

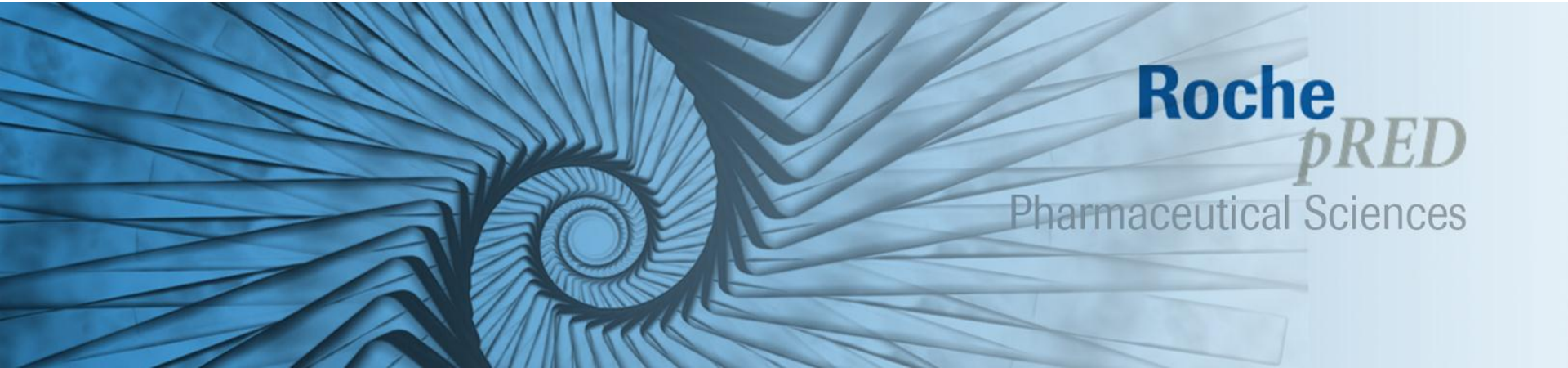
Lisbon, 24th of February 2014



6th Open Scientific EIP Symposium on Immunogenicity of Biopharmaceuticals

Challenges for the determination of cutpoints

*Sabine Bader, Nicole Justies, Thomas Emrich,
Kay-Gunnar Stubenrauch, Julia Heinrich*

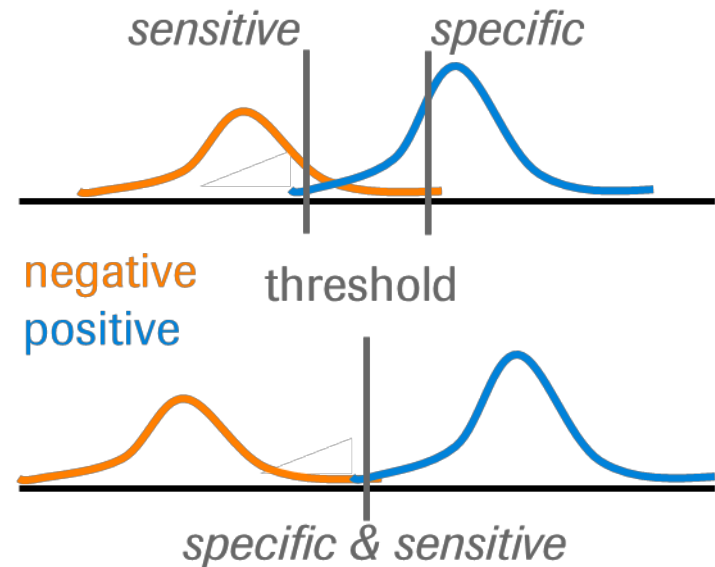


ADA screening assay cutpoint

Introduction

Anti-drug antibody (ADA) assays

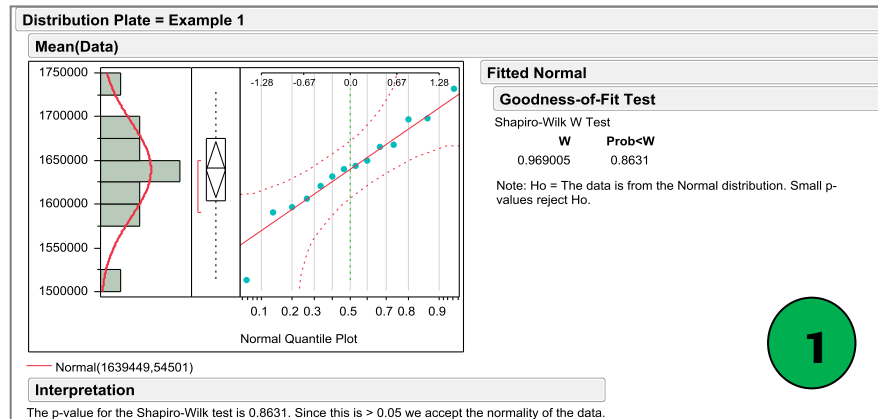
- **Aim**
 - Find a cutpoint that allows to distinguish between *ADA positive* and *negative* samples
- **Common proceeding**
 - Define cutpoint by characterization of a *negative* sample population with a 95 % quantile:
 - 5 % of negative cases will be false positive;
 - few positive cases shall be missed.



