ELISA-Based Neutralizing Antibody (NAb) Detection as a Fit-for-Purpose Alternative to Cell-Based Assays for Biotherapeutic Immunogenicity Assessment

KRISHGEN BioSystems
OUR REAGENTS, YOUR RESEARCH

Authors: Dr Sneha Hande, Krisha Jain, Dr Kalpesh Jain

ABSTRACT

Introduction/Rationale
Neutralizing antibodies (NAbs) are a critical determinant of immunogenicity in biotherapeutics, with the potential to block mechanism of action, alter pharmacokinetics, and reduce clinical efficacy. While cell-based assays remain the gold standard for NAb detection due to their functional relevance, they are often limited by high variability, cell-line dependency, and complex validation requirements. To address these challenges, we evaluated ELISA-based NAb assays as fit-for-purpose alternatives that recapitulate key ligand–receptor interactions in a controlled and reproducible format.

Methods
Competitive ligand-binding ELISAs were developed and applied across multiple clinically relevant biotherapeutics, including monoclonal antibodies (Pertuzumab, Denosumab, Evolocumab, Panitumumab), enzyme replacement therapy (Imiglucerase), and peptide therapeutics (Semaglutide). Each assay quantified NAb-mediated inhibition of drug–target interaction through measurable signal reduction. Reference NAbs were spiked across defined concentration ranges, and assay performance was benchmarked against published cell-based data. Analytical parameters—including sensitivity, precision, accuracy, and cut-point determination—were evaluated in accordance with ICH M10 and FDA bioanalytical guidelines.

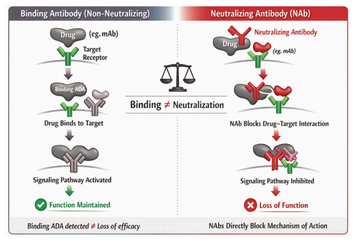
Results
All ELISA formats demonstrated excellent linearity (R^2 > 0.99) and high precision (CV < 10%) across their working ranges, with sensitivity comparable to published cell-based assays. Inhibition profiles showed strong concordance with reported cellular functional data across all biologic classes, confirming mechanistic equivalence in detecting ligand–receptor blockade. Additionally, ELISA-based assays exhibited improved reproducibility, reduced assay complexity, and faster turnaround times.

Conclusion
ELISA-based NAb assays provide a robust, scalable, and cost-effective alternative to cell-based functional assays for immunogenicity assessment across diverse biotherapeutic modalities. By directly modeling ligand–receptor inhibition, this platform maintains biological relevance while enabling greater standardization and throughput, supporting its use as a fit-for-purpose approach in early-stage development and translational immunology workflows.

INTRODUCTION AND OBJECTIVE

herapeutic biologics, including monoclonal antibodies, peptides, and enzyme replacement therapies, have transformed the treatment landscape across oncology, metabolic disorders, and autoimmune diseases. However, these agents can elicit immunogenic responses, leading to the formation of anti-drug antibodies (ADAs). Among these, neutralizing antibodies (NAbs) are of particular concern because they directly interfere with the drug's mechanism of action by blocking critical ligand–receptor interactions, potentially reducing therapeutic efficacy and altering pharmacokinetics. Importantly, the presence of binding ADAs does not always correlate with functional impact, making the detection of NAbs essential for a biologically meaningful assessment of immunogenicity.

Cell-based assays are widely regarded as the gold standard for NAb detection because they measure functional biological activity within a cellular context. However, these assays are inherently complex and often limited by variability arising from differences in cell lines, receptor expression levels, and culture conditions. In addition, they require extensive optimization, exhibit longer turnaround times, and present challenges in standardization and inter-laboratory reproducibility. These limitations can hinder their routine application, particularly in early-stage development and high-throughput settings.



In this study, we developed and evaluated ELISA-based competitive ligand-binding assays to detect neutralizing antibodies by measuring inhibition of drug–target interactions. The approach was assessed across multiple biotherapeutic modalities, including monoclonal antibodies, peptides, and enzyme-based therapeutics, to demonstrate broad applicability.

