Lisbon, 24th of February 2014

6th Open Scientific EIP Symposium on
Immunogenicity of Biopharmaceuticals

Challenges for the determination of cutpoints

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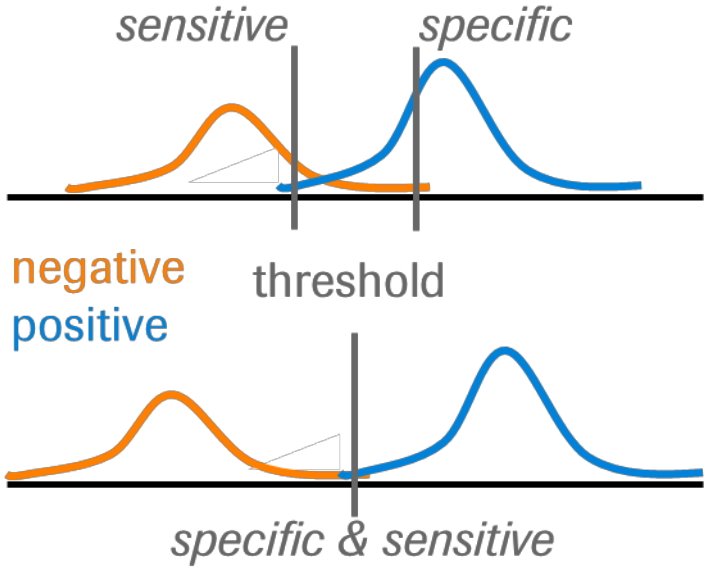
ADA screening assay cutpoint
Introduction

Anti-drug antibody (ADA) assays

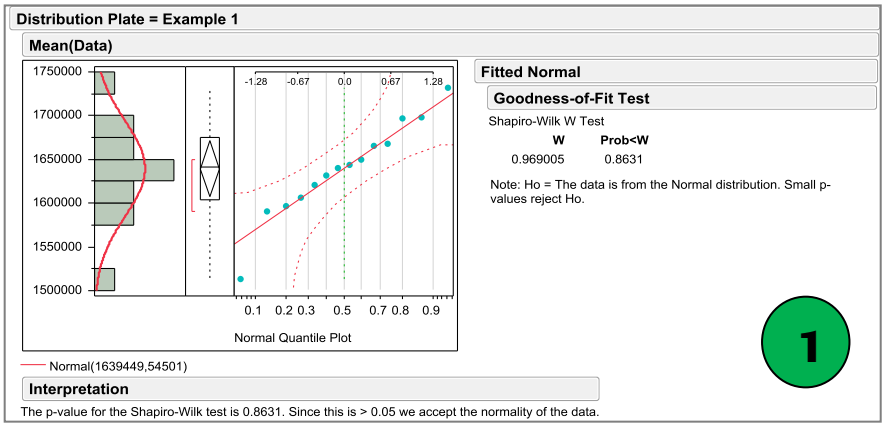
- **Aim**
  - Find a cutpoint that allows to distinguish between ADA *positive* and *negative* samples
- **Common proceeding**
  - Define cutpoint by characterization of a *negative* sample population with a 95 % quantile:
    - ➢ 5 % of negative cases will be false positive;
    - ➢ few positive cases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

