Success and Failure of Generic Assays: Case Studies and a Fit-for-Purpose Strategy for Non-Clinical ADA Support

17th Open Scientific EIP Symposium, 2026

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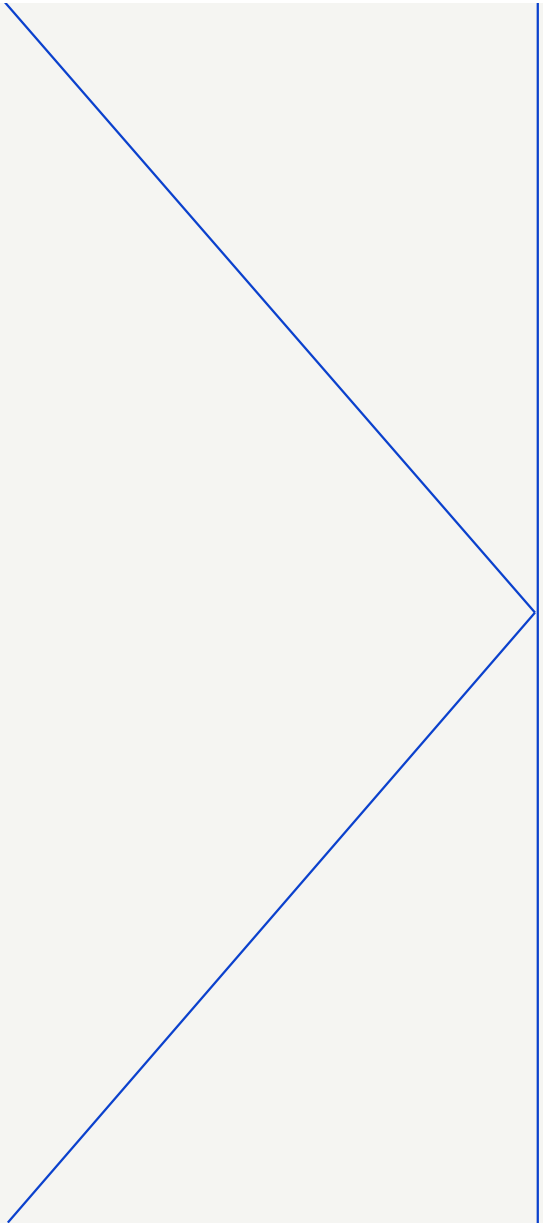
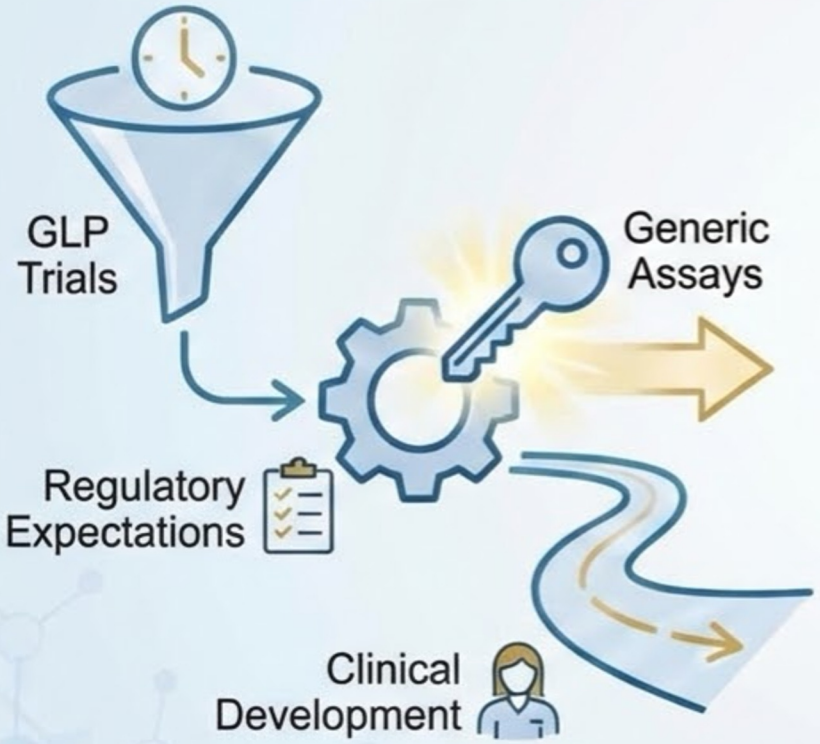
Roche pRED

Agenda



- Generic assay implementation and case studies
- Testing Strategy considerations
- Fit for purpose validation implementation
- Summary

Optimising Pre-clinical Bioanalytical Delivery: Utilizing Generic Assays



- Timely data delivery from GLP trials is a non-negotiable requirement to allow for swift transition into clinical development.
- Consequentially, Sponsors are under constant pressure to streamline bioanalytical and toxicology workflows >
- Here a simple solution to satisfy a big chunk of our portfolio, is by deploying **“one size fits all” generic PK and ADA methods**.