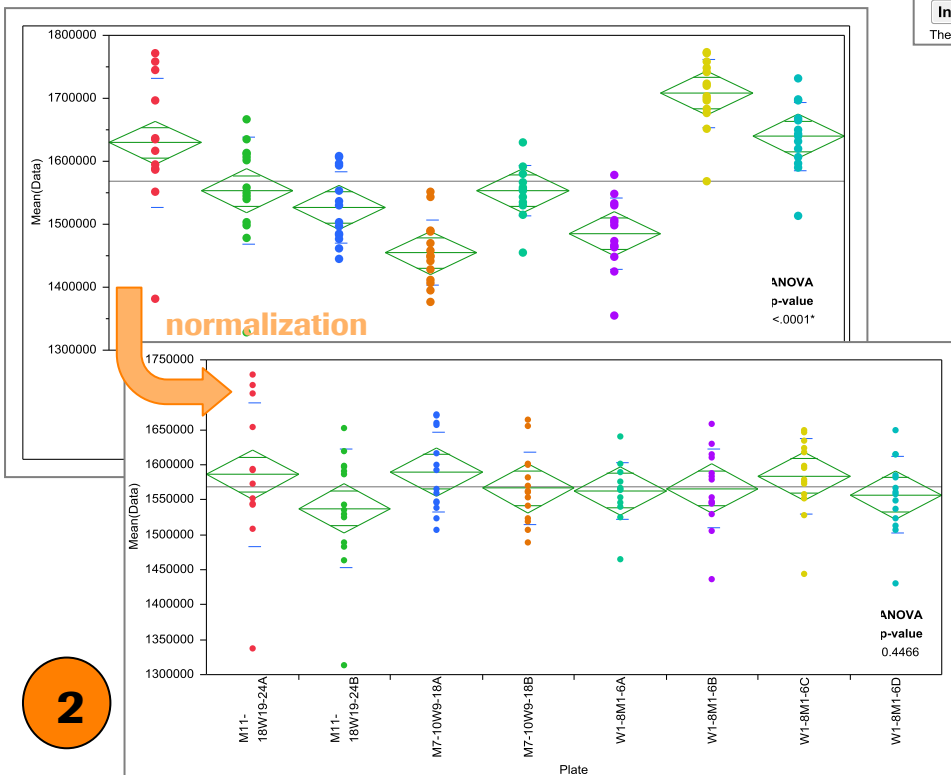
ADA screening assay cutpoint

Ideal case

1. Data show a **normal distribution**
2. Plate normalization leads to **equal means and variances**

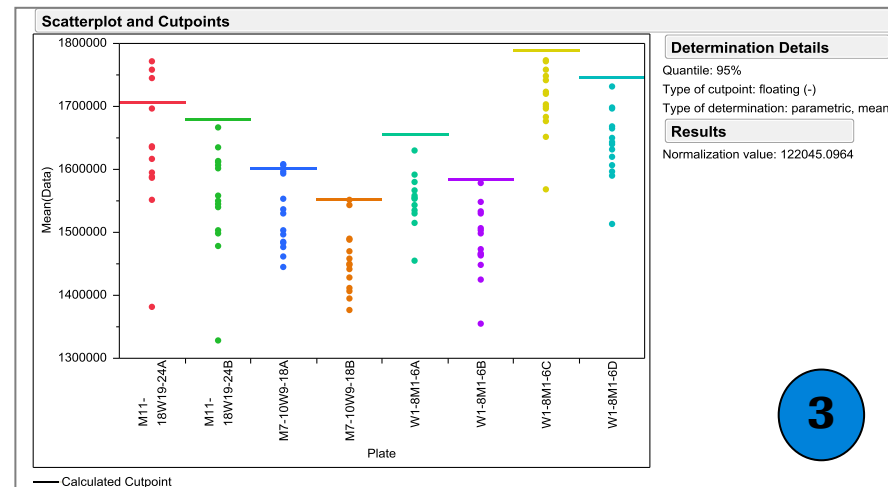


1



2

3. Determination of **parametric cutpoint** based on mean and standard deviation



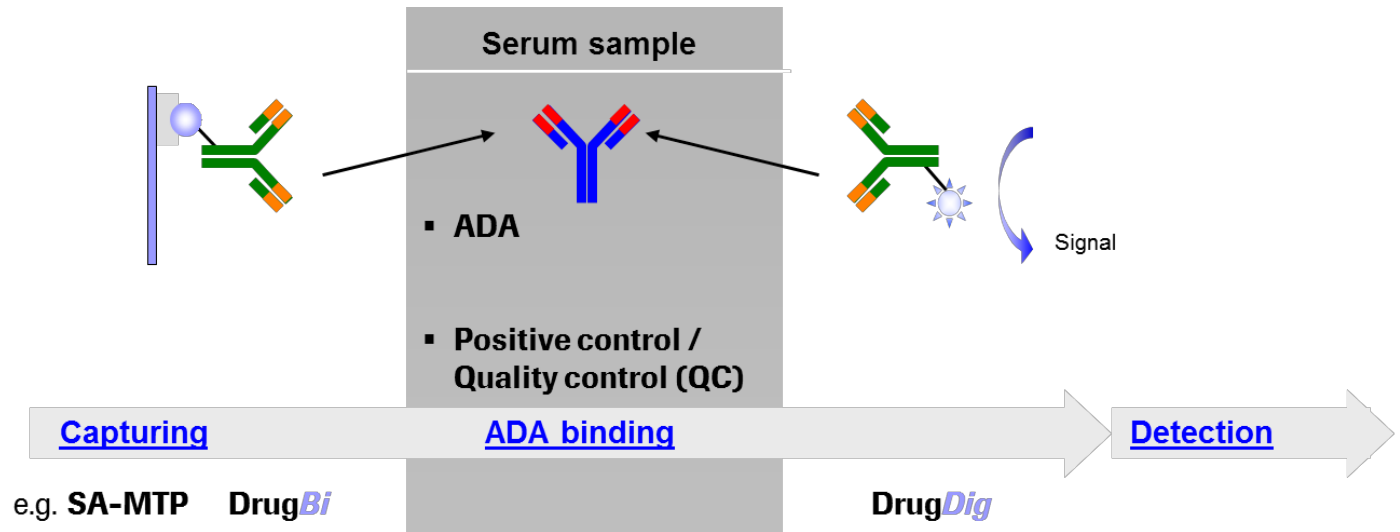
3

Roche ADA assays

Assay format

Bridging assay

- Sandwich immunoassay



- **Diagnostic assay**
 - ➔ **small background aspired**
- **Highly specific capturing surface** (e.g. streptavidin – biotin interaction) & **high-quality assay components**
 - ➔ **extremely low** levels of **unspecific binding** of biological matrix components

Roche ADA assays

Assay data

Aspired low matrix effect leads to new challenges in data analysis

