Case Studies of cross-validation – taking an unbiased perspective

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Session 20: Regulatory Updates

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When do you consider it a different method?

RESOLIAN BIOANALYTICS

6.2. Cross validation

Cross validation is required to demonstrate how the reported data are related when multiple bioanalytical methods and/or multiple bioanalytical laboratories are involved.

Cross validation is required under the following situations:

- Data are obtained from different fully validated methods within a study.

- Different format, different platform
- Existing format, different platform?
- Existing format*, same platform?
  - Could be just orientation change or addition of biotin-conjugations (for eg)

*binding event

Existing binding event, same platform

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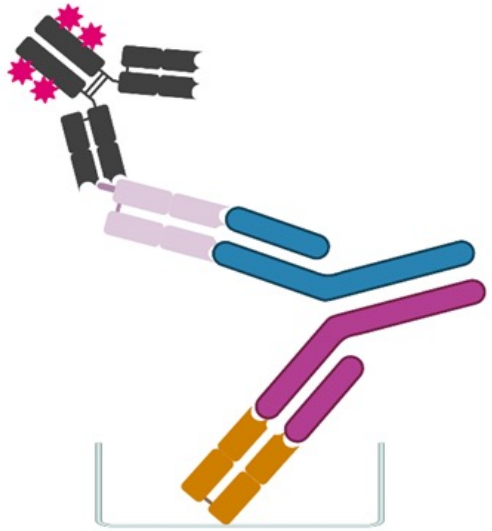
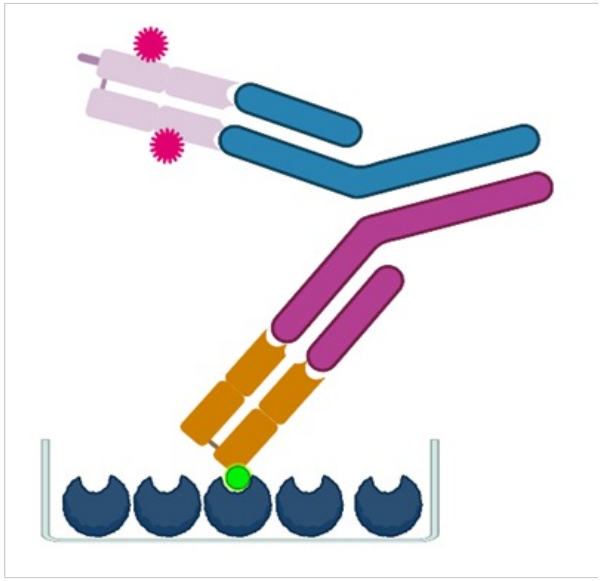


	Original	2.0
Instrument:	MSD	MSD
Plate	Standard Bind	Streptavidin Gold
MRD	10 fold	40 fold
Assay buffer	standard	standard
Capture	Fab α-domain 1	Fab α-domain 1 - BIOTIN
Detection	Fab α-domain 2-V5 (1°) SULFO-TAG α-V5 (2°)	SULFO-TAG Fab α-domain 2-V5
Range	100 – 6400 ng/mL	400 – 16000 ng/mL
Dilution Lin	Up to 5000-fold	Up to 1000-fold
Regression model	5-PL $1/Y^2$	5-PL $1/Y^2$



Cross-validation process

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- 50 samples (within stability) with acceptable data generated using the original method were re-tested with the 2.0 method in a single run.
- Our internal statistician was provided with the reportable values, after correction of any dilution factor.
- Sponsor was keen, if bias was evident, to explore how a correction factor could help alleviate this.