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Roche's generic Immune complex ADA assay

- Roche pRED established the generic immune-complex assay in 2009 (!) mainly used in non-reg analysis.

he Appeal:

- Unlike Bridging assays - no need for labeled drug
  - Excess drug added for ADA complexes to form
  - Inherently drug tolerant . . .
- Standardized protocols across different programs
  - 100-MRD
  - Fixed Cap/Detect and complexing drug concentrations

egulated implementation:

- Deployment into GLP studies: savings in program BioA R&D time internally and streamlined externalisation at CROs
  - Even with MIAD - saving of ~2 weeks internally
  - CRO's familiar with the method!
- Assay still needs a full validation per drug molecule . . .

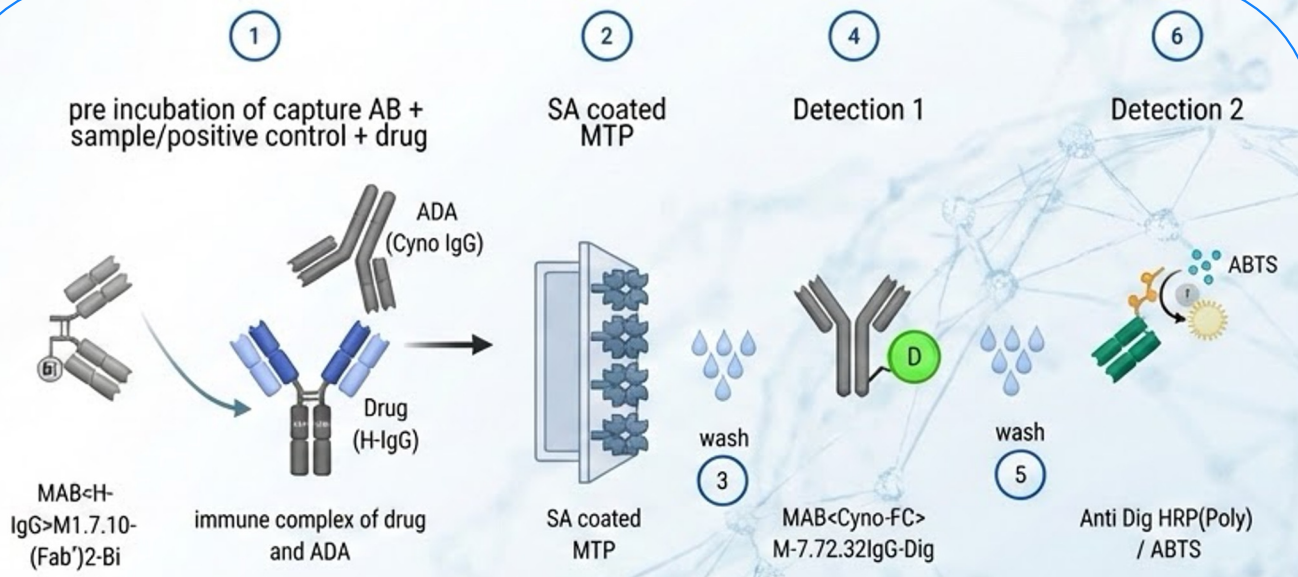


Figure 1: Schematic description of ELISA test procedure for the drug/ADA immune complex assay.

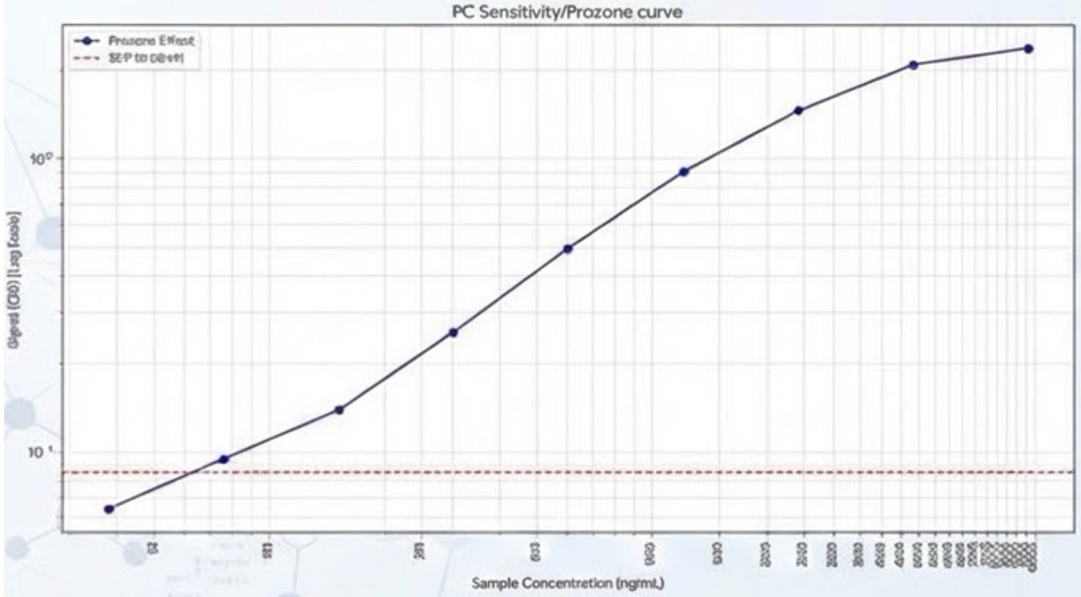
Stubenrauch K, et al. *J Pharm Biomed Anal.* 2010;52(2):249-254

