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Just How Low-a-Level of ADA can you Detect? And Just Because You Can, Does It Mean You Should?

Nick White, PhD

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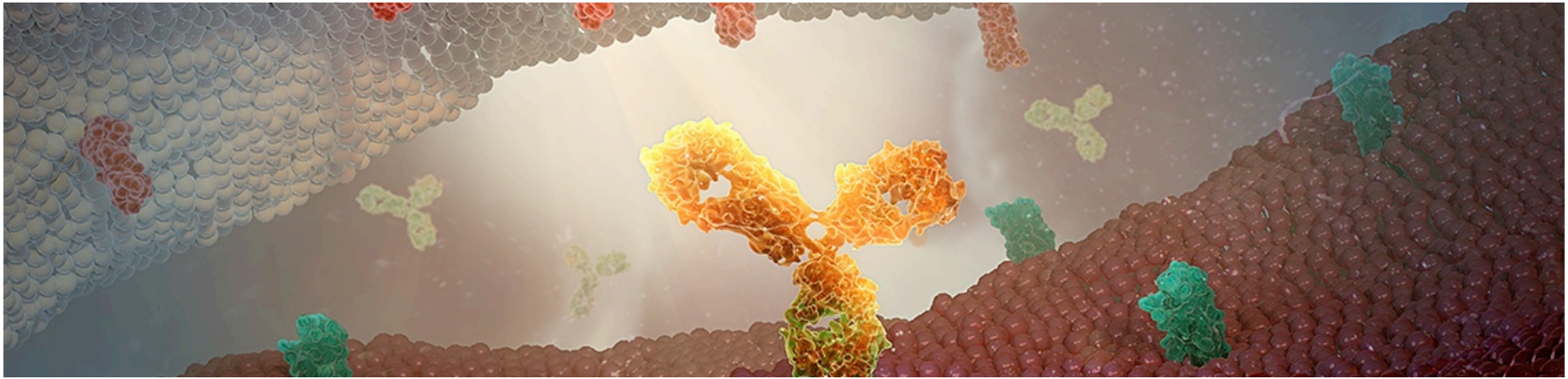


Immunogenicity - Defined

EMA: “Therapeutic proteins are recognised by the human immune system. This recognition is often followed by an immune response to therapeutic proteins. This potentially harmful immune response is complex and, in addition to ADA formation, involves T cell activation and innate immune responses”
https://www.ema.europa.eu/documents/scientific-guideline/guideline-immunogenicity-assessment-therapeutic-proteins-revision-1_en.pdf

FDA: “Immunogenicity is defined as the propensity of the therapeutic protein product to generate immune responses to itself and to related proteins or to induce immunologically related adverse clinical events”
https://www.fda.gov/downloads/Drugs/Guidances/UCM192750.pdf

The ADA Mediated Effects of a Patient's Immune Response can be Highly Variable

- **Safety**
  - ADA may cause high sensitivity reactions from Type I (Allergy/Immediate), to Type II ('Antibody-Mediated'), then Type III (Immune Complex Mediated) and finally Type IV (T-Cell Mediated)
  - ADA neutralise activity of an endogenous equivalent resulting in deficiency syndrome
- **Altered Pharmacokinetics**
  - Adjustments in dose levels and changes in apparent clearance
- **Altered Pharmacodynamics (Efficacy)**
  - Changes in drug effects, so the biotherapeutic no longer affects target in an efficacious manner
- **None**
  - Despite ADA prevalence, clinical effects maybe minimal

ADA Bioanalysis has Evolved over the Past Two Decades; Brief History of the Whitepapers & Regulations

- Findlay *et al*., J. Pharm. Biomed. Anal. 21 (2000) 1249–1273; first proposed Bioanalytical Methods to detect antibodies to macromolecules
- A.R. Mire-Sluis *et al*., JIM 289 (2004) 1–16; Standardised recommendations for the design & optimisation of ADA immunoassays
- Guideline on Immunogenicity Assessment of Biotechnology-derived Therapeutic Proteins; EMA Draft 2006, Effective 2008
- G. Shankar *et al*., Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 1267–1281 formed the basis of most ADA method validation designs
- Assay Development for Immunogenicity Testing of Therapeutic Proteins; FDA 2009
- Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products; FDA 2016
- Guideline on Immunogenicity assessment of therapeutic protein; EMA Draft 2015, Effective 2017
- Ishii-Watabe, Shibata, Nishimura *et al.* Bioanalysis (2018) 10(2), 95–10 Immunogenicity of therapeutic protein products: current considerations for anti-drug antibody assay in Japan