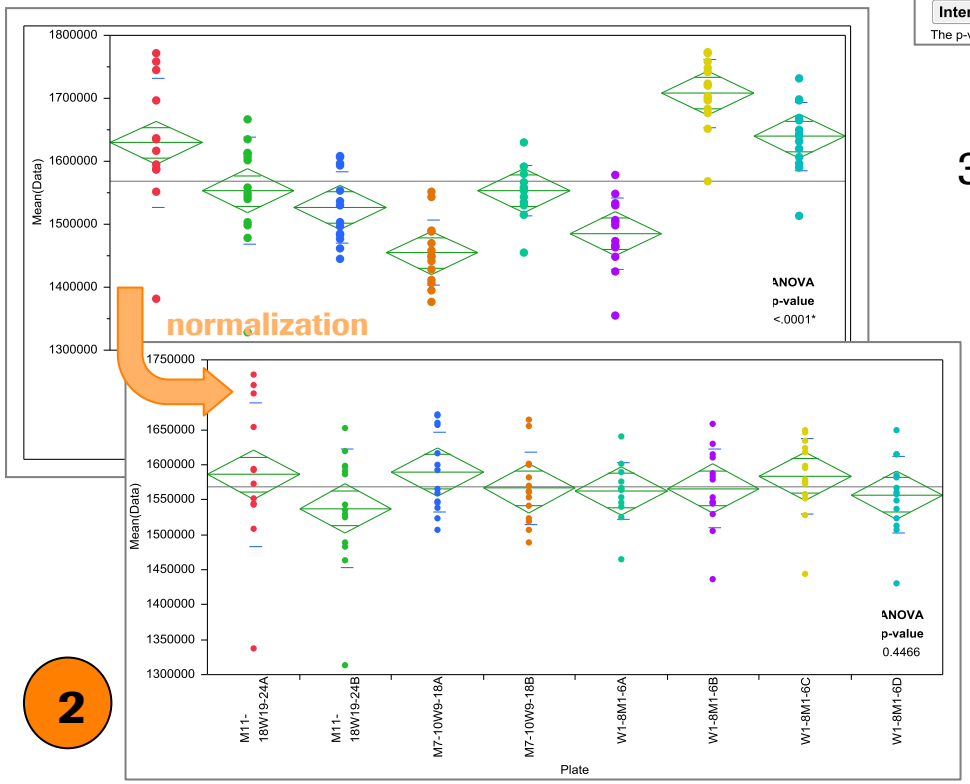


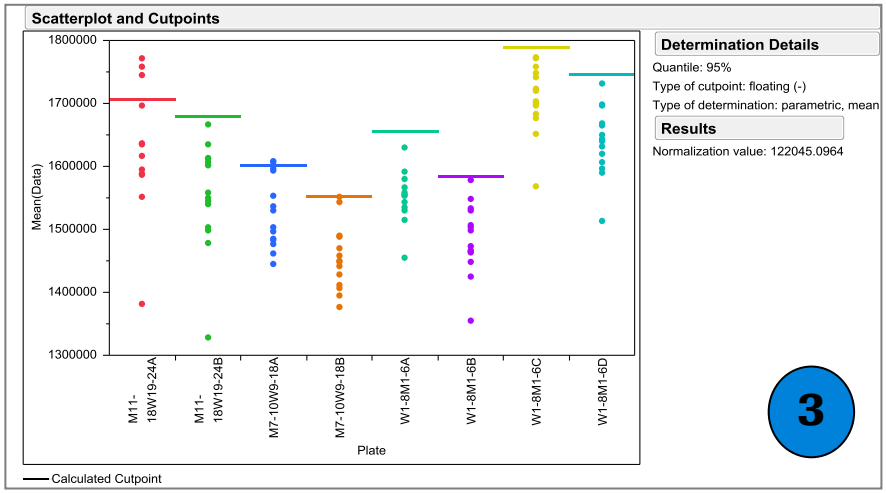
ADA screening assay cutpoint

Ideal case

1. Data show a **normal distribution**
2. Plate normalization leads to **equal means and variances**
3. Determination of **parametric cutpoint** based on mean and standard deviation