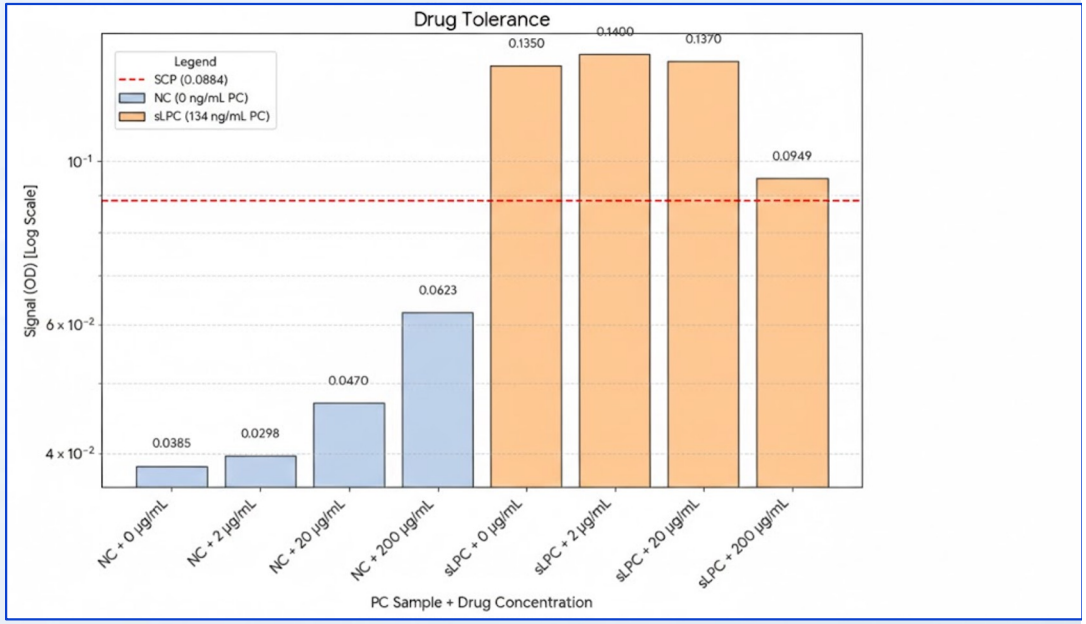
Jordan, G., Staack, R.F. Advancing Quantitative ADA Detection Through **Model Informed Assay Development (MIAD)**. *AAPS J* 28, 54 (2026). https://doi.org/10.1208/s12248-026-01204-3

IC Case Study 1: mAb - Cyno Validation

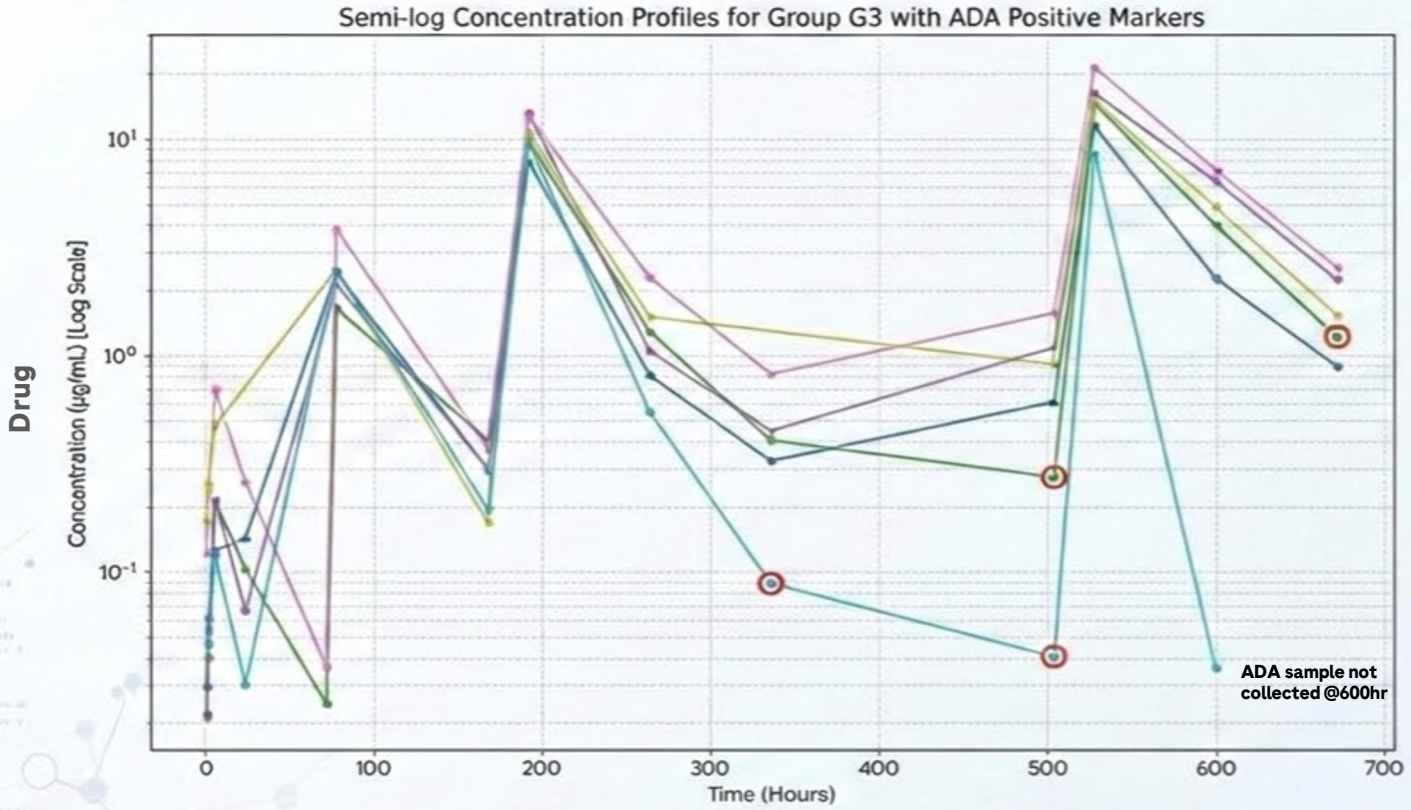
- Study: 4 week repeat dose in Cyno, no recovery period
- Validation: 11 runs over 10 days
- Validation procedure close to clinical approach

