Biosimilar ADA assessments using the Gyrolab platform

Sam Willcox
Immunochemistry Department, Harrogate, U.K.

EBF European Bioanalysis Forum
6th Young Scientist Symposium

September 2020

COVANCE

Outline

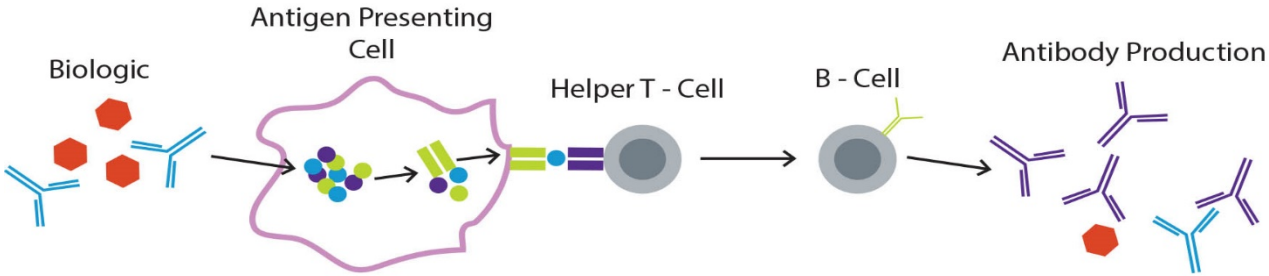
1. Anti-drug antibodies (ADA) and testing strategy
2. Drug Tolerance/Interference and Methods to negate this.
3. ADA testing on the Gyrolab
4. Development of a Pembrolizumab ADA assay & Challenges Encountered
5. Summary and Conclusions

COVANCE

What are anti-drug antibodies (ADA)?

- Large molecule biotherapeutics have complex structures
  - Potential to be recognized as ‘foreign’ by the human / animal immune system, leading to production of antibodies against the biotherapeutic, known as anti-drug antibodies (ADA)
- Clinical effects of ADA are highly variable, ranging from benign to severe adverse effects

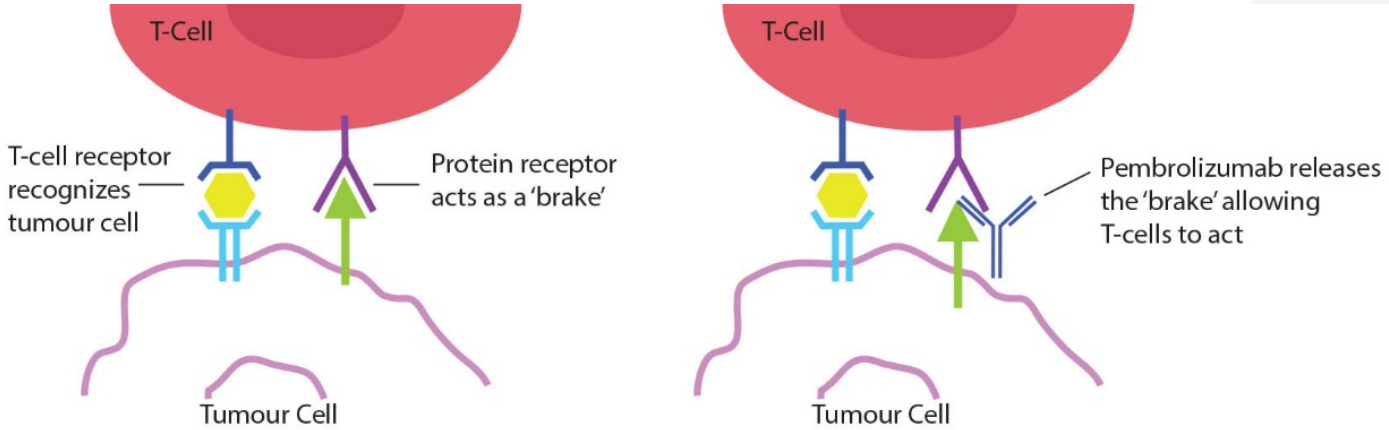
Generation of ADAs



COVANCE

Pembrolizumab (Keytruda®)

- Model mAb: Monoclonal IgG^4 Antibody
- Target: Programmed Cell Death 1 Transmembrane Protein (PD-1)
- Immune Checkpoint Inhibitor
- Top 5 Bestselling Biotheraputics in 2019 – Frequently used in a number of combination therapies



Schematic showing Pembrolizumab interaction with PD-1 on a tumour cell. This releases the immune system 'brake' to allow for T-Cell effector function.