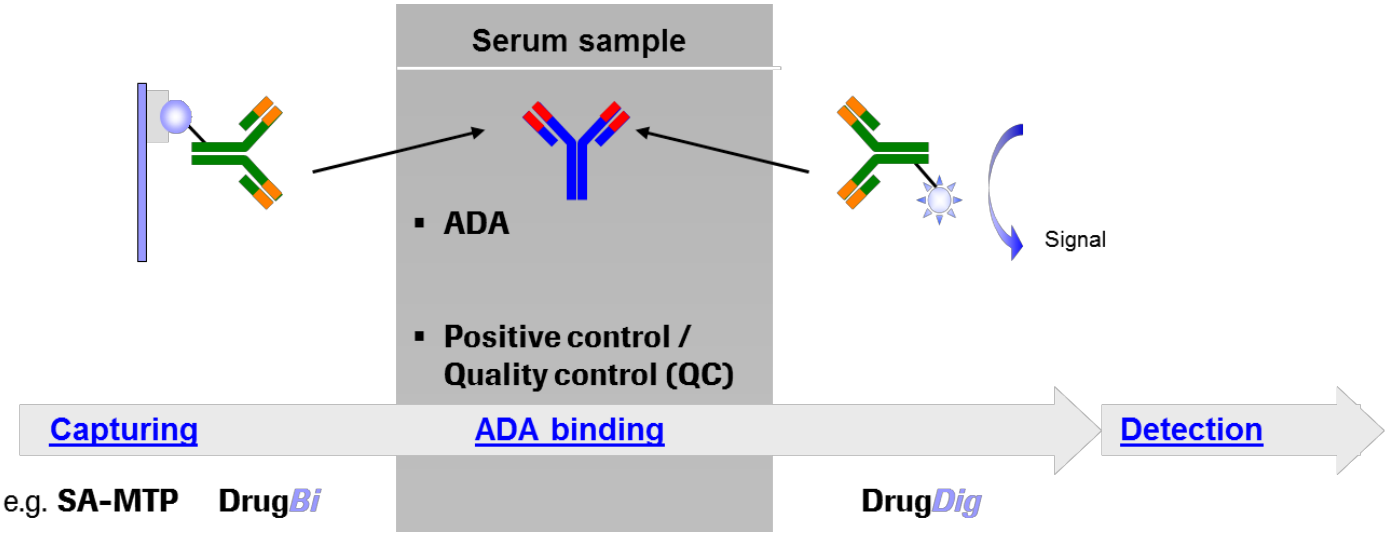




Roche ADA assays
Assay format

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**

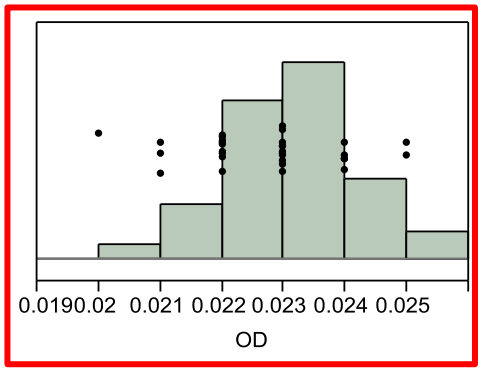
- ➢ Measuring samples that are negative by definition with very low technical noise

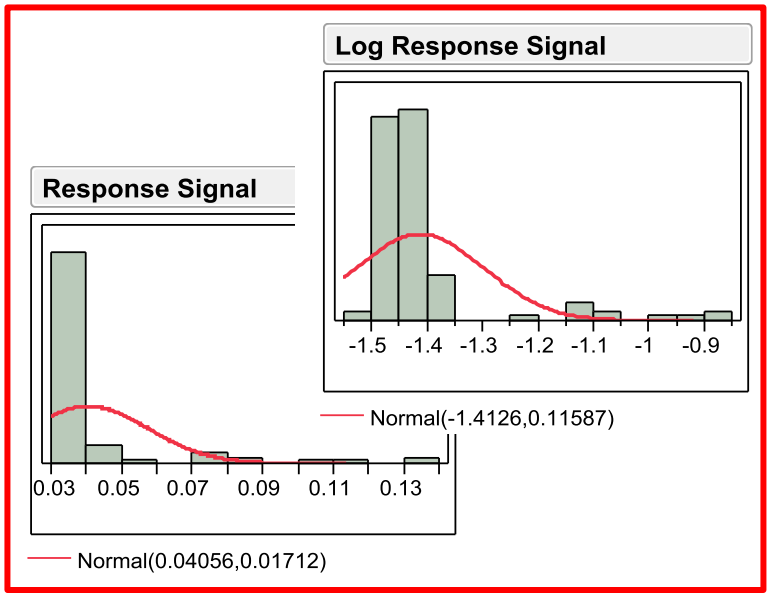
➔ Standard reader settings can lead to **binned data** due to number of decimals