- DT needed at ~25ug/mL
- Sensitivity <100 ng/mL
- SCP ~2.2 S/N (3 batches of 40 individuals)





IC Case Study 1: mAb - GLP Tox study data



Clear correlation between loss of exposure with detection of ADA

Similar performance in validation/study analysis in several molecules (sensitivity, DT, CP, study data correlation).

However, we had a couple of concurrent cases where there were issues:

Case Study 2: Half-life extended BsAb drug in GLP Tox

- Study: 10-week repeat dose Tox in Cynos, no recovery.
- In-house: IC assay showed sensitive PC-drug binding.
- Timeline pressure: Quickly deployed generic IC assay at RegBA.
- Validation failed: Drug intolerance in absence of ADA/PC led to false positives.
- **Cause:** High drug accumulation (up to 10 mg/mL) likely oversaturated assay reagents, causing false positives.

~Only 1ug/mL excess drug is added to the assay to form ADA-complexes

	PC conc (ng/mL)	Drug (mg/mL)	Signal (OD)	Result	Signal/Noise*
Negative Control (0 ng/mL)	0	0.00	0.045	neg	1.00
	0	0.100	0.066	neg	1.47
	0	1.00	0.142	**pos**	3.16
	0	2.50	0.141	**pos**	3.13
	0	5.00	0.130	**pos**	2.89
	0	7.50	0.114	**pos**	2.53
	0	10.0	0.111	**pos**	2.47
Plate Cut point = 1.92					

Case Study 2: Solutions? Leverage study data

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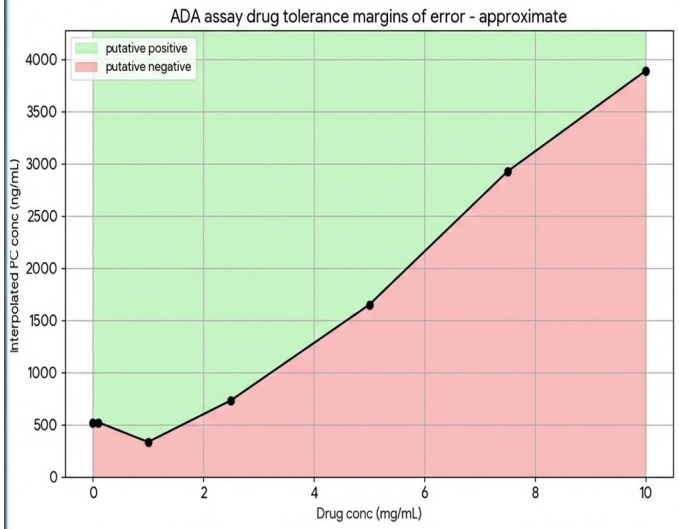
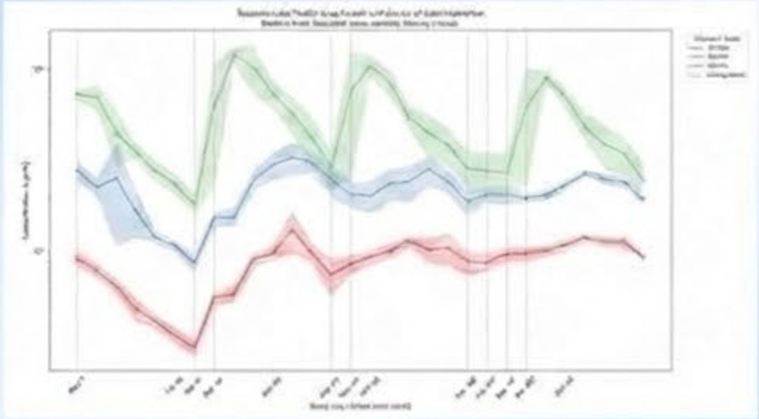
Stick with IC assay:
Arbitrarily amend SCP to mask drug interference > SCP at 4 S/N