Our objective was to establish ELISA-based assays as fit-for-purpose alternatives to traditional cell-based methods, enabling standardized, high-throughput, and reliable functional assessment of immunogenicity in early-stage development.

Figure 1: Schematic - ADA Binding vs nAb Antibody Interactions

PLATFORM DESIGN AND FUNCTIONAL VALIDATION

ASSAY DESIGN AND DEVELOPMENT

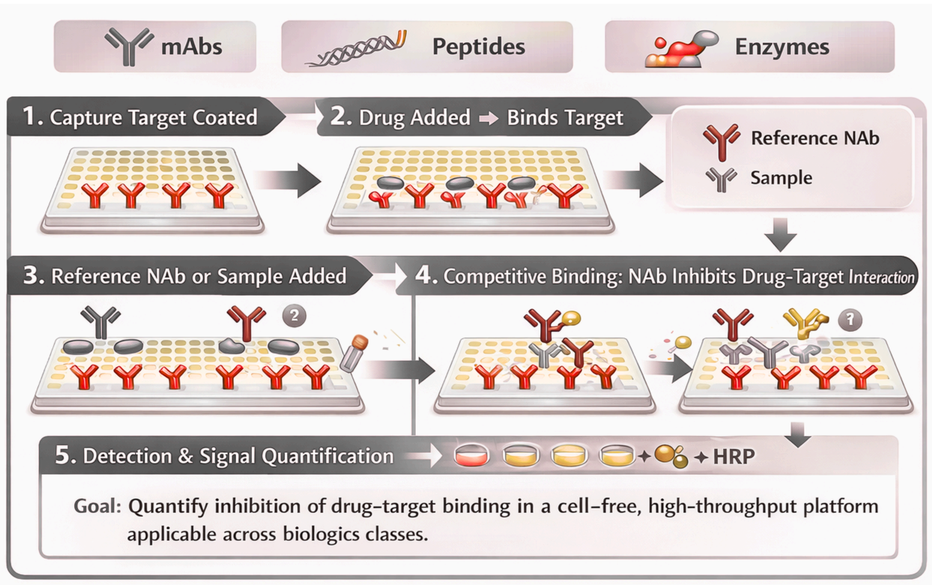


Figure 2: Development of a competitive ELISA-based neutralizing antibody assay. Schematic representation of the assay workflow used to detect neutralizing antibodies (NAbs) across multiple biotherapeutic modalities. Target proteins are immobilized on the microplate, followed by incubation with the therapeutic drug. In the presence of NAbs (reference or sample-derived), drug–target interaction is inhibited through competitive binding. The resulting decrease in signal intensity is proportional to the level of neutralizing activity, enabling quantitative assessment of functional inhibition in a cell-free format.

The assay is based on immobilization of the biological target, followed by binding of the therapeutic agent in the presence or absence of neutralizing antibodies. When NAbs are present, they competitively block the interaction between the drug and its target, resulting in a reduction in detectable signal.

This platform was applied across multiple biotherapeutic classes, including monoclonal antibodies, peptide therapeutics, and enzyme-based drugs, demonstrating its versatility. The assay design enables sensitive and reproducible detection of functional neutralization in a controlled, cell-free system, making it suitable for high-throughput immunogenicity assessment.

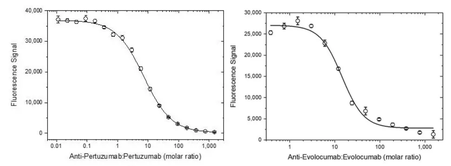


Figure 3: Neutralizing antibody inhibition of Pertuzumab binding (left) and Evolocumab binding (right)

A clear sigmoidal, dose-dependent inhibition profile was observed for both Pertuzumab and Evolocumab assays, demonstrating effective neutralization of drug–target interactions.

The consistency of inhibition curves across biologics confirms the assay principle's ability to capture functional NAb activity in a reproducible and mechanistically relevant manner.