- ➢ 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**
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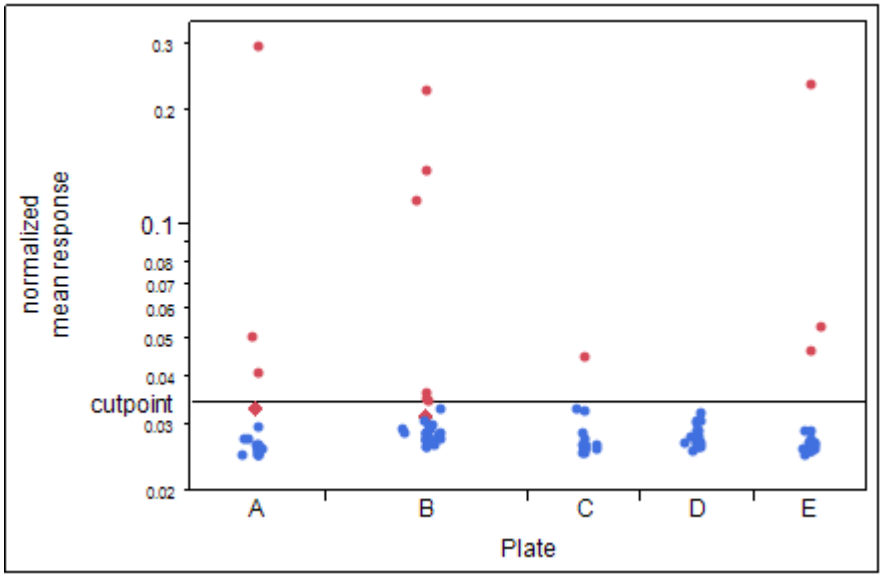
    - **Screening cutpoint** **empirical 95 % quantile**
    - **Confirmatory cutpoint** **empirical 99 % quantile** (or even 99.9 %)
- **Challenges with nonparametric cutpoints**
  - **Limited sample size** leads to **strong influence of maximum value**
    - Sample size between 3x15 (preclinical) and 3x100 (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!**

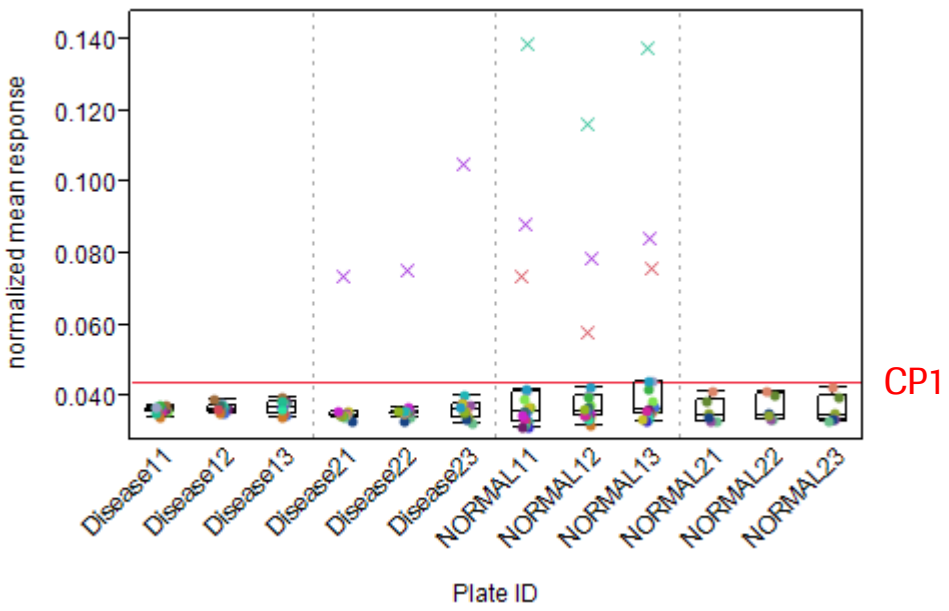
normalized response = response – plate-specific negative control + average negative control across plates