Key Guideline Changes have Occurred in the Past Few Years

Parameter	2009 Draft Guidance	2016 Draft Guidance
Precision	• Not specified	• Inter- and intra-assay % CV ≤ 20%
False Positive Rate (FPR)	• Screen: 5% • Confirm: Not specified	• Screen: No less than 5% false positives • Confirm: No less than 1% false positives
Cut Point Calculation	• Point estimate (50% confidence)	• Screen: 90% confidence interval • Confirm: 80% confidence interval
Sensitivity	• Screen: ≤ 250 ng/mL	• Screen: ≤ 100 ng/mL
	• Confirm: Not specified	• Confirmatory assay at least as sensitive as screen

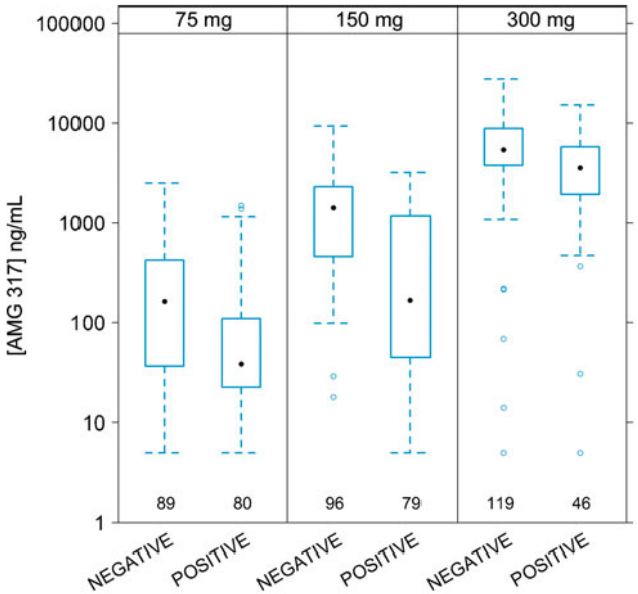
- EMA Immunogenicity guideline doesn't cite sensitivity requirements in their 2017 document

Changes in ADA Assay Sensitivity Over the Years

- 2009 FDA Guidance; '*Based on data from completed clinical trials, FDA recommends that screening assays achieve a sensitivity of approximately 250 – 500 ng/mL. Such antibody concentrations have been associated with clinical events'.*
- 2010 – EBF OS; Susan Kirshner '*A sensitive (250 – 500 ng/mL) screening assay with a low but defined false positive rate (5%) should initially be used*'.
- 2016 FDA Guidance; '*FDA recommends that screening and confirmatory ADA assays achieve a sensitivity of at least 100 ng/mL of undiluted matrix'.*

What Drove the FDAs Change in Sensitivity?

- Zhou *et al*. AAPS J 15(1)p30-40 2013; suggests that ADA against AMG317 (anti-IL4R) at 100 ng/mL reduced drug exposure and did not affect safety in there Phase 2 trial
  - <100 ng/mL level arbitrarily set on RLU between PC and Plate Cut Point
  - 100 to 500 ng/mL level arbitrarily set on RLU
  - >500 ng/mL arbitrarily set on RLU
  - Why was exposure affected?
    - AMG 317 trough levels were low ~100 ng/mL - 1000 ng/mL
  - **Conclusion**; This 100 ng/mL ADA finding can only be generalized to drugs with molar exposure similar to AMG317