COVANCE

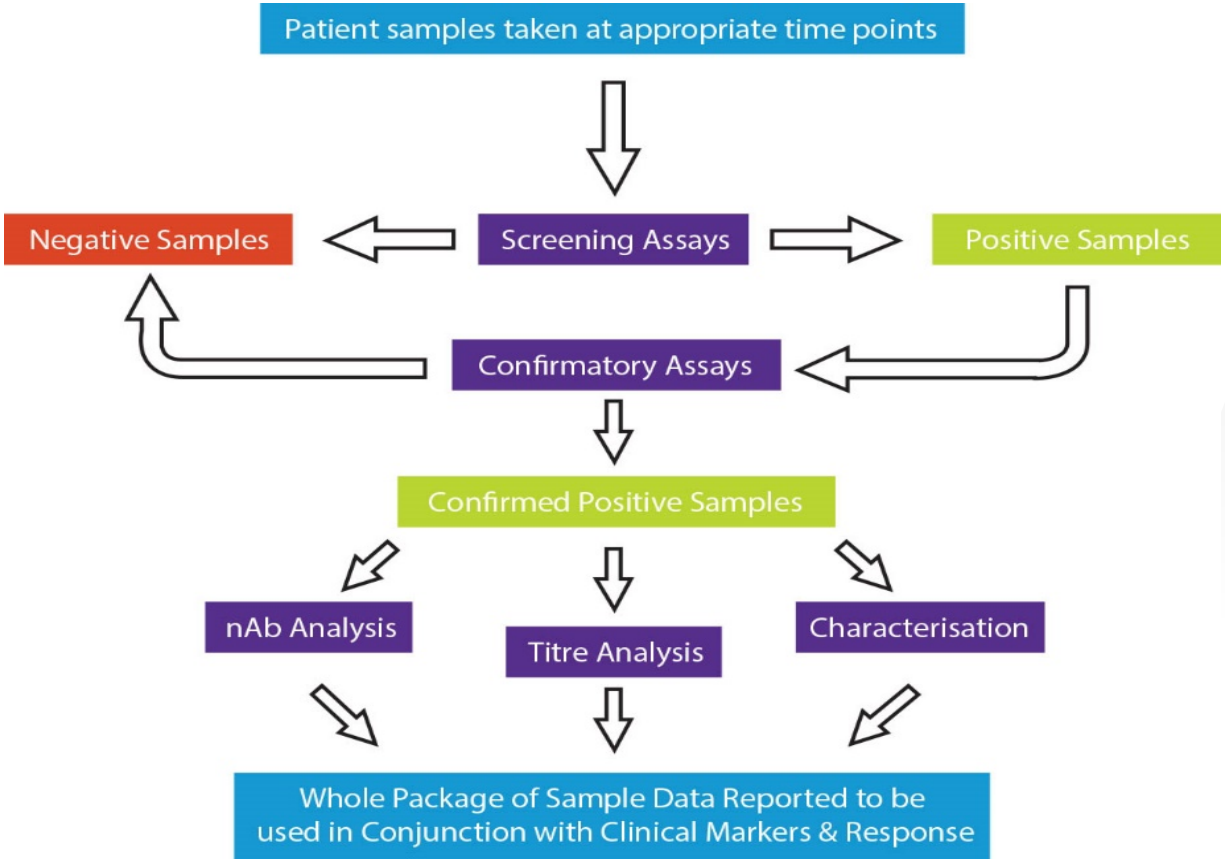
ADA testing

Important parameters to assess in development and validation of ADA methods:

- **Sensitivity**
  - Regulatory expectation that assay achieves a sensitivity of 100 ng/mL relative to a positive control reference material
- **Drug tolerance**
  - Need to ensure we can detect the ADA in the presence of biotherapeutic levels that will be present in study samples
- **Selectivity**
  - Need to robustly detect ADA across different individuals
- **Stability**
  - Want to ensure that routine sample handling doesn't impact the result in the assay
  - But stability of positive control may not represent that of samples

COVANCE

ADA Strategy – Qualitative not Quantitative



COVANCE

Clinical Testing for ADA

- Historically ELISA industry standard for ADA Detection.
  - Pros: Easy, Cheap, Minimal Equipment, Non-proprietary
  - Cons: Drug Tolerance, Sensitivity – New FDA guidelines

  More recently the Meso Scale Discovery™ (MSD) platform has become widely used
  - Pros: Sensitivity, Drug tolerance
  - Cons: Cost, Proprietary, Low Background Signals = Low Cut-point Factors.

  Gyros Previous ADA Solutions: Worked - But Not Competitive.
  - Offline Sample Processing
  - 48 Channel Mixing CD

COVANCE

Testing ADA on the Gyrolab

- Semi-Automated Immunoassay Platform on a Nanolitre Scale
- New Mixing CD 96 Increases Sample Throughput
- Aim of the Project to Evaluate new CD Type.

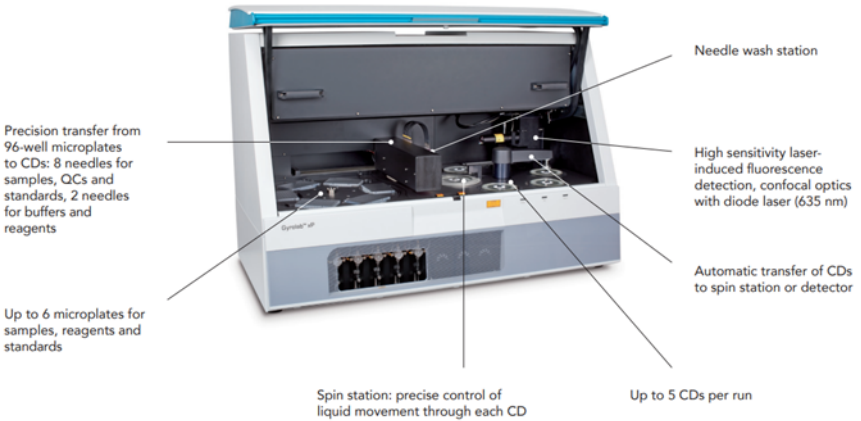
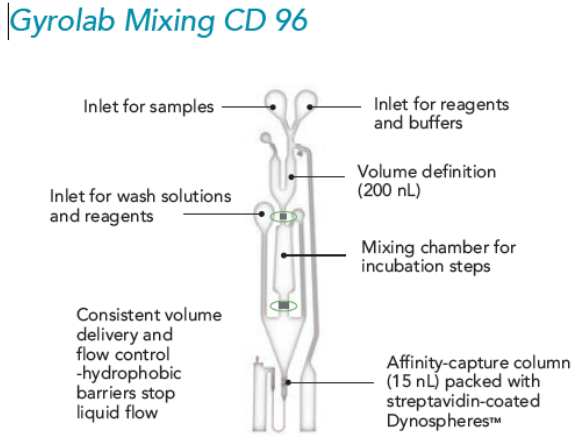