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

normalized response = response – plate-specific negative control + average negative control across plates

Case study – mAb XY

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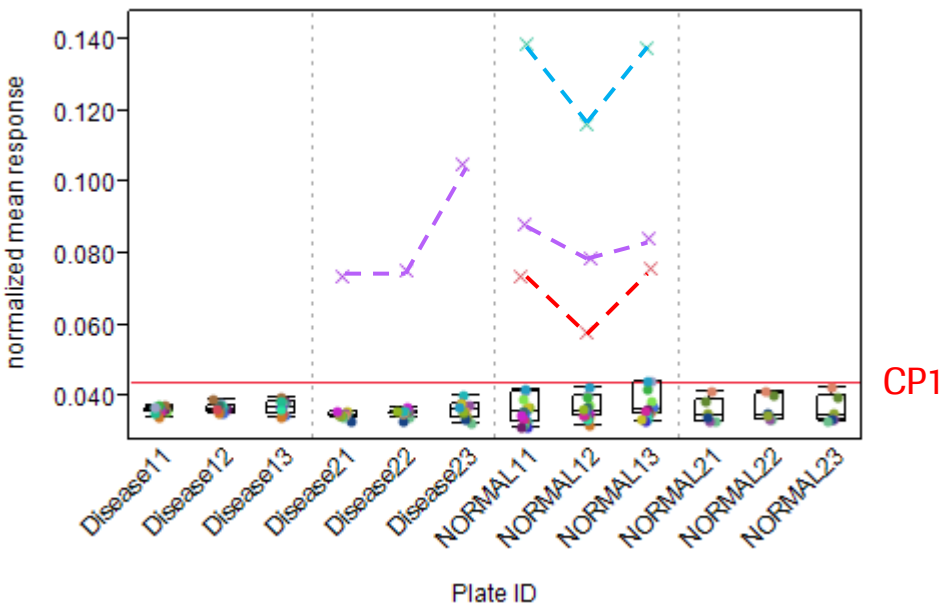
12 outliers (x) were identified in the validation study data and **excluded** for screening cutpoint calculation.

- 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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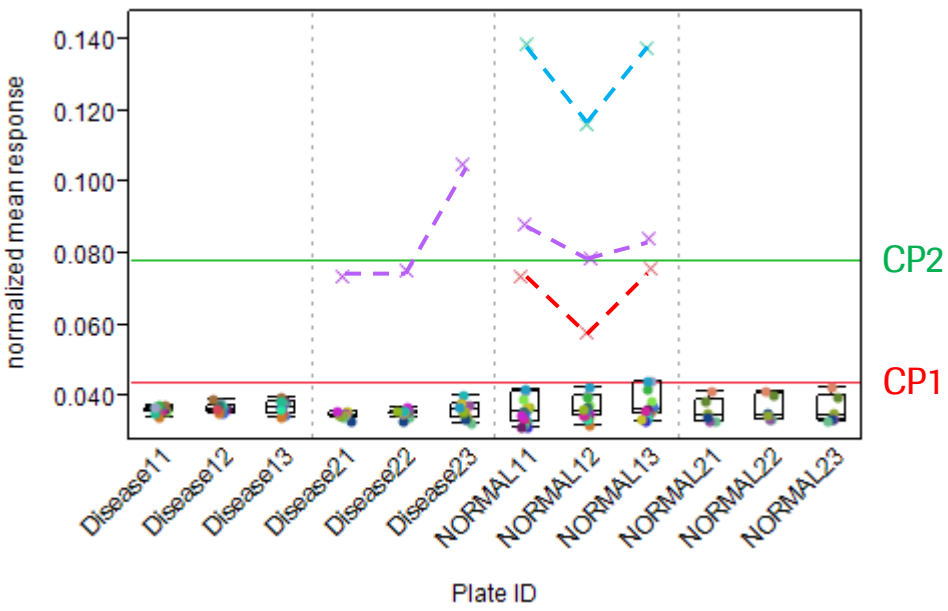
2. Re-evaluated approach (CP2)