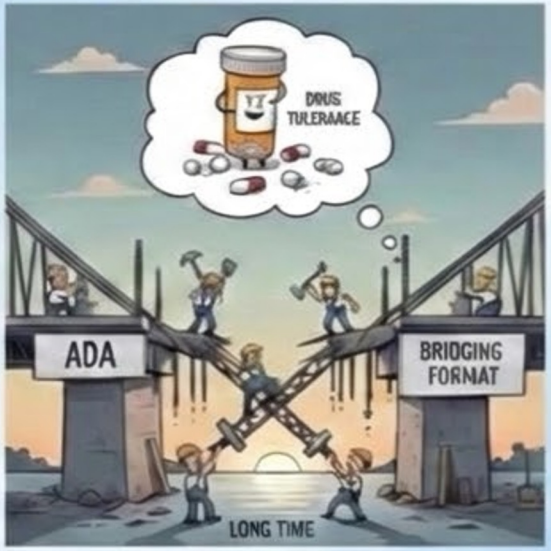


Leverage PK/PD/Safety data to understand impact of ADA within study



ICH S6 R2 - ADA testing when:
(1) evidence of altered PD activity;
(2) unexpected changes in exposure in the absence of a PD marker; or
(3) evidence of immune-mediated reactions

Redevelop ADA to a bridging format?



Performed in the background "in case"

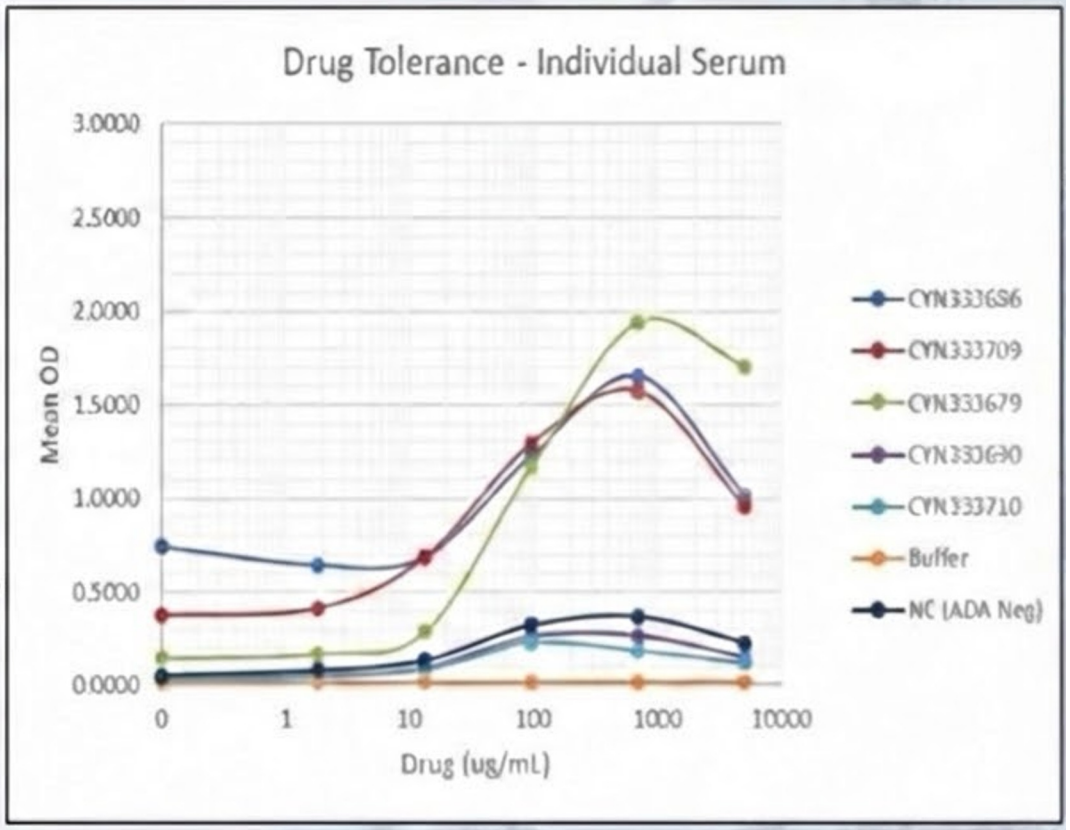
Case Study 3: BsAb drug in a Cyno GLP Tox study

Study: Repeat dose over 10 weeks, 8 week recovery period

Validation fell over during drug tolerance run . . . intolerant to drug in absence of ADA/PC

Significant false positives observed @>10 ug/mL. C_{tough} est. up to 5 mg/mL

Cause? High individual variability & over saturation causing the FP's.



Case Study 3: Solutions?