Image courtesy of GyrosProtein Technologies



Automated acid dissociation takes place in the mixing chambers of the Gyrolab Mixing CD

COVANCE

Drug Tolerance & Acid Dissociation

- Antibody therapeutics are dosed at high concentrations and have a long half life.
- Often multiple dosing so ADA sampling performed prior to drug clearance

- High drug tolerance is a challenge due to low sensitivity requirements – usually a tradeoff between the two.
- Acid treatment of samples to Drug-ADA complexes most commonly used technique to improve DT.
- Manually performed this contains multiple assay steps in quick succession
- Mixing CD automates this process.

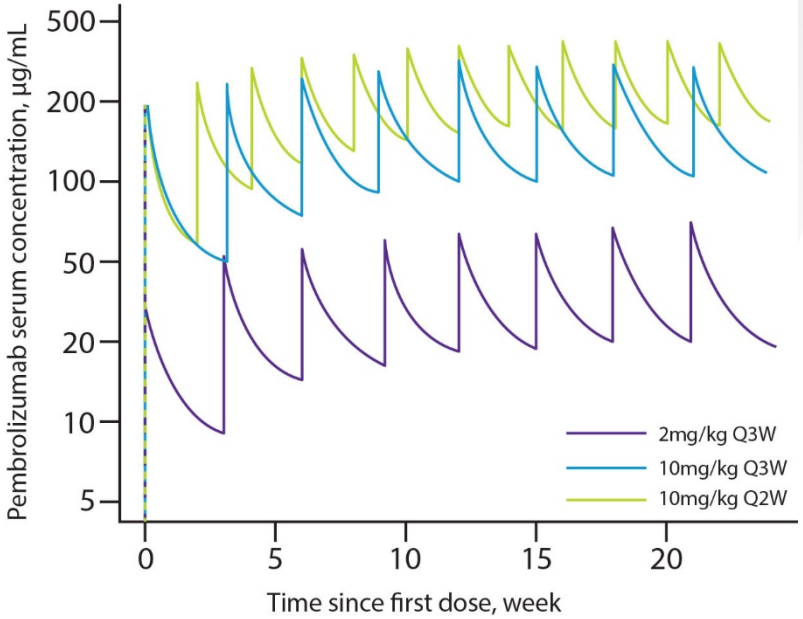
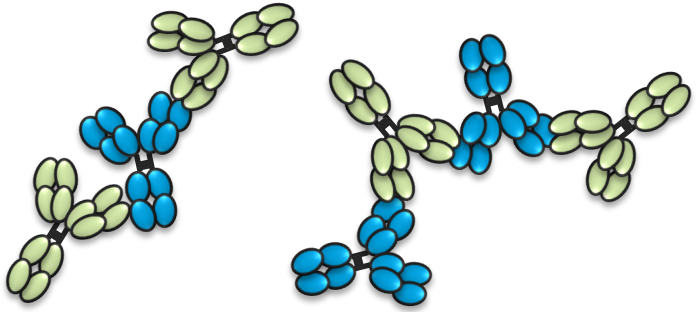


Image Source: Ahamadi et al. CPT: Pharmacometrics & Systems Pharmacology (2017) 6(1): 49-57

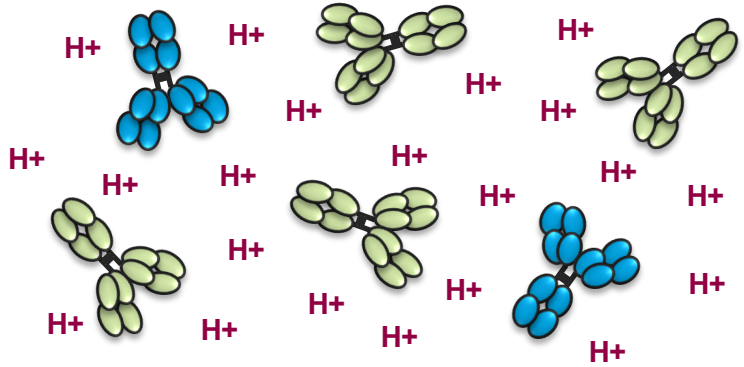
COVANCE

Overcoming Drug Interference With Acid Dissociation

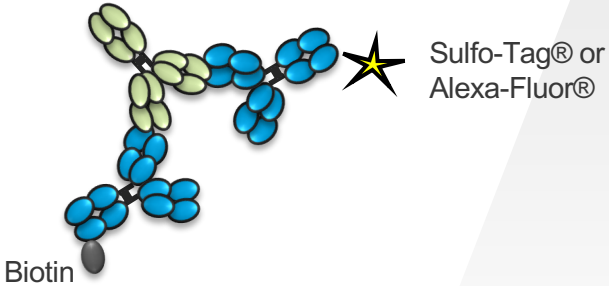
1. Sample containing **Pembrolizumab** and **ADA**



