Development of a Highly Sensitive, Drug-Tolerant, and Target-Tolerant Competitive Ligand Binding Assay for Detecting Neutralizing Antibodies Against Omalizumab Using Dynabeads Affinity Purification

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Introduction: Neutralizing antibodies (Nabs) are the subset of ADA (Anti-Drug Antibody) that prevents the binding of therapeutic protein product with its target thus reducing the efficacy of the drug. Detection of neutralizing antibody is important part of product's immunogenicity analysis and it is also a regulatory expectation. Development of sensitive and drug tolerant assay to detect Nab is very challenging due to presence of drug it self in the samples and multiple sample purification steps involved in the method. Additionally, soluble target can produce false-positive result.

Method Principle:

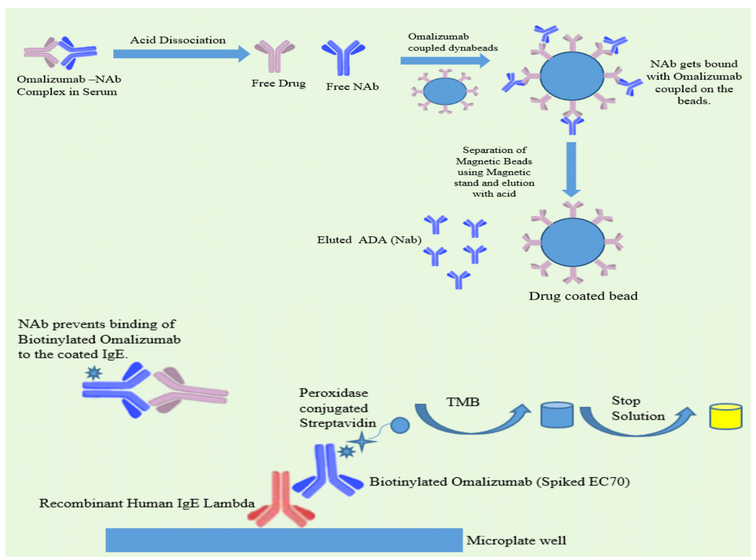


Figure 1: Diagrammatic representation of assay principle. Assay involves affinity purification using Dynabeads followed by detection using biotinylated drug in ELISA.

Method Challenges	Mitigation Strategy
Leaching of drug from drug coupled Dynabeads	Multiple washes of drug coupled beads before using
Presence of high amount of therapeutic drug and drug target in the sample	Affinity purification of samples along with acid dissociation of sample introduced Sample and Omalizumab coupled beads volume for affinity purification optimized.
Higher EC50/EC70 concentration	Biotinylated drug used to achieve lower EC50/EC70

Inter Run Precision Data

Precision Statistics	NC	LPC (166 ng/mL)	MPC (1000 ng/mL)	HPC (10000 ng/mL)
Mean S:N Ratio	1.000	0.857	0.509	0.322
Inter-Assay Precision	1.9	4.2	12.2	15.8
n (replicate)	18	18	18	18

Inter run precision evaluated from 03 batches performed by two different analyst on two different days. 02 batches performed using biotinylated test drug and 01 batch performed using biotinylated reference drug.

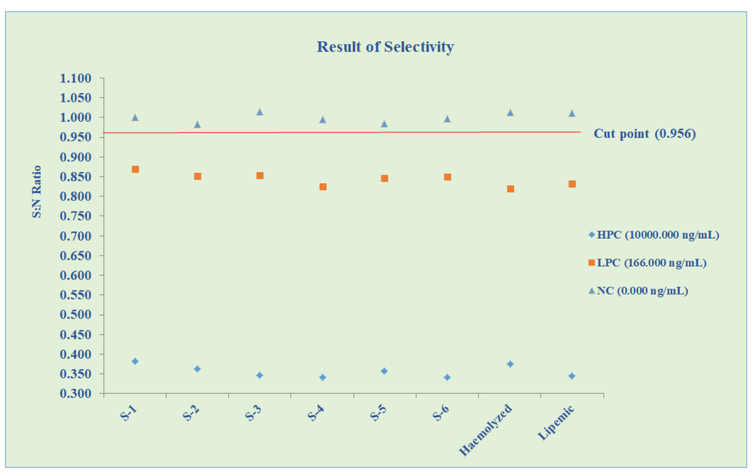


Figure 2: Data of selectivity performed using different lot of human serum including haemolyzed and lipemic serum .