Cross-validation - outcomes

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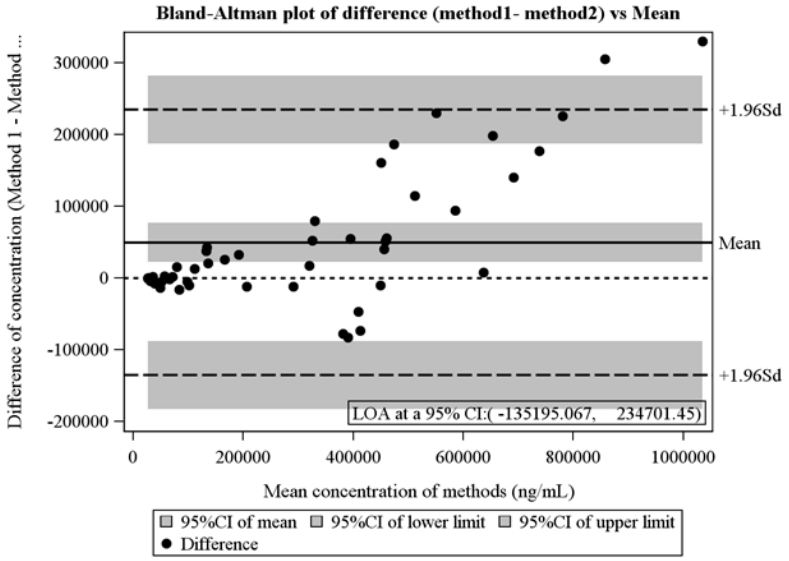
➢ Bland-Altman

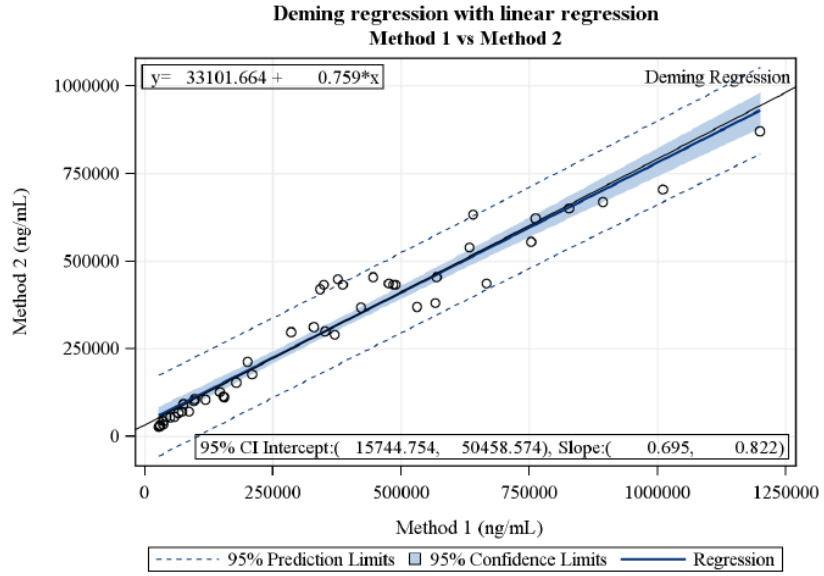
does not directly provide a numerical relationship or correction factor between the two methods. It primarily focuses on assessing the agreement and identifying any systematic biases or trends between the measurements



t-test p-value of 0.0007.
These results suggests that there is not a good level of agreement between paired data.

➢ Deming Regression

considers both the measurement errors and the uncertainties associated with both methods. It estimates the slope (proportional bias) and intercept (fixed bias) of the relationship between the two methods, allowing for the determination of a correction factor".