No (biological) **outlier exclusion**



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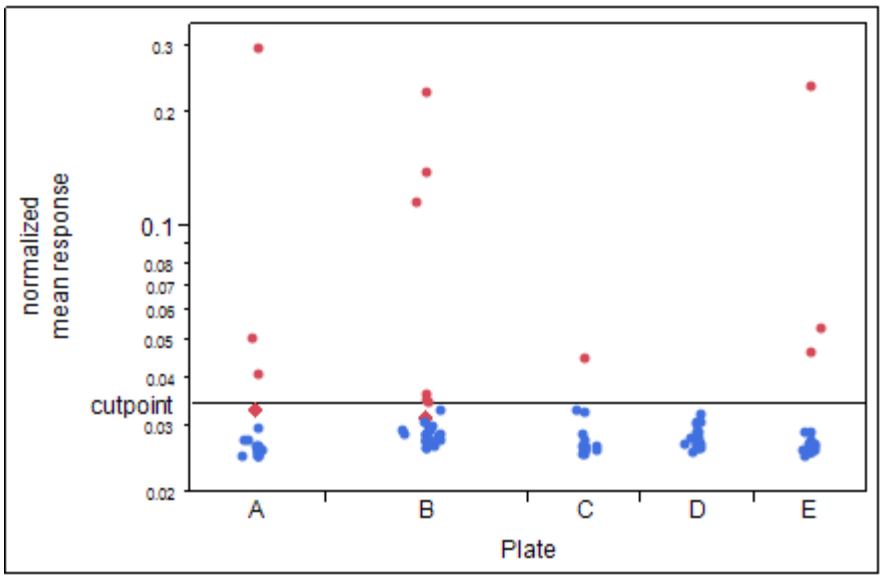
Case study – mAb XY

Re-evaluated screening cutpoint

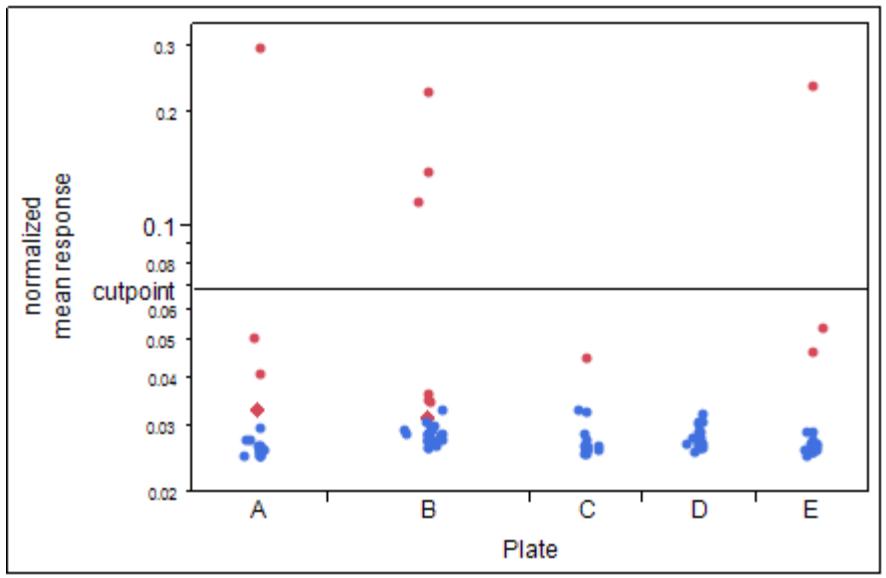
Back to study data



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**

normalized response = response – plate-specific negative control + average negative control across plates