→ Small ODs close to instrument level

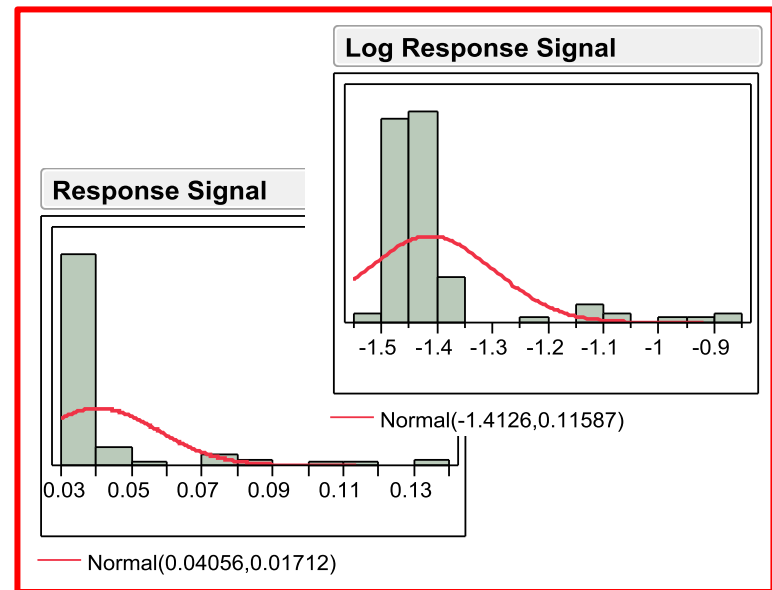
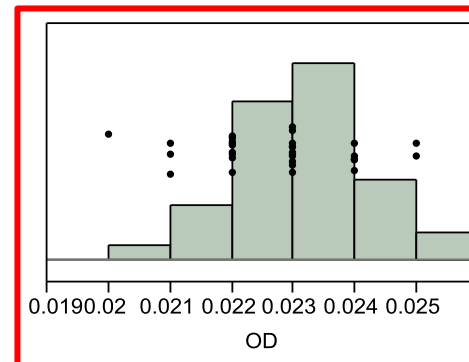
- Measuring samples that are negative by definition with very low technical noise

→ Standard reader settings can lead to **binned data** due to number of decimals

- Data not continuous

→ Data show **no normal distribution**

- Mostly neither normal nor log normal distribution – even after outlier exclusion
- **Rules out standard parametric and ‘robust’ methods for cutpoint determination**



Roche ADA assays

Cutpoint determination

- **Nonparametric cutpoint calculation**
 - **Due to skewed distribution** of data close to instrument level
 - **Screening cutpoint** **empirical 95 % quantile**
 - **Confirmatory cutpoint** **empirical 99 % quantile** (or even 99.9 %)