PRECISION

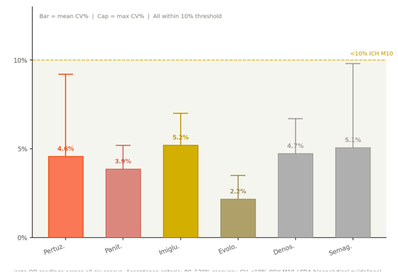


Figure 10: Average %CV across standards for n=15 assay runs.

Mean CV% per biologic with the cap showing the maximum observed CV. Clean at-a-glance comparison — Panitumumab is tightest (mean ~3.6%), all nAbs are well within threshold.

ADA VS NAB FOR IMMUNOGENECITY

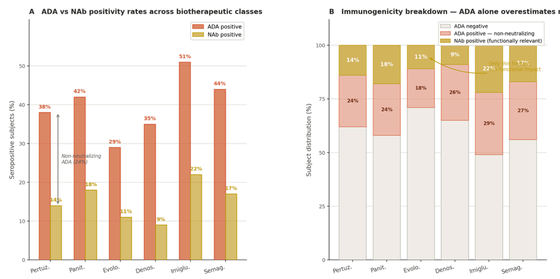
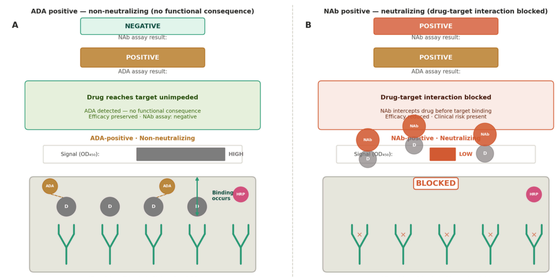


Figure 4: ADA positivity overestimates functional immunogenicity, nAb supports decision making

Across six biotherapeutic classes, NAb-positive rates were consistently 2–3× lower than ADA-positive rates, underscoring that ADA alone cannot distinguish clinically relevant immune responses from benign binding events. Neutralizing antibody-specific detection is therefore essential for accurate immunogenicity risk stratification.



Both scenarios yield a positive ADA result, yet only Panel B represents a clinically meaningful immune response. In Panel A, non-neutralizing ADA binds the drug without blocking target engagement, leaving efficacy intact and the NAb assay negative. In Panel B, neutralizing antibodies intercept the drug prior to target binding, abolishing the therapeutic interaction and triggering a positive NAb readout. This distinction — invisible to ADA screening — underlies the clinical and regulatory necessity of fit-for-purpose NAb assay development.

CROSS-PLATFORM APPLICABILITY

The nAb assay demonstrates a robust, reproducible, and concentration-dependent response with strong curve fitting (R^2 > 0.96), confirming reliable detection of neutralizing antibodies.

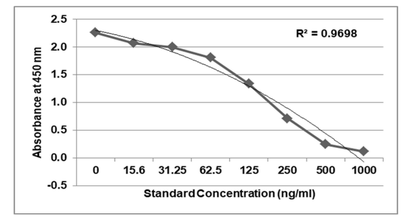


Figure 5: mAb and nAb dose response curve

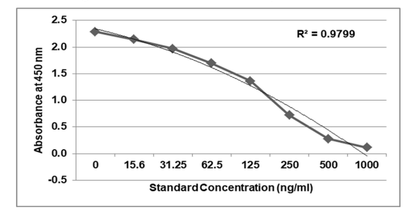


Figure 6: Peptide (drug) + NAb dose response curve

The comparable dose-dependent response across formats confirms the assay's robustness and its ability to consistently model ligand–receptor inhibition.