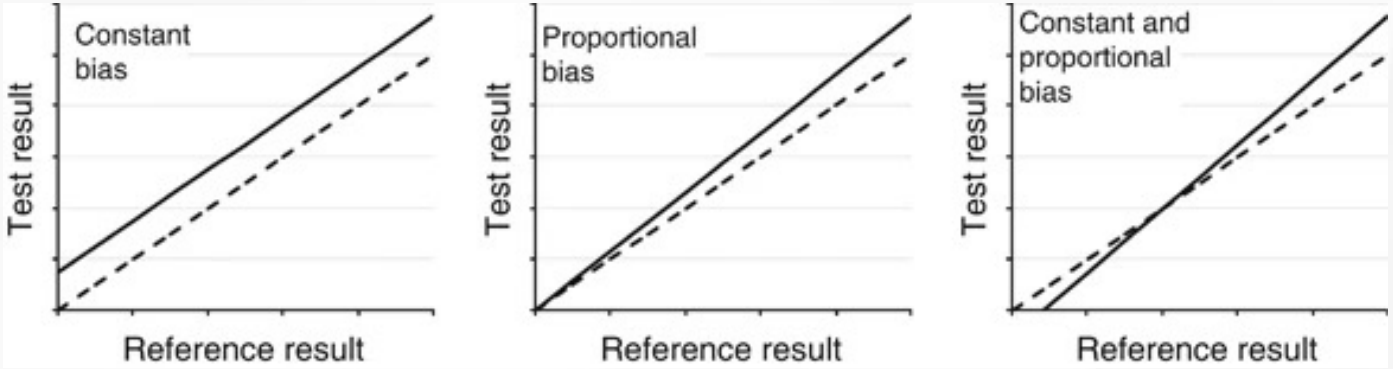


Widely scattered data points indicating variability.

The intercept of the best-fit line is 33101.66, significantly different from zero indicating a systematic difference.

The slope of the best-fit line is 0.76, which suggest that there is a proportional bias.

How can it be so different ?



Back to the BA scientist to have a think about the data…

Original		2.0	
Dilution	#	Dilution	#
400	23	10	18
1000	9	100	32
2000	14		
5000	4		

How can it be so different ?

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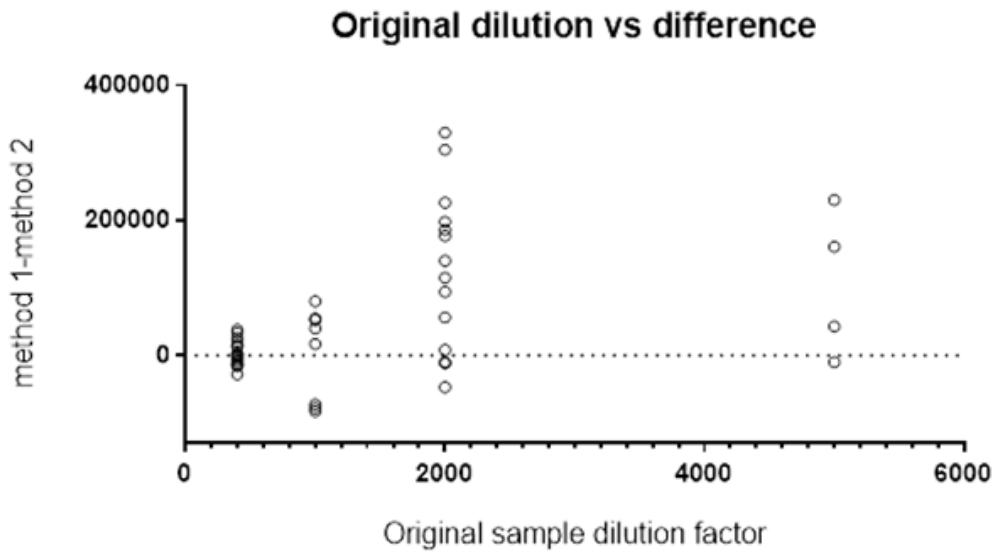