Roche ADA assays

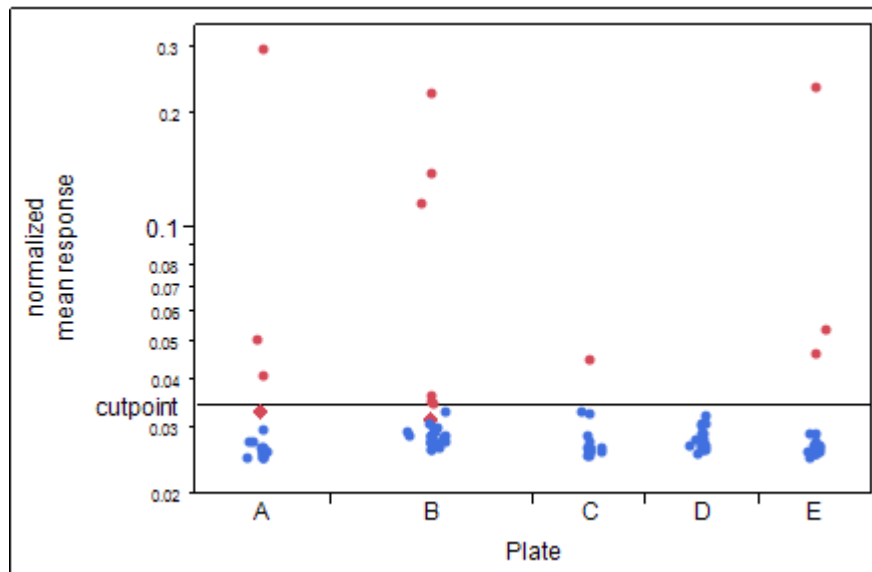
Cutpoint determination

- **Nonparametric cutpoint calculation**
 - **Due to skewed distribution** of data close to instrument level
 - **Screening cutpoint** **empirical 95 % quantile**
 - **Confirmatory cutpoint** **empirical 99 % quantile** (or even 99.9 %)
- **Challenges with nonparametric cutpoints**
 - **Limited sample size** leads to **strong influence of maximum value**
 - Sample size between 3x15 (preclinical) and 3x100 (clinical)
 - Cutpoint can correspond to the maximum of all observed values – potentially compromising robustness as based on only one sample
 - This can result in a deviation of the aspired percentage of false positive samples
 - **Report „actual“ quantile** (e.g. 98 % quantile) – otherwise claiming to be more strict than actually the case
 - Resulting **cutpoint depends on applied software** as algorithms vary

Case study – mAb XY

Unexpected high amount of positives in study data

Pre-dose data of 120 healthy volunteers (phase I study)



Screening cutpoint was statistically evaluated to lead to **5% false positives** in validation data.

Study data: 12.5 % screening positive samples (15/120)

(10.8 % without two borderline cases with only one out of two replicates above cutpoint but mean below)

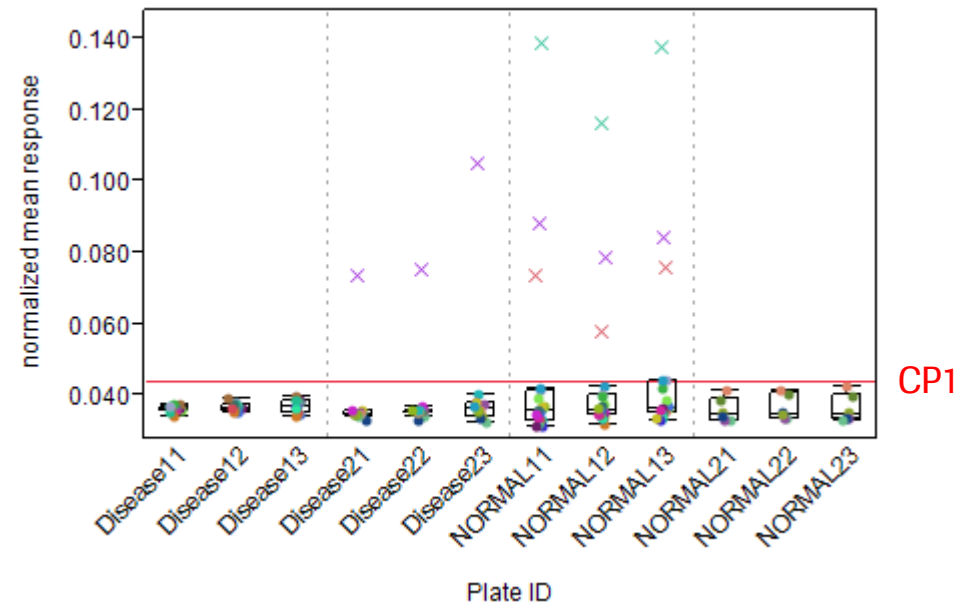
→ Percentage of positives unexpectedly high in set of pre-dose samples of healthy volunteers!