Zhou *et al*. AAPS J 15(1)p30-40 2013

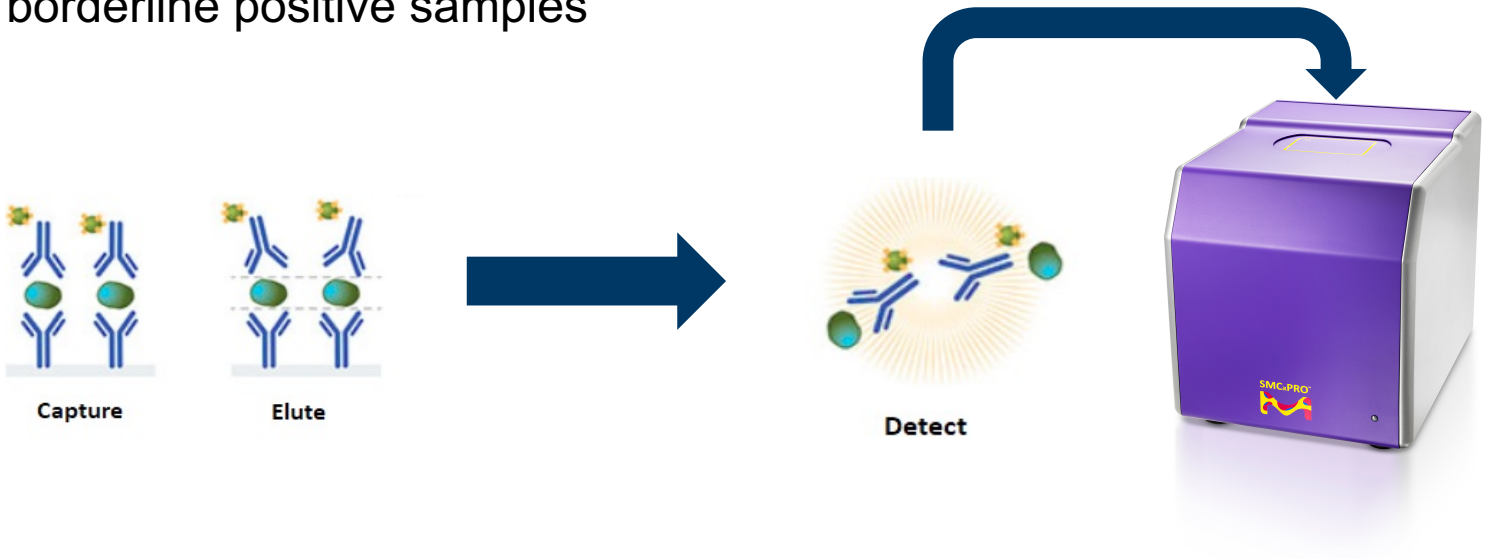
Familiarity May Drive Assay Platform Choice Decisions

- Majority of ADA Assays are run on an ECL immunoassay (ECLIA) format
  - Plate carbon electrode plate offers greater binding capacity than ‘standard’ polystyrene plates
  - Signal amplification from multiple levels of excitation per label leads to larger dynamic range
  - Typically offers increased sensitivity and drug tolerance over ELISA

- ☠ Single vendor reliance on specific reagents and equipment

SMCxPRO™ Offers a Viable Alternative to ADA Assessments

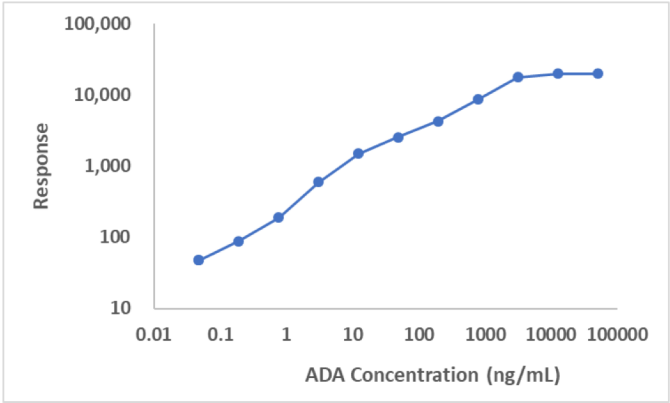
- Literature searches indicate that this technology has not been utilised in the detection of ADA complexes to date
- Could this platform be a viable alternative to improve signal-to-noise and limit borderline positive samples



Working with MilliporeSigma FAS Assay Development was Rapid and Straight Forward

Instrument Install

Reagent Conjugation



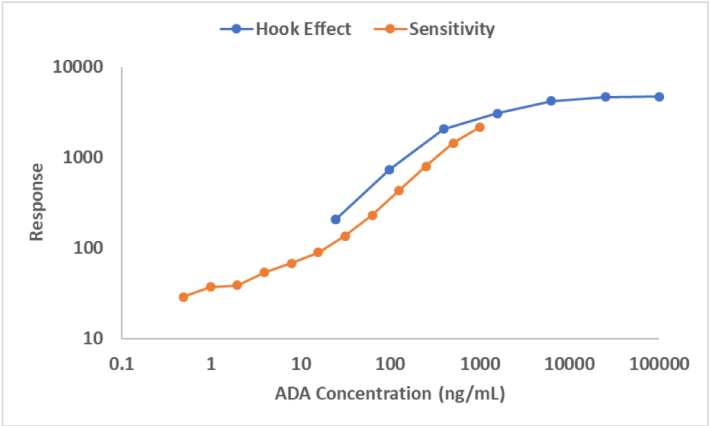
- PAb Surrogate Control
- Curve prepared in Assay Buffer

Chequerboard & MRD Assessment

- By the second run the following conditions were optimised:
  - MRD (1 in 5 [20% Matrix])
  - 0.025 µg/mL Biotin-Drug: 0.025 µg/mL Alexa-Drug
  - Higher MMX concentrations = higher background