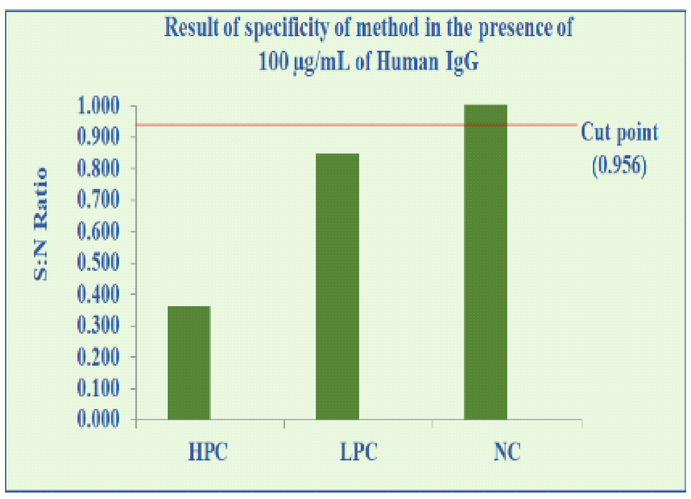


Figure 3: Results of specificity evaluated in the presence of 100 µg/mL of Human IgG

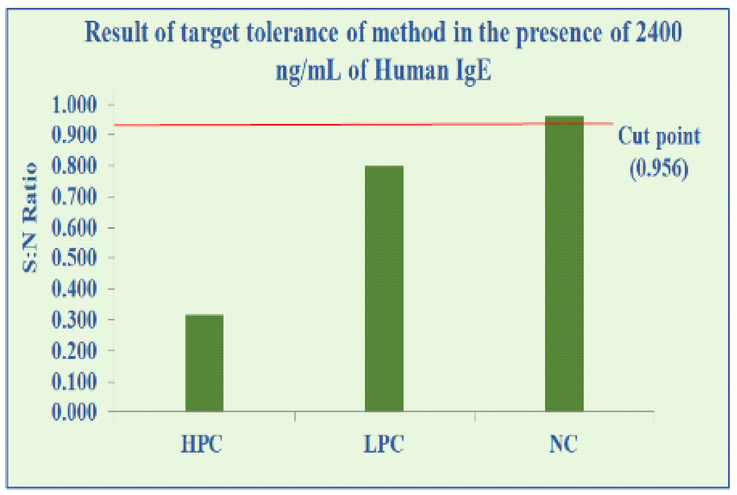
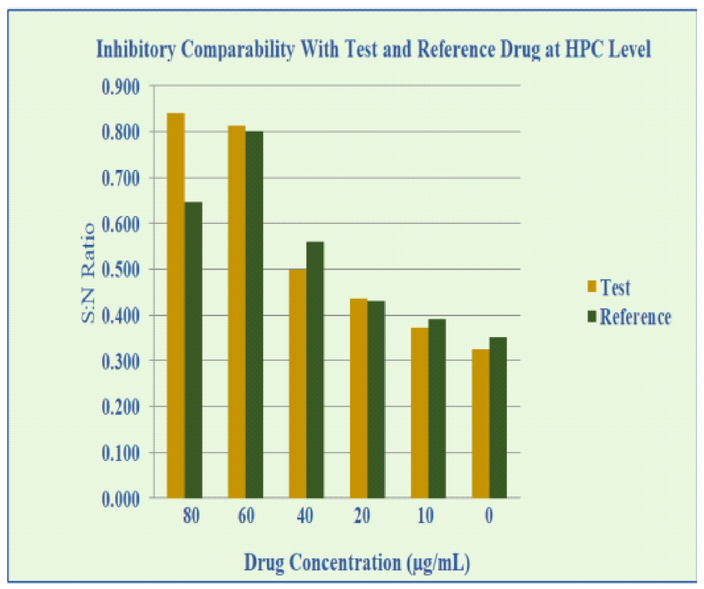