Case study – mAb XY

Re-evaluation of validation data

1. Original approach (CP1)

12 outliers (x) were identified in the validation study data and **excluded** for screening cutpoint calculation.



Validation study data

- 50 samples (25 disease, 25 healthy)
- measured on triplicate plates

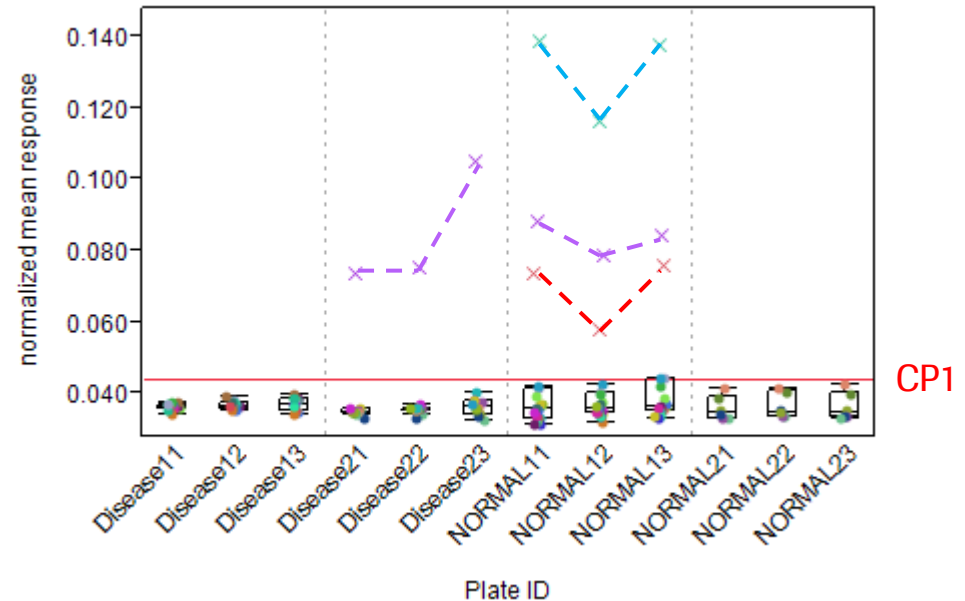
Case study – mAb XY

Re-evaluation of validation data

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12 outliers (x) were identified in the validation study data and **excluded** for screening cutpoint calculation.

- However: **biological not technical outliers !**
- They **reflect part of the negative population** that we aim to characterize, and are therefore **not to be excluded** from screening cutpoint calculation (unless samples assumed to be positive).



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Case study – mAb XY

Re-evaluation of validation data

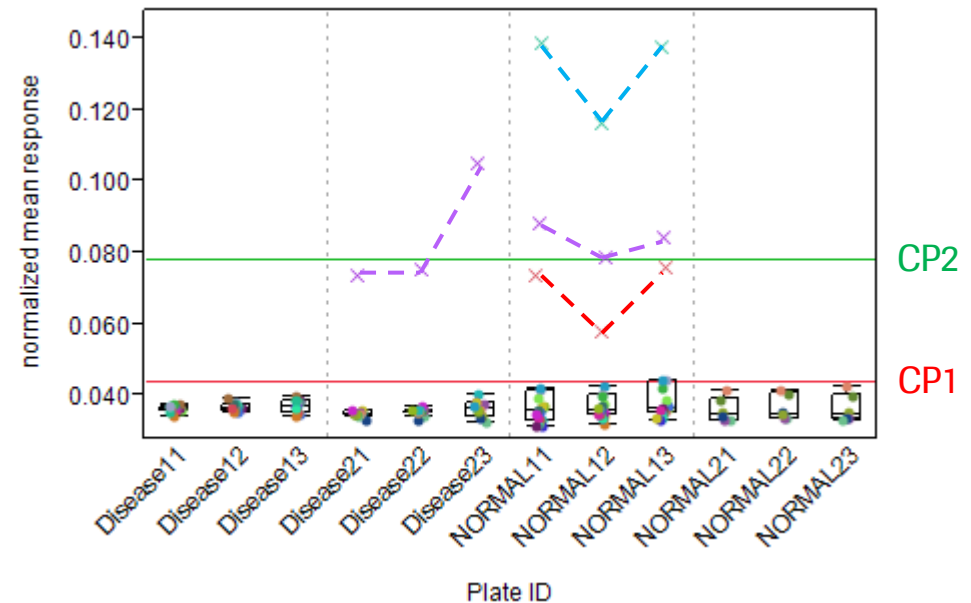
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2. Re-evaluated approach (CP2)