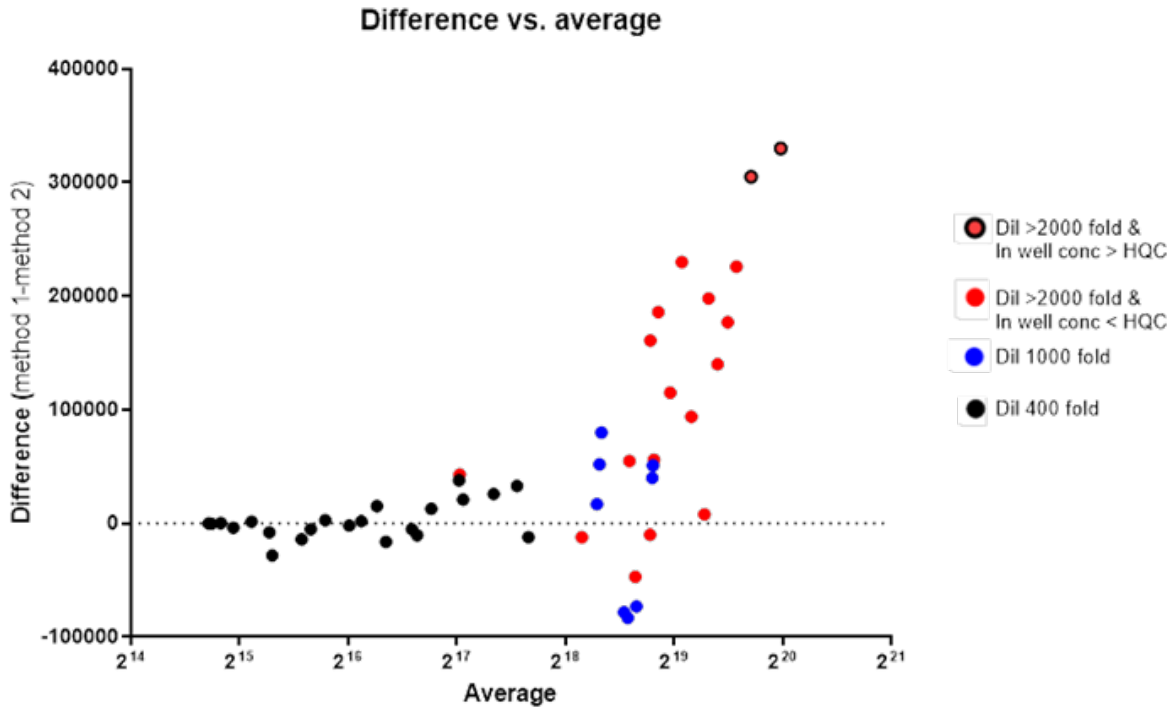

How was it resolved ??

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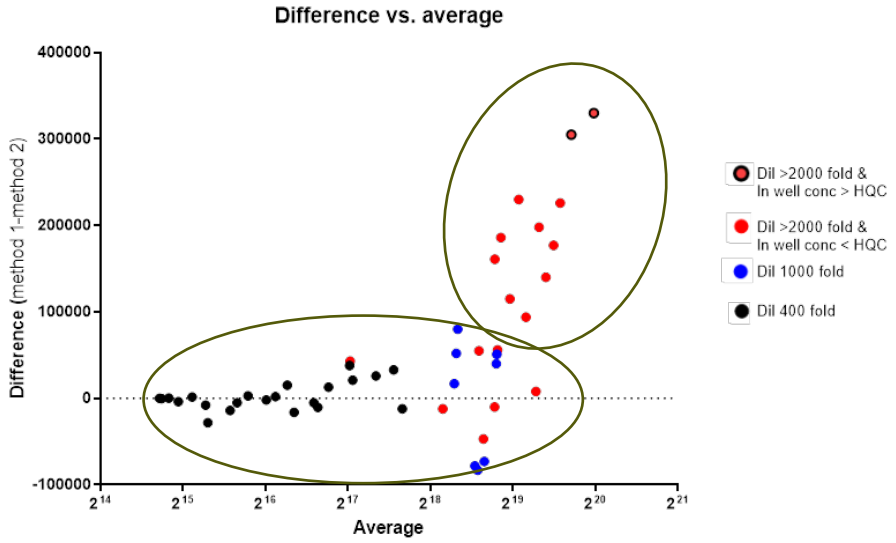
- Statistician looked at the bias of two separate concentration groups, high and low concentration samples:
  - Group 1 (< 500,000 ng/mL):
    35 samples; good agreement was observed between the two methods within this concentration range. no significant difference observed in the absolute difference and the percentage difference was not significant
  - Group 2 (> 500,000 ng/mL):
    In this group, there 12 samples, and a clear proportional bias was observed between the two methods within this concentration range



