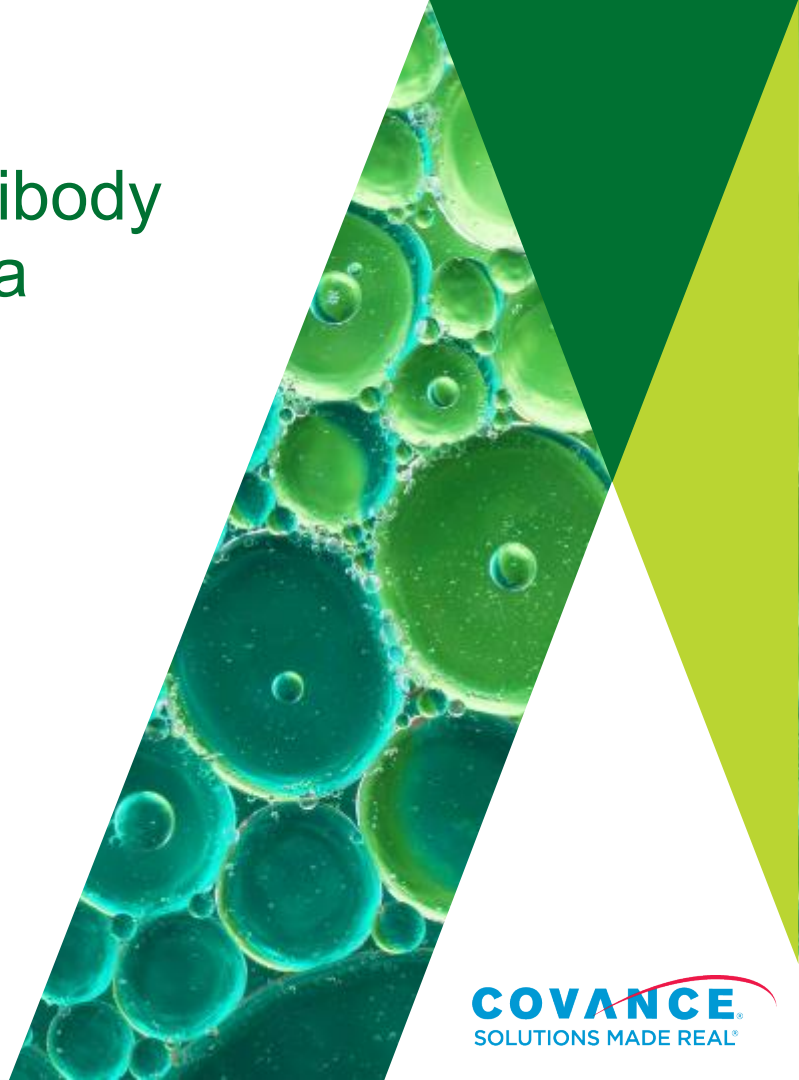
No Cut-Point, No Cry

Validating preclinical anti-drug antibody (ADA) assays without generating a statistical cut-point

Laure Queyrel, Dipl. Ing.



21 November 2019 - 12th EBF Open Symposium

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Agenda

1. Introduction to preclinical immunogenicity assays
2. Summary of previous EBF interventions
3. Our arbitrary cut-point approach
4. Experience with clients
5. Case studies
6. Summary & Conclusion

Introduction

- Main purpose of preclinical immunogenicity assays:
  - Interpretation of pharmacokinetic/pharmacodynamic (PK/PD) data
  - Pre-clinical anti-drug antibody (ADA) data not used for safety assessment
- ADA assay validations are time consuming due to statistical cut-point generation
- ADA assay output: Positive/Negative result (if titer not performed)
- Industry going towards a simplified approach for preclinical ADA assays

Can we develop and validate preclinical ADA assay differently to clinical assays?