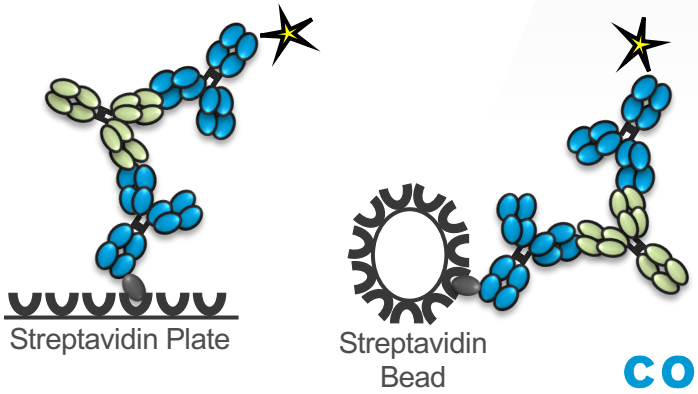
2. **Acid** treatment to dissociate complexes



3. Neutralize, then add **Labelled-Pembrolizumab** to form **ADA bridge-complex**



4. Capture **ADA bridge-complex** on solid state, then detect



COVANCE

Advanced Techniques for Overcoming Drug Interference

- **ACE** – Affinity Capture Elution
- **SPEAD** – Solid Phase Extraction with Acid dissociation
- **PandA**[1]– Precipitation and Acid dissociation

All Involve some acid dissociation but with further ‘clean up’ steps

- **BEHD**[2] – Bead Extraction and Heat Dissociation
- **HISDA**[3] – High Ionic Strength Dissociation

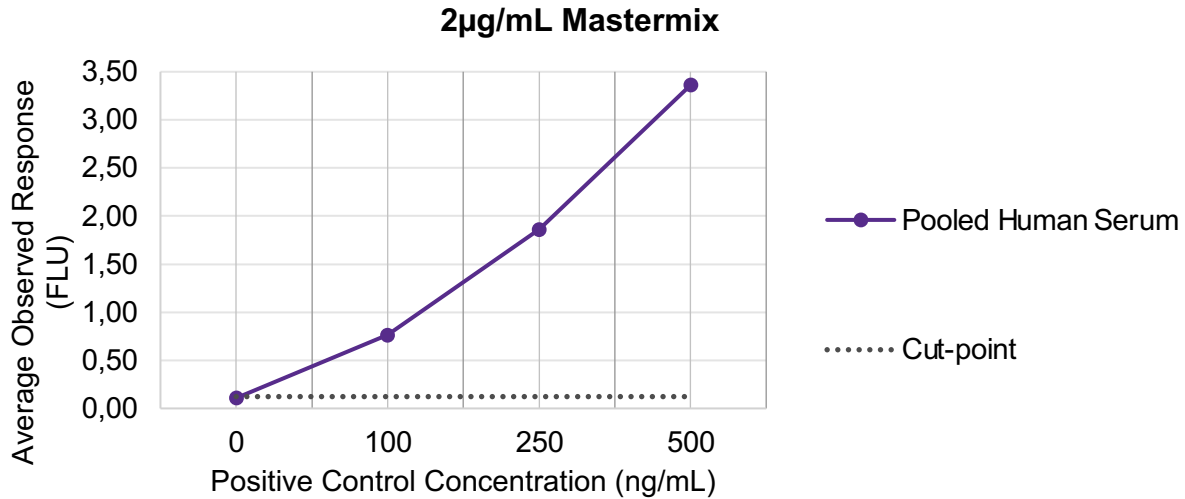
Dissociation but with alternative approaches

[1]Jad Zoghbi et al, A breakthrough novel method to resolve the drug and target interference problem in immunogenicity assays J Immunol Methods 2015 Nov;426:62-9.
[2]Weifeng Xu et al, Bead-extraction and heat-dissociation (BEHD): A novel way to overcome drug and matrix interference in immunogenicity testing, J Immunol Methods 2018; 462:34–41
[3]Gregor Jordan et al. High ionic strength dissociation assay (HISDA) for high drug tolerant immunogenicity testing, Bioanalysis Journal June 2020

COVANCE

Assay Optimization and Challenges Observed

- Followed Suggested Assay Protocol –
- Acid Buffer pH 2.6 Glycine HCl – Neutralization Buffer– 2M Tris-HCl pH8.0
- Master-Mix Concentrations of 2, 8 & 32µg/mL.
- MRD – 1 in 5

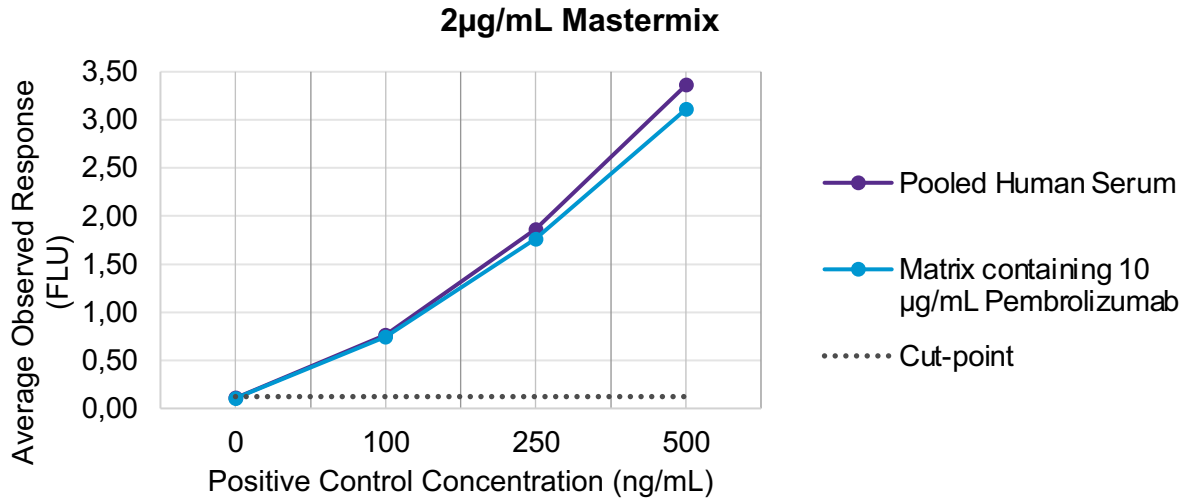


Sample	Signal: Noise Ratio
2 µg/mL Master Mix	
Matrix Blank	-
100 ng/mL ADA	7.06
250 ng/mL ADA	17.16
500 ng/mL ADA	31.11