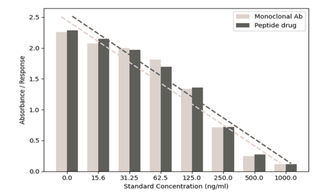
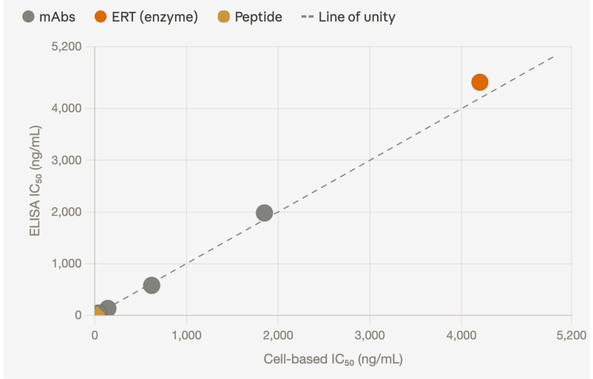


Figure 7: Comparative dose response curve

COMPARISON TO CELL-BASED ASSAYS



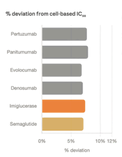
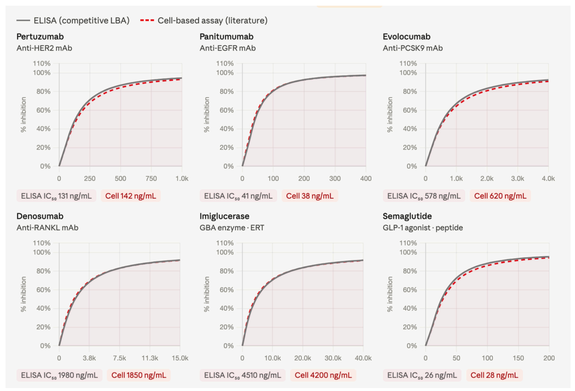


Figure: Comparison of ELISA-based and cell-based assay sensitivity across biotherapeutic classes. Deviation = $|IC_{50}(ELISA) - IC_{50}(cell)| / IC_{50}(cell) \times 100$.



Comparison of ELISA-derived neutralization profiles with representative cell-based assay performance showed close agreement across multiple biotherapeutic classes. IC50 values differed by less than 10% from corresponding cell-based values, supporting mechanistic concordance between ligand–receptor inhibition measured by ELISA and functional activity observed in cellular systems.

CONCLUSION

ELISA-based neutralizing antibody assays provide a robust, reproducible, and mechanistically relevant approach for functional immunogenicity assessment across diverse biotherapeutic classes. By directly measuring inhibition of drug–target interactions, these assays distinguish functional neutralization from simple ADA binding and therefore offer greater biological relevance than conventional binding immunogenicity assays.

The consistent dose-dependent inhibition observed across representative monoclonal antibody, peptide, and enzyme-based therapeutics supports the platform's broad applicability. Together, these findings position competitive ELISA-based NAb assays as fit-for-purpose alternatives to more variable and complex cell-based methods.

Parameter	Cell-based assay	ELISA-based NAb assay
Assay variability	× High	✓ Low
Throughput	× Low	✓ High
Cell-line dependency	× Required	✓ None
Turnaround time	× Days–weeks	✓ Hours
Standardization	× Difficult	✓ High
Validation complexity	× High	✓ Low
Biological relevance	× Direct	✓ Equivalent
ICH M10 compliance	× Challenging	✓ Fit-for-purpose

Key Takeaways

- Platform-agnostic applicability
- R^2 >0.99 linearity, CV <10% precision
- 80–120% recovery confirmed
- IC_{50} concordance <10% deviation
- No cell-line dependency
- Clinically relevant sensitivity
- Fit-for-purpose validated

About Krishgen Biosystems:

Established 2003, Krishgen is an immunoassay manufacturer based out of Mumbai, India. Our key products include assays for mAbs, bispecific antibodies, antibody-drug conjugates, peptides as well as a wide range of cytokine and biomarker ELISA across various species.

Our products are well validated, sensitive, robust and competitively priced. As of April 2025, Krishgen ELISA have been cited in 5000+ publications worldwide. ***Learn more about Krishgen and ELISA we offer at www.krishgen.com.***