- The PK modeller applied the Deming equation to some of the data in the original study to give an estimate of typical Cmax concentrations - lowers concentration by <24%

- The stakeholder concluded that the data could not be combined, nor could a correction factor be applied

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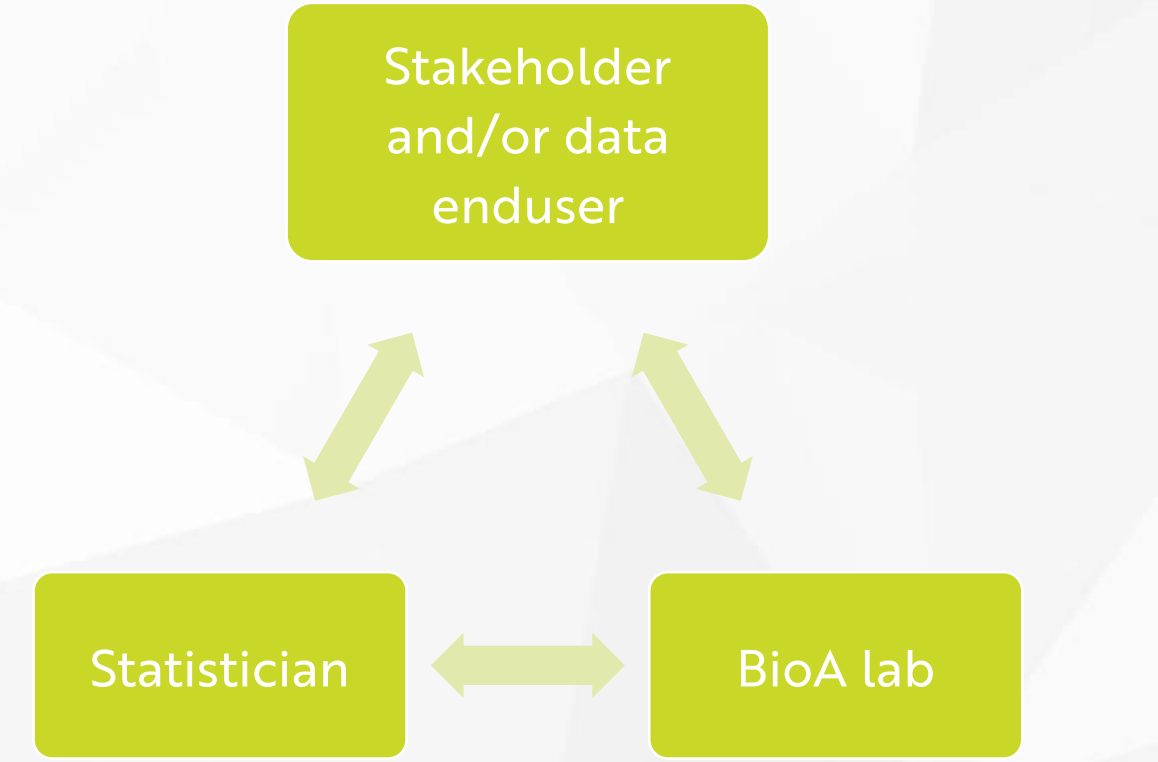
- Different format, different platform?
- Do the calibration ranges overlap?
- Are the same dilutions applied to the samples?

Had this not been considered a ‘different’ method, or the cross-validation been conducted with only QCs then the outcome would have been quite different

Acknowledgements



Sophia Li – statistics
Kim Deadman – PK

Tom Wilford - scientist

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