No (biological) **outlier exclusion**



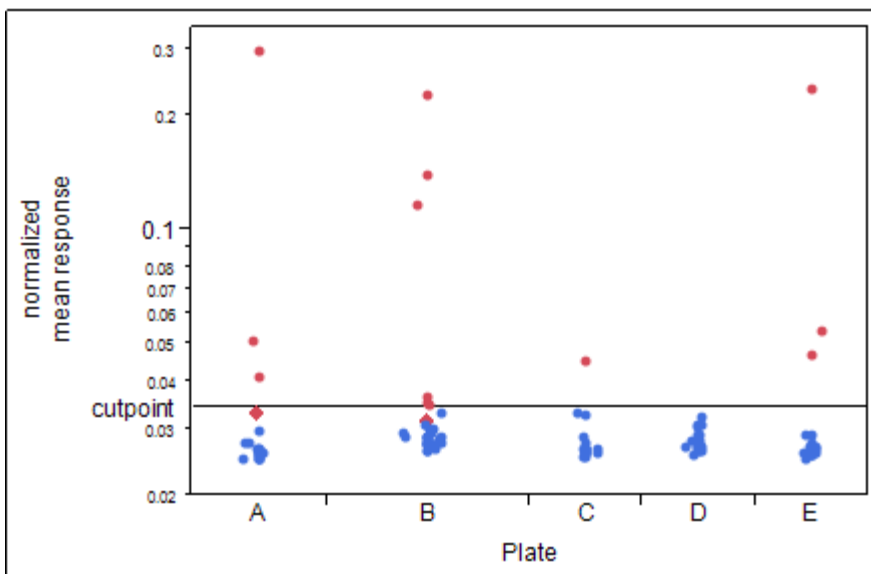
Validation study data

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Case study – mAb XY

Re-evaluated screening cutpoint

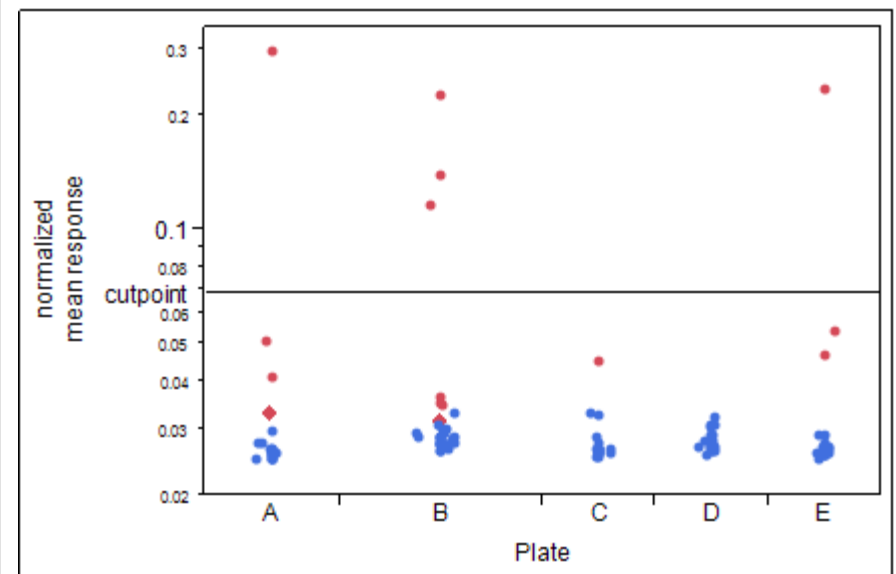
Back to study data



Original approach (CP1):

12.5 % screening positive samples (15/120)

- Percentage of positives unexpectedly high in pre-dose samples of healthy volunteers!



Re-evaluated approach (CP2):

4.2 % screening positive samples (5/120)

- Percentage of positive samples now in expected range

Case study – mAb XY

Re-evaluated screening cutpoint

Change of validation parameters after re-evaluation

Validation parameter	Validation result CP1	Validation result CP2
Mean NC signal (OD) during validation runs	0.0374	0.0374
Normalization value (additive normalization)	0.006	0.0339
Assay sensitivity	0.288 ng/mL	1.64 ng/mL
Drug tolerance factor	80	13
= ratio of drug concentration and lowest positive control concentration giving a signal above the cutpoint	→ 250 ng/mL ADA can still be found with 20 µg/mL drug	→ 250 ng/mL ADA can still be found with 3.25 µg/mL drug

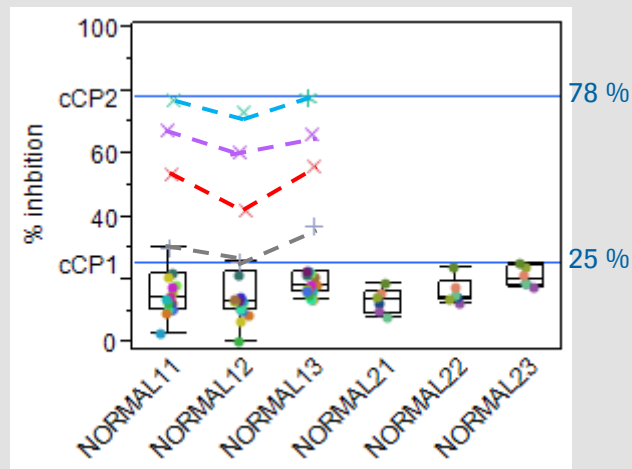
Case study – mAb XY

Comparable issue for confirmatory cutpoint

Validation data of 25 healthy samples

1. Original approach (cCP1)

12 outliers (x) were identified in the validation study data and **excluded** for confirmatory cutpoint calculation.