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

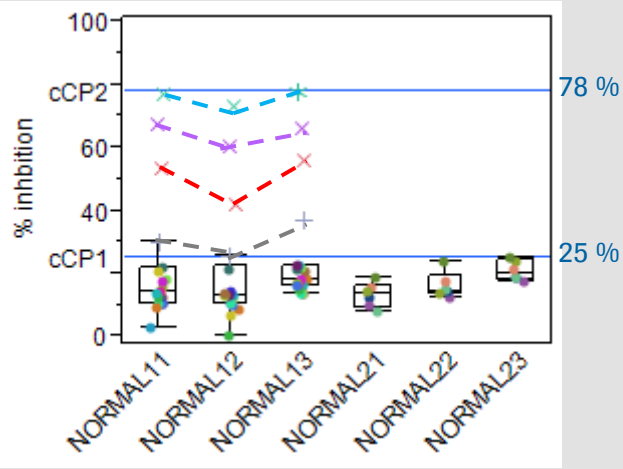
Case study – mAb XY

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

Comparable issue for confirmatory cutpoint

Validation data of 25 healthy samples

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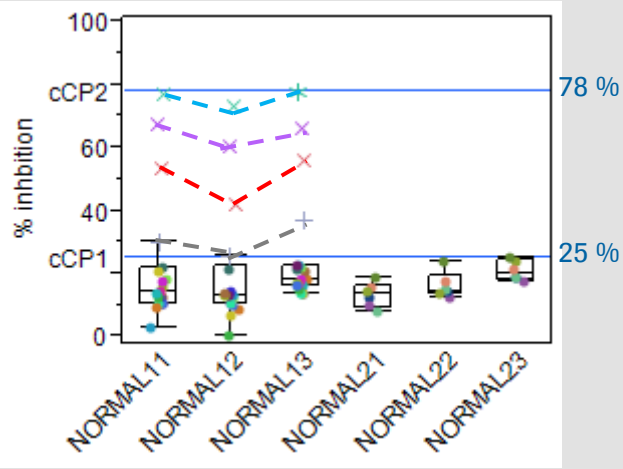

2. Re-evaluated approach (cCP2)

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

Comparable issue for confirmatory cutpoint

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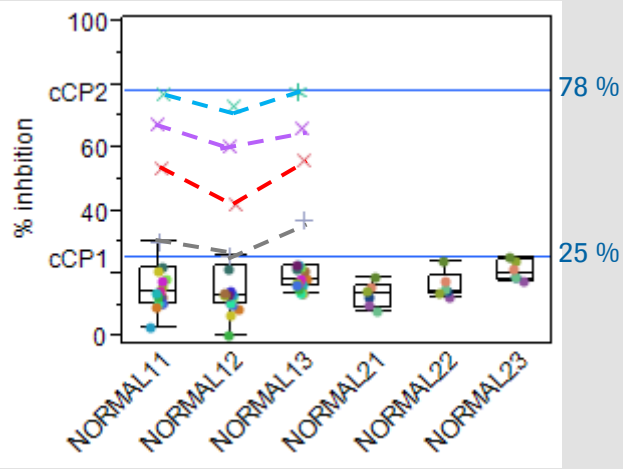

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Case study – mAb XY

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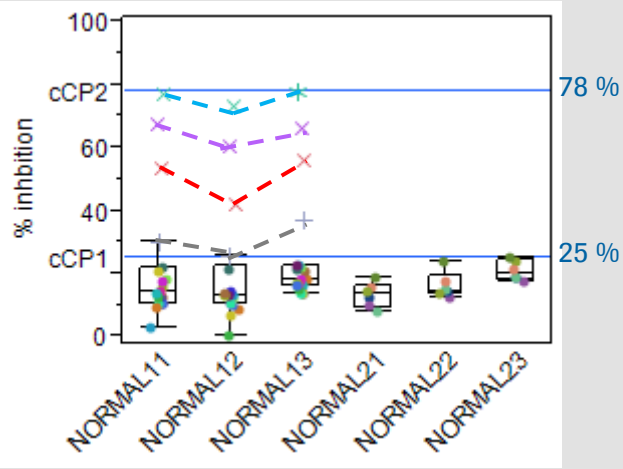