COVANCE

Assay Optimization and Challenges Observed

- Followed Suggested Assay Protocol –
- Acid Buffer pH 2.6 Glycine HCl – Neutralization Buffer– 2M Tris-HCl pH8.0
- Master-Mix Concentrations of 2, 8 & 32µg/mL.
- MRD – 1 in 5

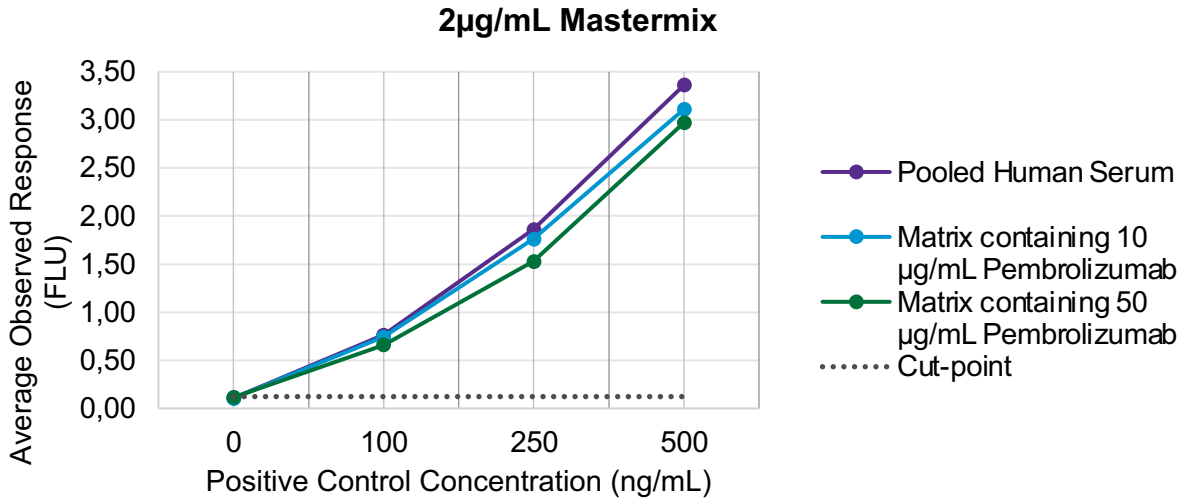


Sample	Signal: Noise Ratio
2 µg/mL Master Mix	
Matrix Blank	-
100 ng/mL ADA	7.06
250 ng/mL ADA	17.16
500 ng/mL ADA	31.11

COVANCE

Assay Optimization and Challenges Observed

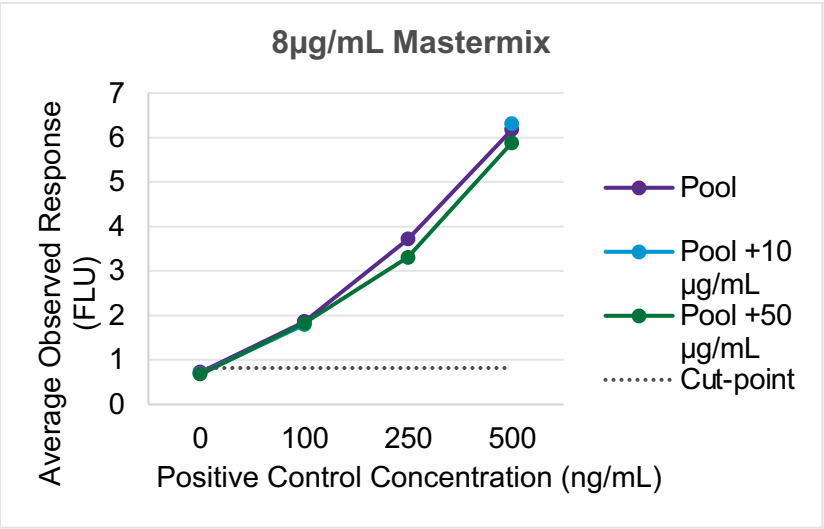
- Followed Suggested Assay Protocol –
- Acid Buffer pH 2.6 Glycine HCl – Neutralization Buffer– 2M Tris-HCl pH8.0
- Master-Mix Concentrations of 2, 8 & 32µg/mL.
- MRD – 1 in 5