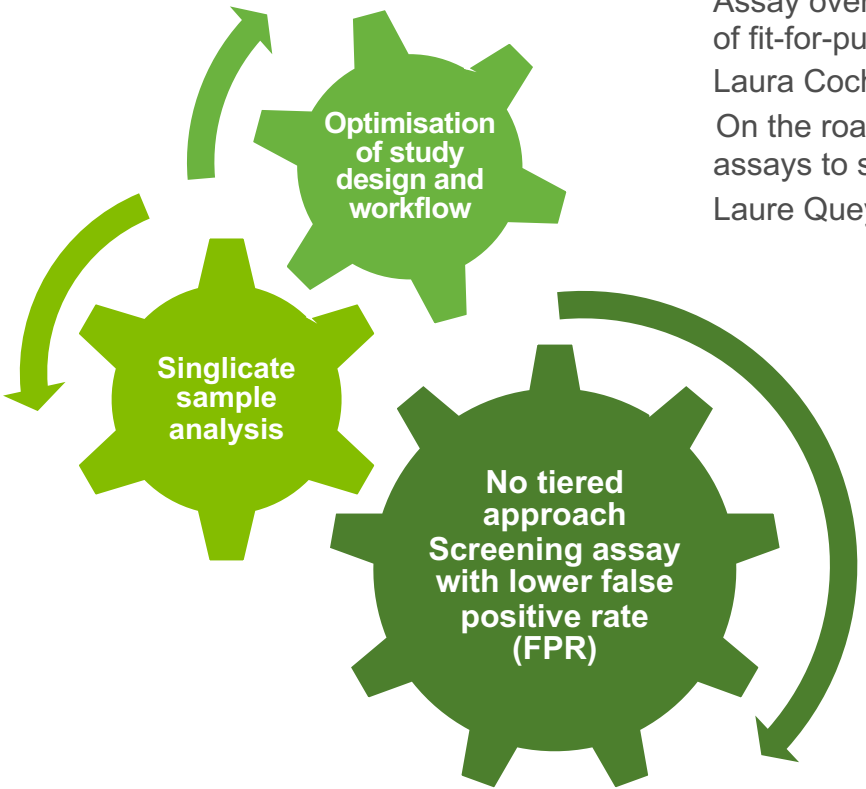
Risk-Based Strategy Discussed By EIP

- European Immunogenicity Platform issued proposed strategy in 2015
- Risk-based approach to immunogenicity testing mentioned by regulator but recommendations for tailored immunogenicity testing strategy are missing

	Low Risk	High Risk
ADA assay format	Screening assay only (99.9th)	Screening assay only (99.9th)
Sample collection	Frequent	Frequent
Samples to be tested	Event driven	Event driven
Execution of testing	Batch wise at the end of the study if required	Batch wise at the end of the study
Neutralization	-	If of added value: PD/CLB or CBA
Characterization	-	-

J Immunol Methods. 2015 Feb;417:1-9. doi: 10.1016/j.jim.2015.01.003. Epub 2015 Jan 17.
A fit-for-purpose strategy for the risk-based immunogenicity testing of biotherapeutics: a European perspective.
Kloks C, Berger C, Cortez P, Dean Y, Heinrich J, Bjerring Jensen L, Koppenburg V, Kostense S, Kramer D, Spindeldreher S, Kirby H.

Previously At The EBF…



Assay overkill: Practical solutions for development and validation of fit-for-purpose pre-clinical immunogenicity assays

Laura Coch - 11th EBF Open Symposium (2018)

On the road to simplified and fit-for-purpose immunogenicity assays to support pre-clinical studies

Laure Queyrel – 6th EBF YSS (2019)

- Fit for purpose assays
- Efficient assay development and validation
- Reduction of time and ressources

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Our Latest Strategy – Cut-Point Control

	Option 1	Option 2	Option 3
Context of use	Clinical ADA mainly	Preclinical ADA (Sponsor's request)	Preclinical ADA (preferred approach)
Approach	Screening + Confirmatory Titer if required	Screening only	Screening only
Cut-point assessment	Balanced design 51 individuals over 3 days x 2 analysts x 3 plates	15 individuals 2/3 days x 2 analysts x 2/3 plates	No statistical cut-point **Arbitrary cut-point instead**
Assay output	Confirmed Positive/Negative (titre if required)	Positive/Negative	Instrument response ratio

- Alternative to statistical cut-point: Arbitrary cut-point at the desired sensitivity (or below)
- Floating cut-point approach: cut-point control (CPC) included on each plate alongside negative control (NC), low and high positive controls (LPC and HPC)
- Instrument response ratio between sample and CPC to compare immune response between plates and between studies

Client Reactions

- “Yes – it will save us money”
- “Yes – we are running out of time”
- “Ok since we are struggling with the cut-point”
- “OK you are the experts”

- “Oh no – let’s stick to the guidelines”
- “No thanks – we have been using this assay for over 10 years and we don’t want to do it differently”

Arbitrary Cut-Point Strategy As Backup

When the statistical cut-point doesn't work

- ADA assay development for a biosimilar using an commercially available ELISA kit
- Development:
  - Cut-point assessed with 15 individuals across 2 plates x 2 days x 2 analysts
  - Results: correction factor (CF) of 0.018 and sensitivity calculated as 16 ng/mL (99% confidence interval)
  - PCs prepared at 50 (CPC), 100 and 600 ng/mL
  - Problems: 2 out of 28 NC above calculated CP and poor precision at the bottom
  - Selectivity in 6 individuals: all individuals above the statistical CP and CPC
  - Coefficient of variation (CV) of blank around 50% and below 20% for CPC for both intra and inter-assay precision: no need for a normalisation
- Validation:
  - CPC approach taken forward
  - No problem with assay validation completed in 6 days

