	154	-0.947	-0.929	-0.911	-1.064	-0.936	0
1	16	12	153	-0.933	-0.920	-0.906	-1.080	-0.920	0
1	8	22	155	-0.956	-0.938	-0.920	-1.107	-0.893	1

Use of Dose Proportionality Methodology: Presentation of results

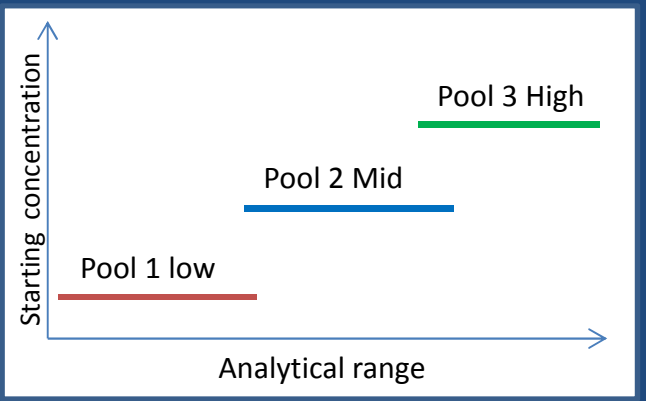
Method with median as reference

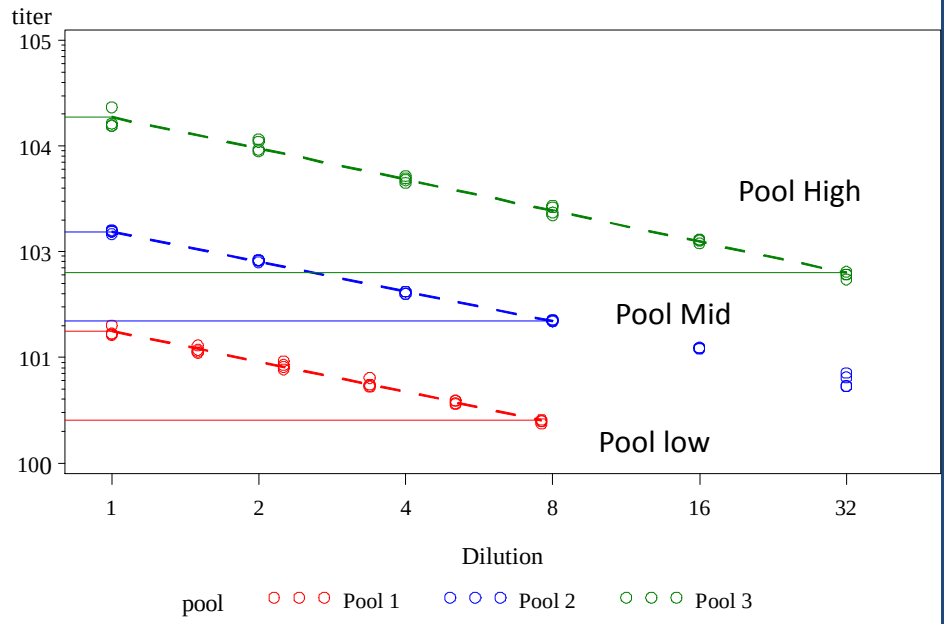


First Dilution	Last Dilution	Range Lower	Range Upper
1	32	6	155

Use of Dose Proportionality Methodology: Presentation of results

When multiple blood sample pools are used in order to cover the analytical range, informative to present results on same graph





Dose Proportionality Methodology: Pros - Cons

Pros

- Methodology adapted to the question
- Increased power compared to 'guideline recommended' method

Cons

- Assumes power model (although methodology available for linear link)
- Need to specify upfront that first dilution is the reference (no hook effect)

Discussion

Thank you for your attention

And

Any question ?

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References

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