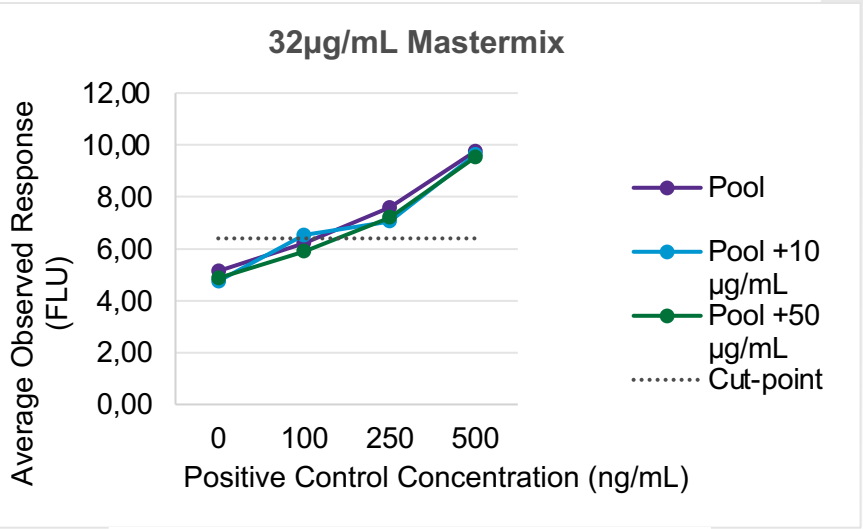


Sample	Signal: Noise Ratio
2 µg/mL Master Mix	
Matrix Blank	-
100 ng/mL ADA	7.06
250 ng/mL ADA	17.16
500 ng/mL ADA	31.11

COVANCE

Assay Optimization



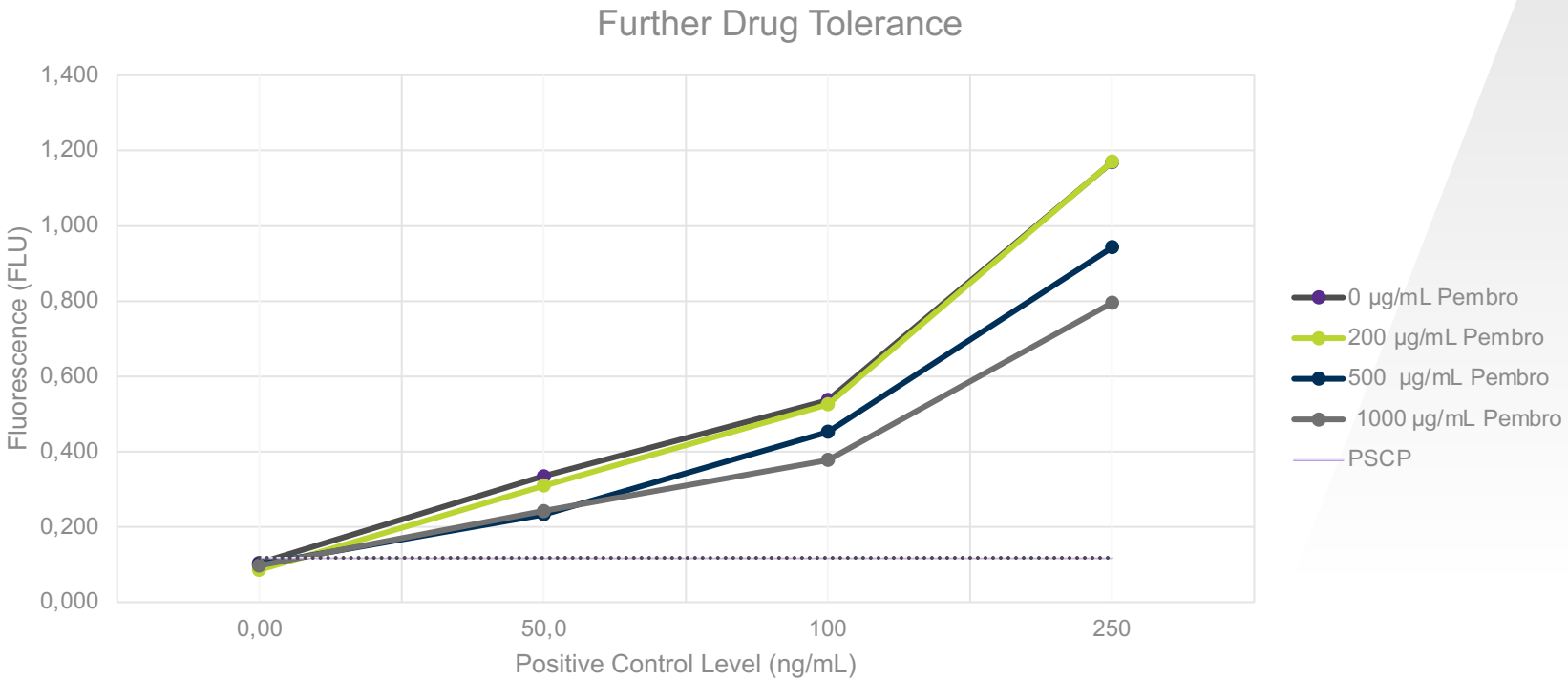


Sample	Signal: Noise Ratio
8 µg/mL Master Mix	
Matrix Blank	-
100 ng/mL ADA	2.57
250 ng/mL ADA	5.13
500 ng/mL ADA	8.53

Sample	Signal: Noise Ratio
32 µg/mL Master Mix	
Matrix Blank	-
100 ng/mL ADA	1.21
250 ng/mL ADA	1.47
500 ng/mL ADA	1.90