2. Re-evaluated approach (cCP2) No outlier exclusion

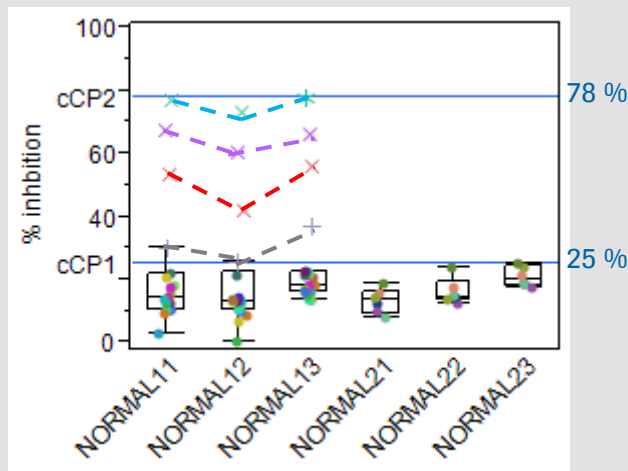
Case study – mAb XY

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No outlier exclusion

Pre-dose data of 120 healthy volunteers

- % confirmed positive samples

		Screening (OD)	
		sCP1 0.043	sCP2 0.071
		12.5 % (15/120) screening positives	4.2 % (5/120) screening positives
Confirmation (% inhibition)	cCP1 25 %	9.2 % (11/120) confirmed positives	4.2 % (5/120) confirmed positives
	cCP2 78 %	4.2 % (5/120) confirmed positives	4.2 % (5/120) confirmed positives

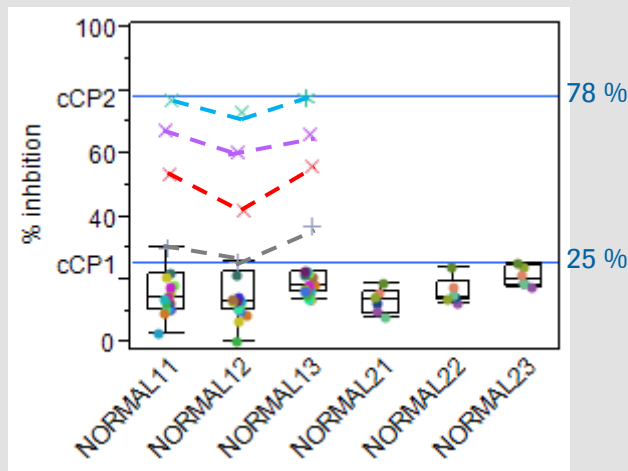
Case study – mAb XY

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For sCP2 , all screening positives are confirmed with both cCPs.		12.5 % (15/120) screening positives	4.2 % (5/120) screening positives
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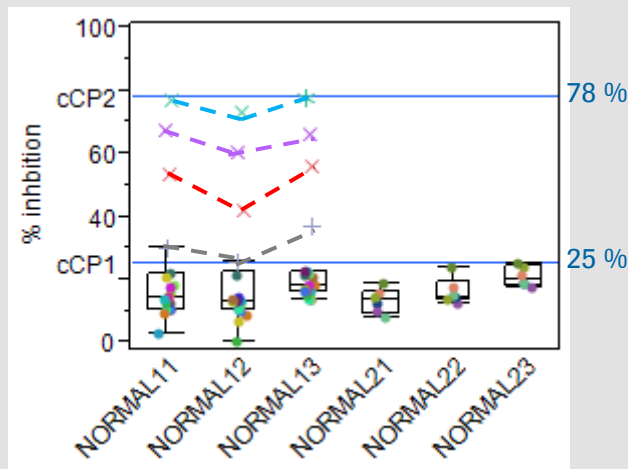
Case study – mAb XY

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For **sCP2**, all screening positives are confirmed with both cCPs.