Develop/Validate bridging format & analyse

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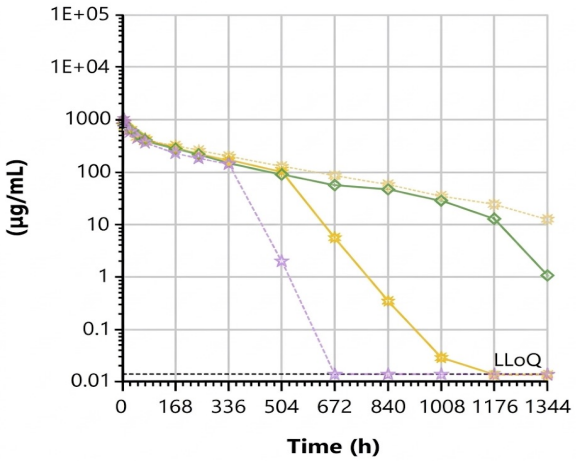
Leverage PK/Safety data to understand impact of ADA within study



DRF study observed faster clearance of PK in some animals, though no immune related safety findings

ICH S6 R2 - ADA testing when:
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(2) unexpected changes in exposure in the absence of a PD marker; or
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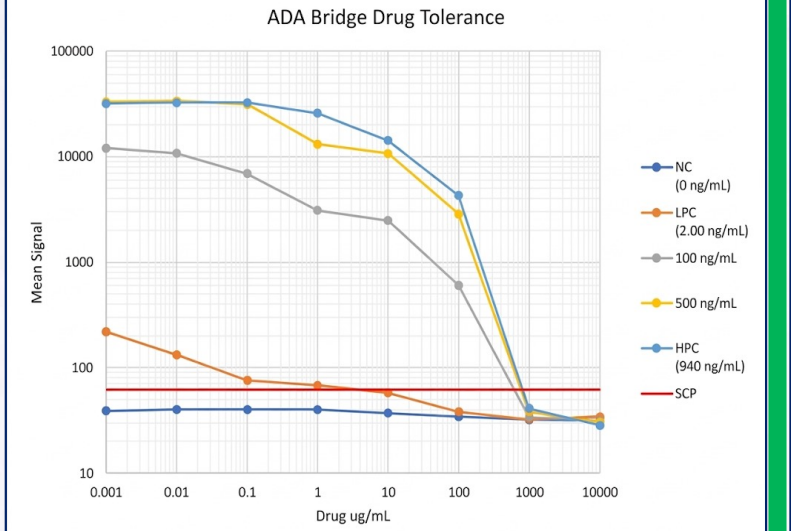
> Redevelop ADA to a bridging format

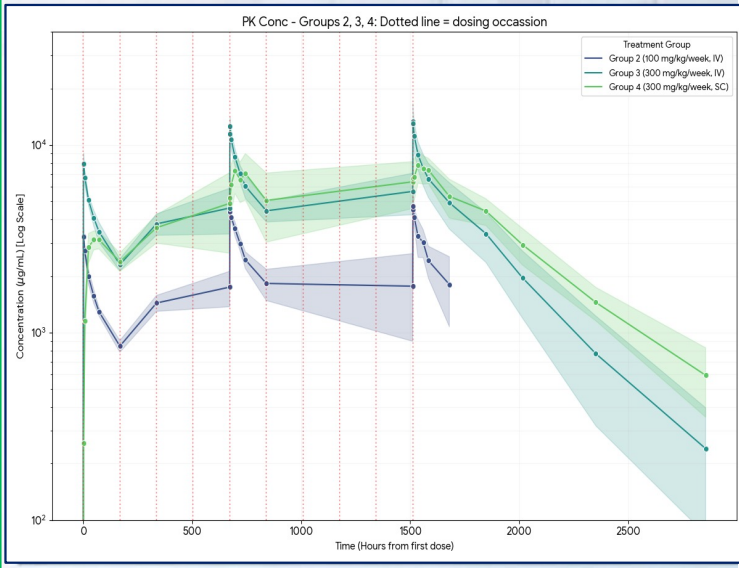


Drug tolerance
500 ng/mL PC @ 700 ug/mL

Target: 5 mg/mL

Deployed due to risk of PK exposure impact, trade off's: inconclusive ADA reporting in some groups in treatment phase until assay DT achieved in recovery phase

Study data:



✓ No immune complex related safety findings, no impact to TK parameters by end of study