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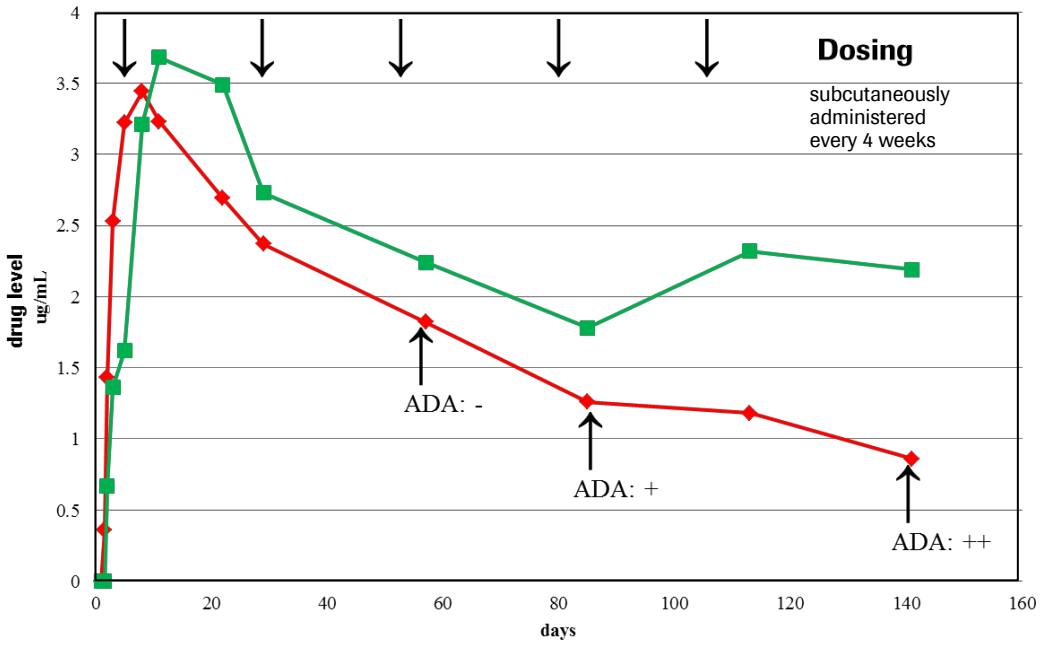
➢ **'Conservative' approach** (cCP1) **chosen to mitigate risk of false negatives**

Case study – mAb XY
'Real' positive samples

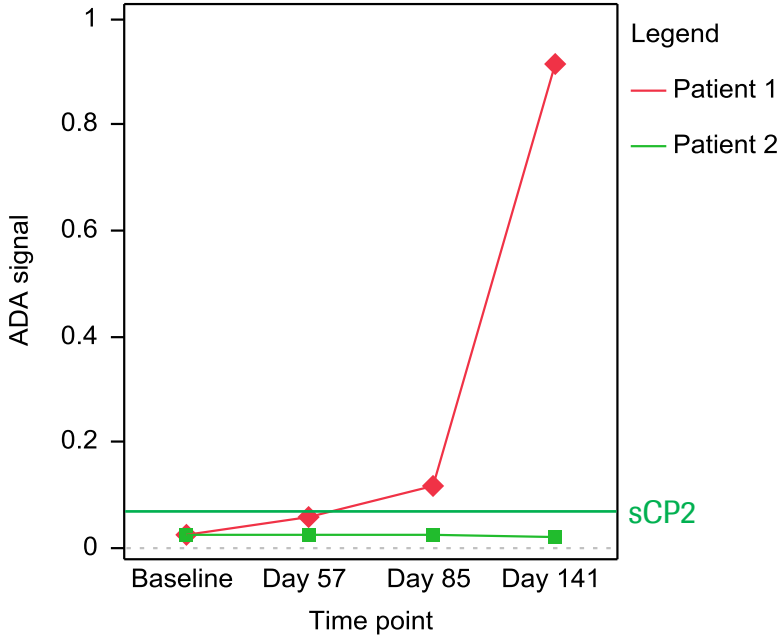
Patient 1: Expected PK profile
Patient 2: PK decrease & ADA increase
(patients from same dose group)

- **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
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    Herbert Birnboeck
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  - **Penzberg**
    Anton Belousov
    Florian Lipsmeier

THANK YOU FOR YOUR ATTENTION!

ANY QUESTIONS?

Doing now what patients need next