Mouse ADA Assay Life Cycle

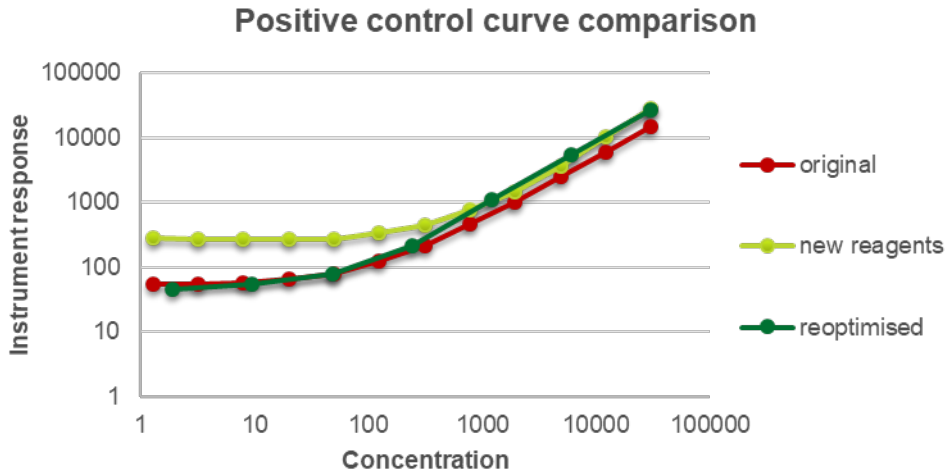
Content of initial validation

- Work started in 2016 with a receptor antagonist for asthma
  - Screening assay only due to low sample volume
- Development study workflow:
  - Reagent optimisation
  - Matrix screen and positive control stock assessment
  - Screening cut-point over 2 days with 2 analysts and 3 plates (5 individuals per plate)
  - Preparation of positive control sample at appropriate levels
  - Intra-inter-assay precision, selectivity, drug tolerance, prozone
- Validation study workflow:
  - Screening cut-point over 3 days with 2 analysts and 3 plates
  - Intra-inter-assay precision, selectivity, drug tolerance, prozone
  - Short-term stability assessment

Mouse ADA Assay Life Cycle

Assay re-establishment

- Assay required a couple of years later but insufficient reagents
- Previous study showed ADA in all dosed animals therefore sensitivity was not a concern
- Approach taken: relabel reagents and reoptimise concentrations before performing 3 inter-assays using CP and LPC levels used in previous validation



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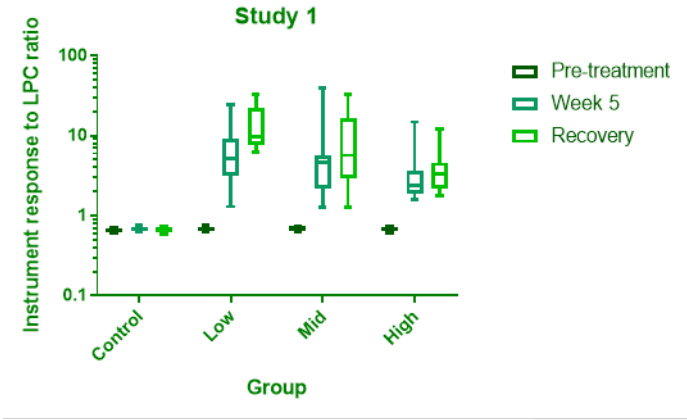
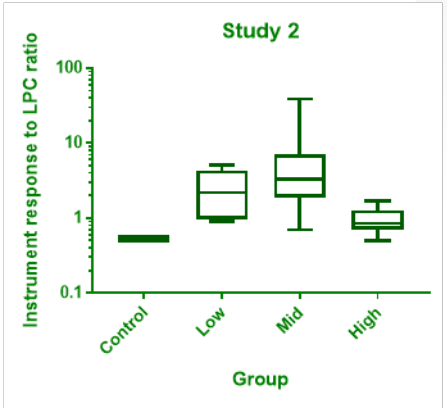
Mouse ADA Assay Life Cycle

How could we have done it better?

- Results of original validation:
  - Sensitivity calculated as 8 ng/mL, LPC prepared at 20 ng/mL but selectivity failed
  - LPC level increased to 30 ng/mL
  - Some NC gave positive results
- Avoid cut-point assessment
- Choose an arbitrary cut point based on S/B ratio
  - Standard at 50 ng/mL only 20% higher than blank
- Presenting results as instrument response ratio between sample and CPC for comparison





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Summary & Conclusions

- Arbitrary cut point has become our preferred approach to validate fit-for-purpose assays to support pre-clinical projects
- Sensitivity of ADA assays is usually not a problem as they tend to be more sensitive than required
- CPC approach benefits:
  - Reduces development/validation time significantly
  - Reduces animal use as cut-point not being assessed
  - Semi-quantitative results if desired
  - Simplifies assay re-establishment (transfer or change of reagents/matrix/instrument)

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