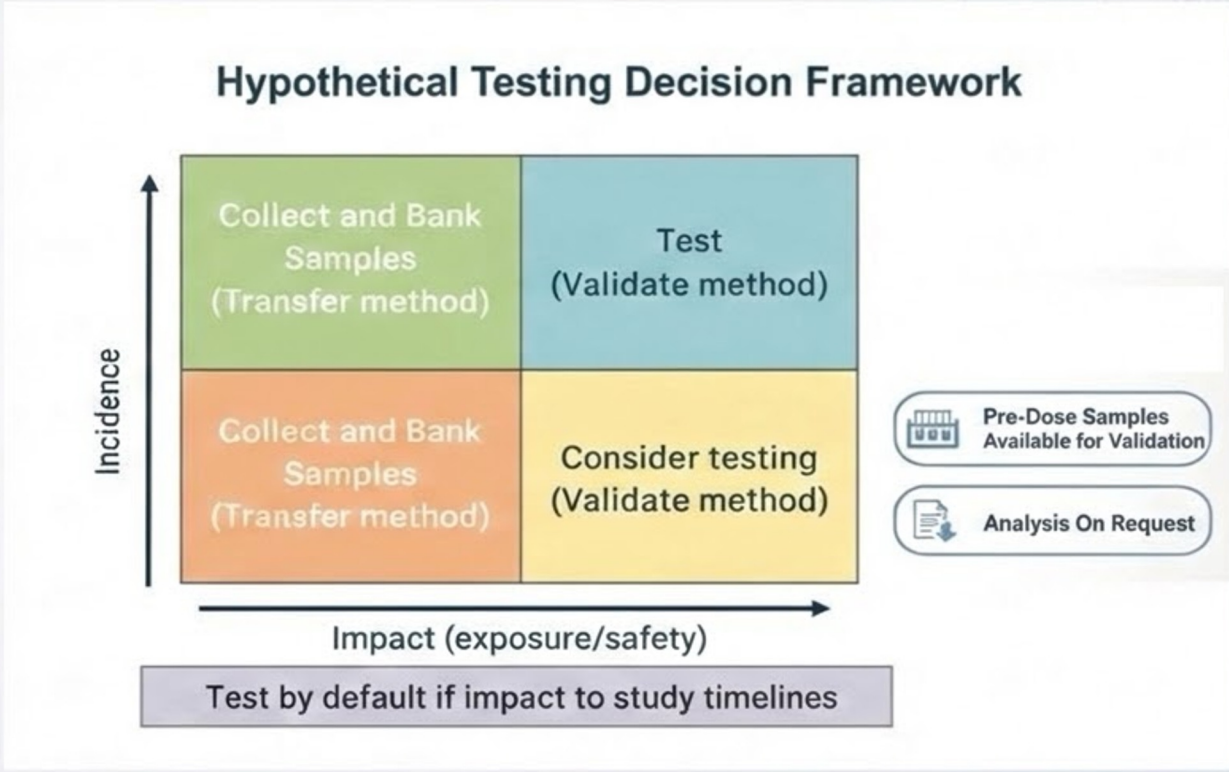
Testing Strategy Considerations

Can the testing strategy be tailored?

Nonclinical Immunogenicity Assessment – When to include and what to include? Anna Laurén, on behalf of the EBF, 2019

Business Risk & Willingness

- Some always include ADA in nonclinical studies due to time limitation
- Some no actions and develop/validate ADA assays only if needed
- Some sponsors and CROs felt that their stakeholders (eg toxicologist) required ADA as a tick box exercise



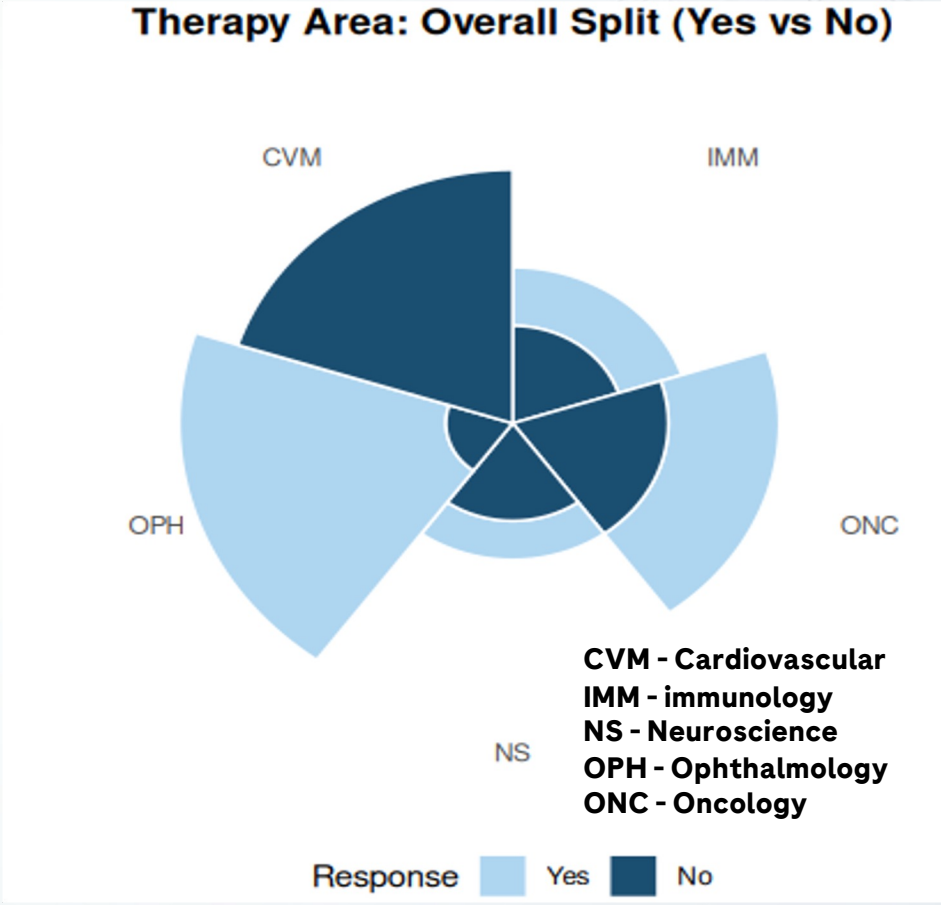
Can the testing strategy be tailored? Portfolio view