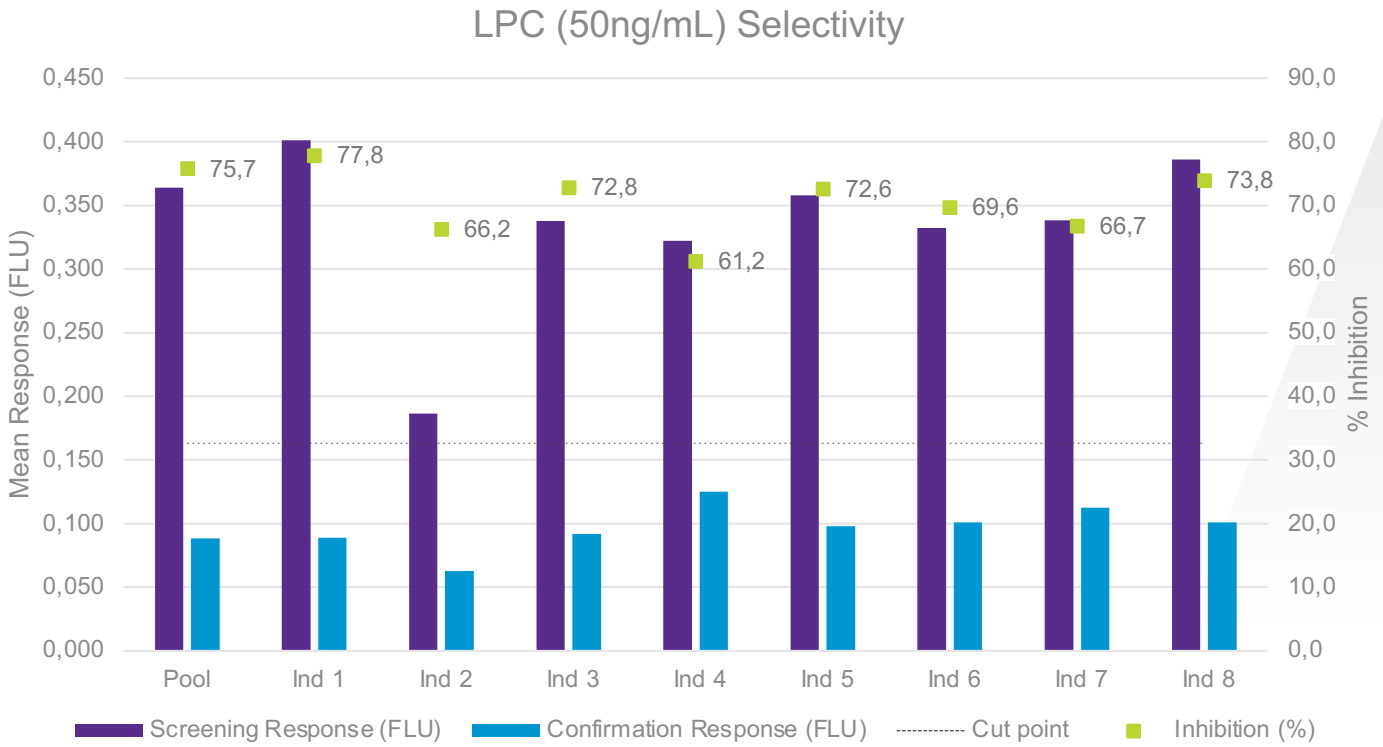
COVANCE

Drug Tolerance



COVANCE

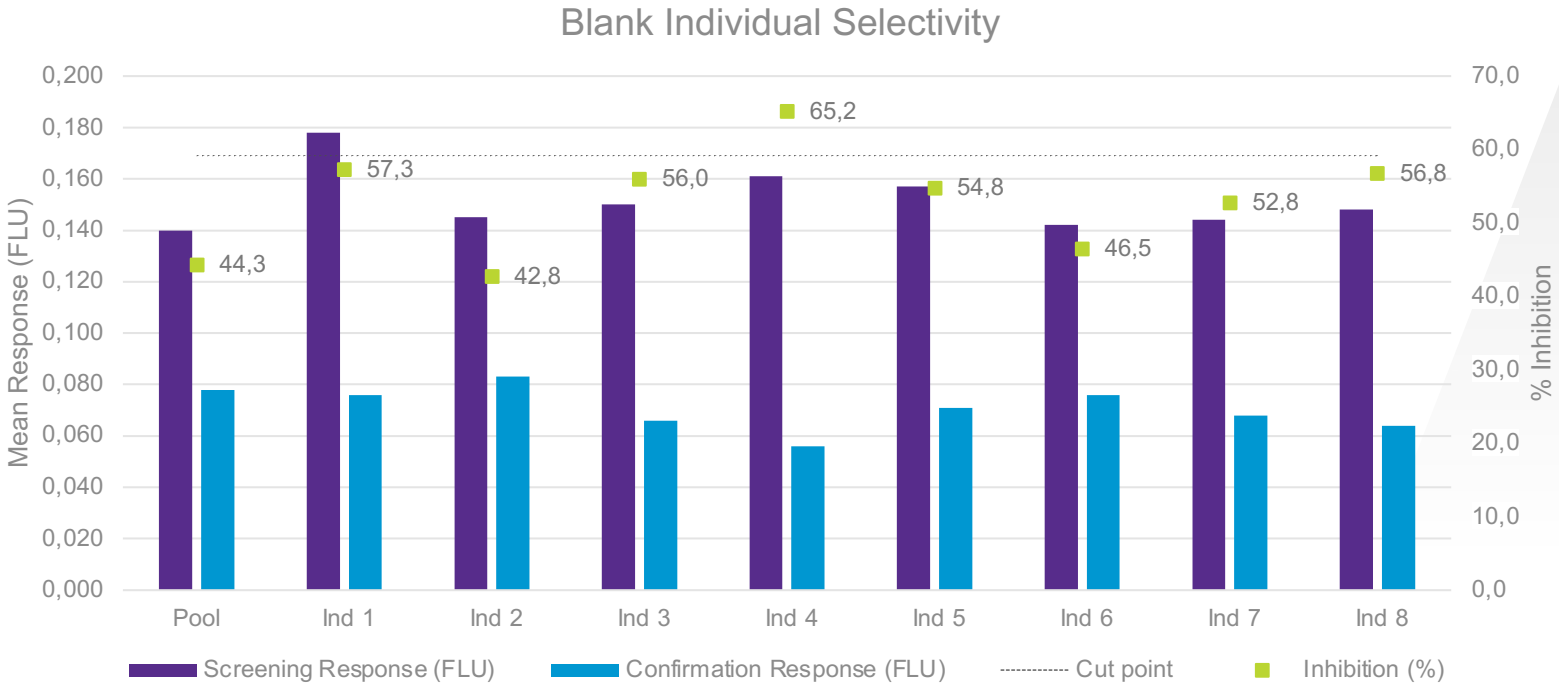
Selectivity



COVANCE

Blank Confirmation Issues

Excess drug (200µg/mL) added to Mastermix Solution

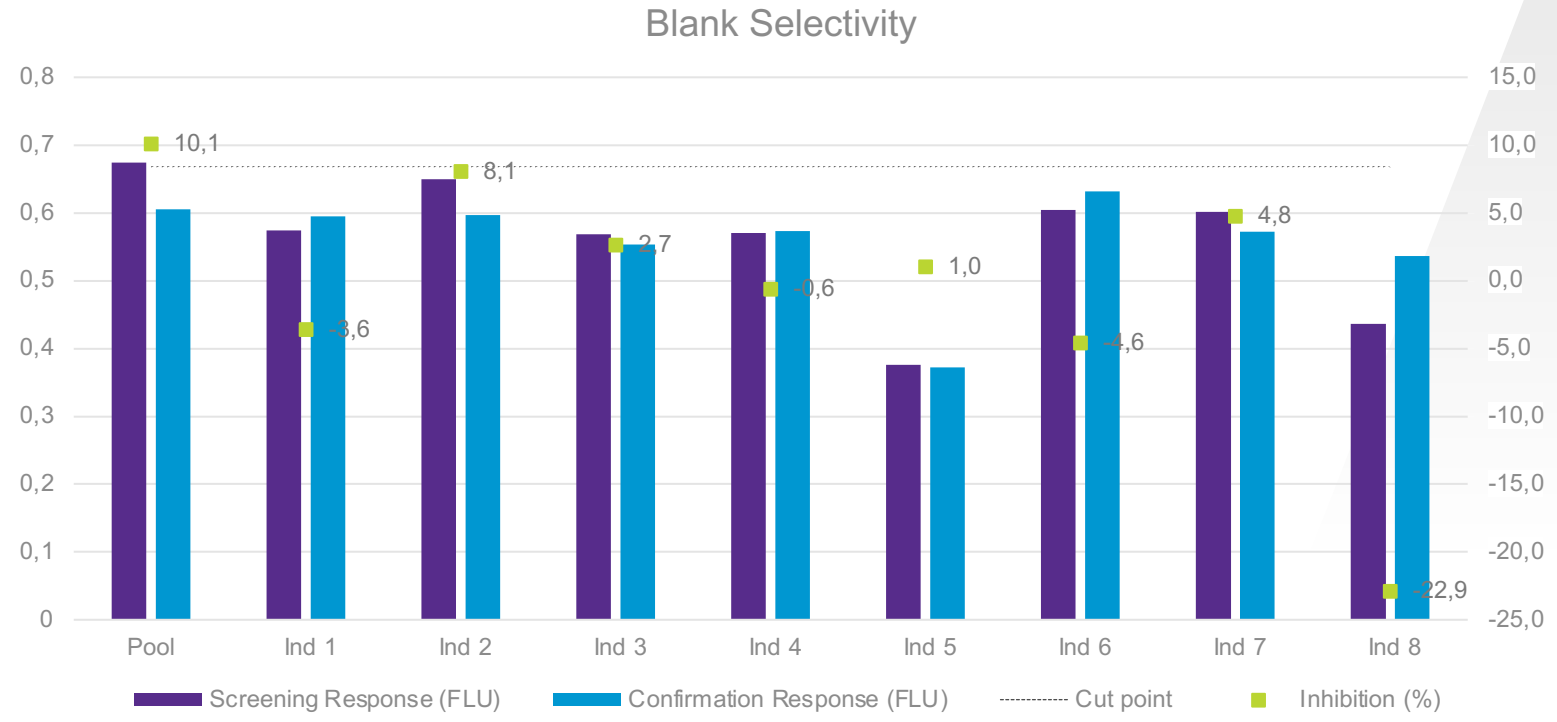


Fixed Confirmation Cut-Point (% Inhibition)	77.5

COVANCE

Solution- Changing Addition of Excess Drug

Method Altered: Excess Drug added to Sample Dilution Buffer



COVANCE

Gyrolab Summary & Conclusions

Proof of Concept Successful:

Sensitivity & Drug tolerance exceeds regulatory expectations

Challenges Remaining:

Are background variation issues inherently the same as other platforms? – Further assessment of cut-points using full balanced design required for full assessment

Gyrolab	
Screening Cut-Point Factor	1.14
MSD	
Screening Cut-Point Factor	1.09

Conclusion:

Results show that the Gyrolab platform is a viable alternative for regulated ADA analysis with added benefits of semi-automation which can improve sample throughput & reduce analytical errors.

COVANCE

Acknowledgements

Hannah Craig
Covance/University of Leeds MSc Biopharmaceutical Development
Sarah Malpas
Robert Nelson
Johannes Stanta
Covance Statistics Group
John Chappell
Gyros Protein Technologies