➤ **‘Conservative’ approach (cCP1) chosen to mitigate risk of false negatives**

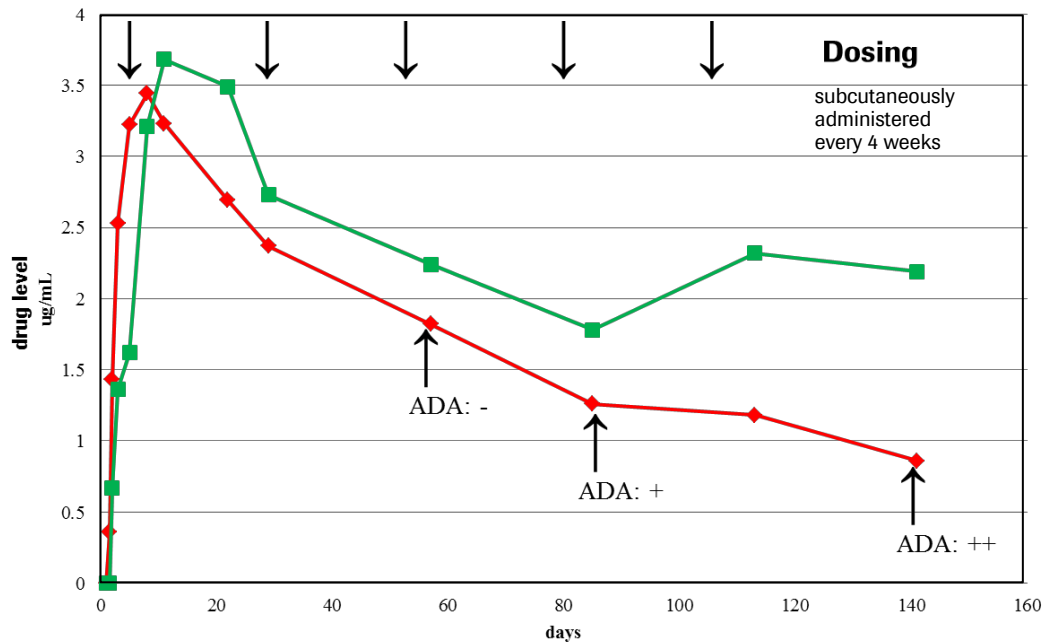
Case study – mAb XY

‘Real’ positive samples

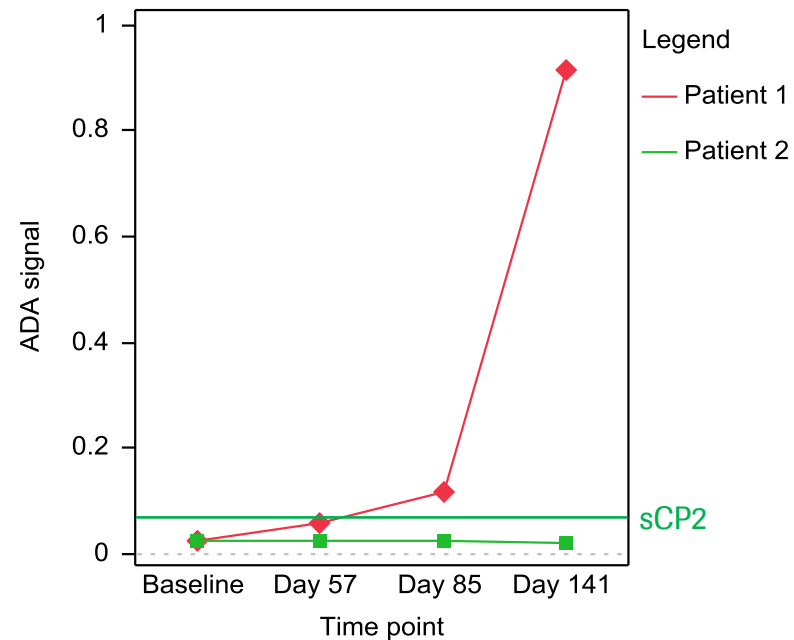
Patient 1: Expected PK profile
Patient 2: PK decrease & ADA increase
 (patients from same dose group)

- **Clinical on-treatment study data of patients**

PK Profile



ADA profile



➤ „Real“ positive samples → ADA signal in different range

Challenges for the determination of cutpoints

Summary

- **Typical challenges**

- Lack of normal distribution which hinders usage of “standard” methods
- Imprecise determination of empirical quantile depending on sample size
- Strong influence of outlier treatment/interpretation on result

- **Statistics can offer only limited support**

- Mainly for ‘ideal’ cases
 - But even then seemingly in irrelevant OD range

- **Solution more on biological / experimental level ?**

- Looking for and assessing different new approaches
 - Increased background, ...
- Potentially go via positive controls
 - As actual positives seem to lie in completely different range anyways

Challenges for the determination of cutpoints

Acknowledgements

- **Bioanalytics**

- **Penzberg**

- Roland Staack

- Martin Schäfer

- Apollon Papadimitriou

- **Basel**

- Corinne Petit-Frère

- Eginhard Schick

- Herbert Birnboeck

- **Biostatistics**

- **Penzberg**

- Anton Belousov

- Florian Lipsmeier

A large orange oval with a white border and a slight drop shadow, containing the text "THANK YOU FOR YOUR ATTENTION! ANY QUESTIONS?".

**THANK YOU FOR
YOUR ATTENTION!
ANY QUESTIONS?**

Doing now what patients need next