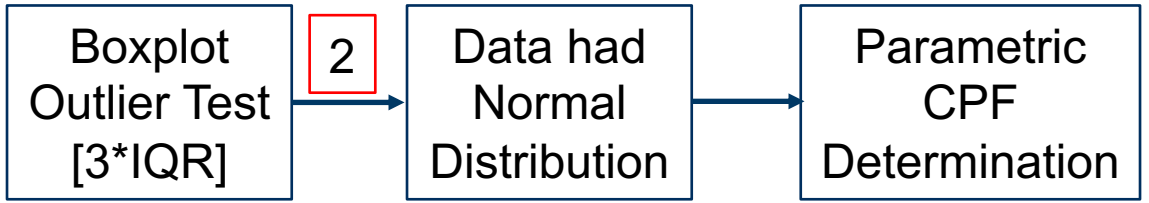
Assay Performance Fulfilled FDA/EMA ADA Validation Guideline Requirements and Were Comparable to Industry Standard Platforms

- Hook effect; Not evident up to 100,000 ng/mL
- Precision; ≤ 20% CV
  - Once familiar with the platform, this improved
- Tolerant of Acid Treatment



- Cut Point Assessment
  - 50 drug naïve cyno samples assessed
  - SCPF 2.98 (1% FPR*)

  * More stringent SCPF for non-clinical



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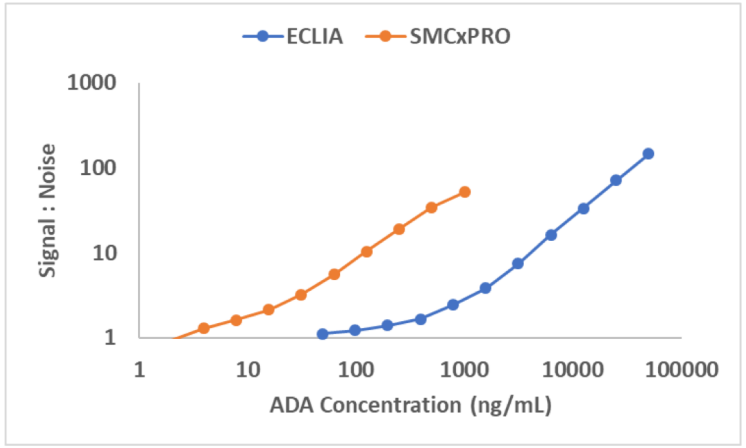

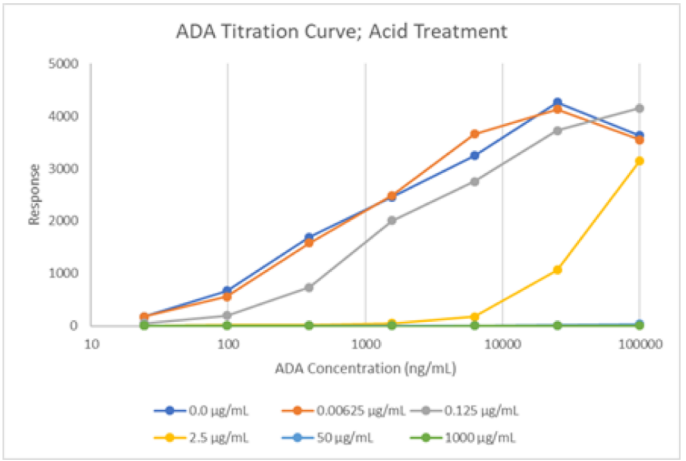

- Using this Rabbit PAb raised to Drug sensitivity was observed at 20 ng/mL
- Drug Tolerance; Detect 97.7 ng/mL PAb surrogate control in 0.125 µg/mL Drug

Potential New ADA Platform: Strengths and Weakness

Pros	Cons
• Performs within HA defined criteria • Open platform with regard to assay optimisation • Off-the-shelf reagents to aid assay development • Acid treatment tolerant • Cost • FAS Support • Bead based or Plate based • Throughput • Efficient assay development workflow	• Reduced tolerance to circulating therapeutic • Hinder assessment of ADA responses • Sample handling requirements; impact on precision • Pushing sensitivity to lower levels adds no value for safety

Closing Remarks and Summaries

- The SMCxPRO™, a plate reader marketed by MilliporeSigma, was evaluated as an alternative technology to existing platforms used to measure immunogenicity
- Whilst the SMCxPRO™ had a lower level of sensitivity for this assay, drug tolerance was poor and could cause false negatives

- **We believe pushing sensitivity so low is not warranted and does not provide any additional information to help interpret the clinical safety impact**

- Just because you can does not always mean that you should!

- The evaluation of immunogenicity should be based on the integrated analysis of pharmacokinetic, pharmacodynamic and immunogenicity to fully understand an individual patient response

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