Evaluating ADA-Mediated Trends: Insights from 55 Roche preclinical studies (2020–2025)

Yes = Safety or PK observations which would trigger ADA assessment

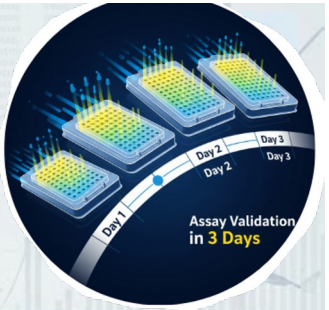
No = No in-study trigger for ADA testing (we did it anyway)

Retrospectively > half the studies did not have ADA analysis triggers



Fit-for-purpose validation

Our approach for a minimal, Fit-for-purpose non-clinical ADA method validation



Strategy in line with EBF recommendation*

Tiers

Runs: -

Procedure: Screening only

Singlicate/ Duplicate?

Runs: -

Procedure: Singlicate

Cut Point

Runs: 0*

Procedure: Use 2 x NC. Statistical CP only if deemed necessary

Sensitivity/ Selectivity/Prozone

Runs: 1

Procedure: Sensitivity curves in 10 individuals (target 1000ng/mL PC), Selectivity at a low PC level is inherently established in the assessment

Precision (Inter/Intra)

Runs: 3

Procedure: Low-Mid-High PC levels

Drug Tolerance Target Tolerance

Runs: 1 (for DT)

Procedure:
DT: Low-High-1000ng/mL PC levels spiked w/expected drug levels

TT: As DT, if needed

* Lauren et al, A strategic approach to nonclinical immunogenicity assessment: a recommendation from the EBF, Bioanalysis (2021) 13(7), 537–549

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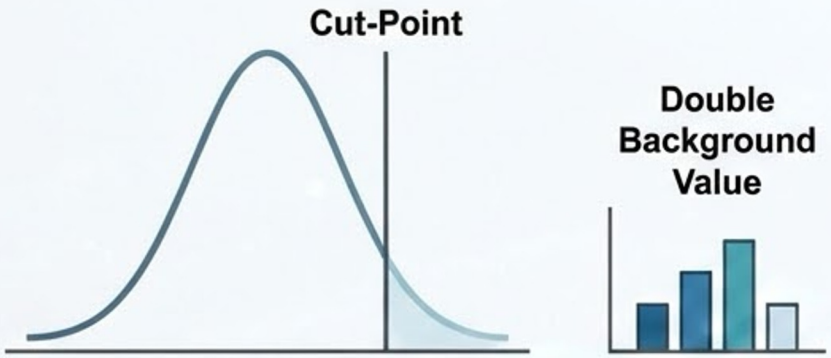
Simplifying the cut point approach

TRADITIONAL STATISTICAL APPROACH (WHERE JUSTIFIED)

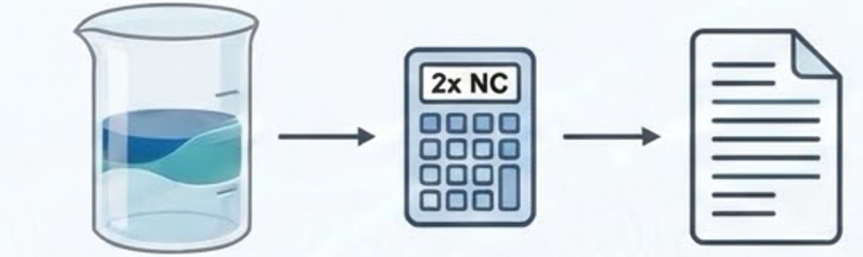


A statistical approach could be used to establish the assay cut-off value, where justified.

Alternatively, real data (e.g. double background value) can be used to determine what will be considered the lowest positive result.

Guideline on Immunogenicity assessment of therapeutic proteins EMEA/CHMP/BMWP/42832/2005 Rev1

NEW SOP APPROACH: SIMPLIFIED & QUALITATIVE



POOLED NC
(Commercial/Individual)

QUALITATIVE RESULT,
with S/N

Main difference is no statistical CP experiment (in most cases). NC is pooled – tested to sit in the variance of individuals thus 2xNC is considered appropriate. If variability is high, a statistical approach can be taken if needed.

Samples report qualitatively and with S/N.

Summary

- **Generic assays** are powerful tools for efficiency allowing R&D focus on more tricky molecules/modalities > *when deployed correctly!*
- **Fit-for-purpose validations** save bench time while maintaining data integrity
- Consider carefully implementing **alternative testing strategies**
- Finding the **balance** is essential for supporting fast-paced non-clinical portfolios

Acknowledgements

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Doing now what patients need next