Figure 4: Results of target tolerance evaluated with 2400 ng/mL of Human IgE



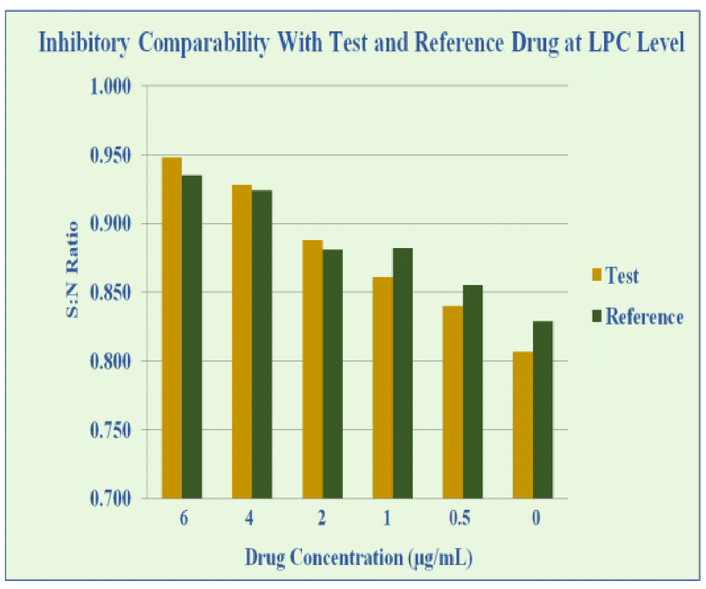


Figure 5 & 6 : Drug tolerance performed with different concentration of test and reference drug. Drug tolerance of 80 µg/mL obtained with both test and reference drug at HPC level and 6 µg/mL with both test and reference drug at LPC level.

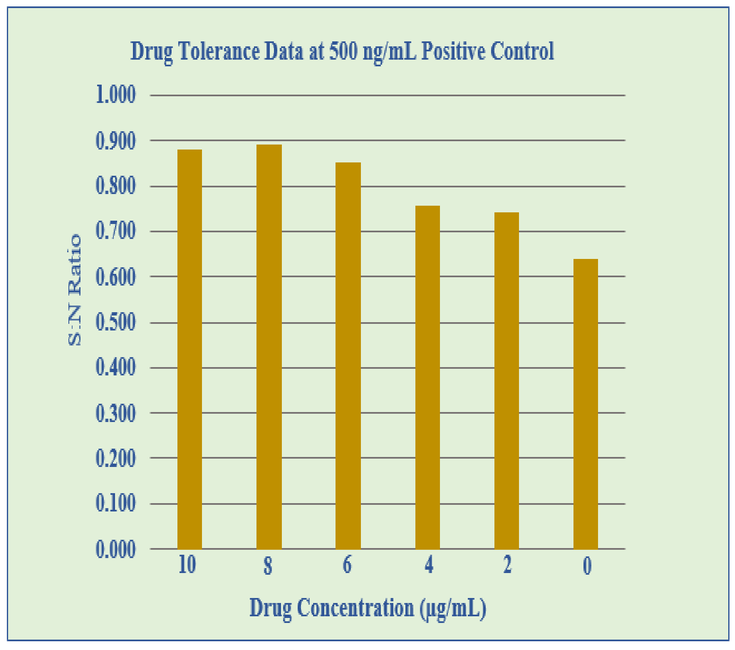


Figure 7: Results of drug tolerance data at 500 ng/mL positive control. 10 µg/mL of drug tolerance obtained at 500 ng/mL PC.

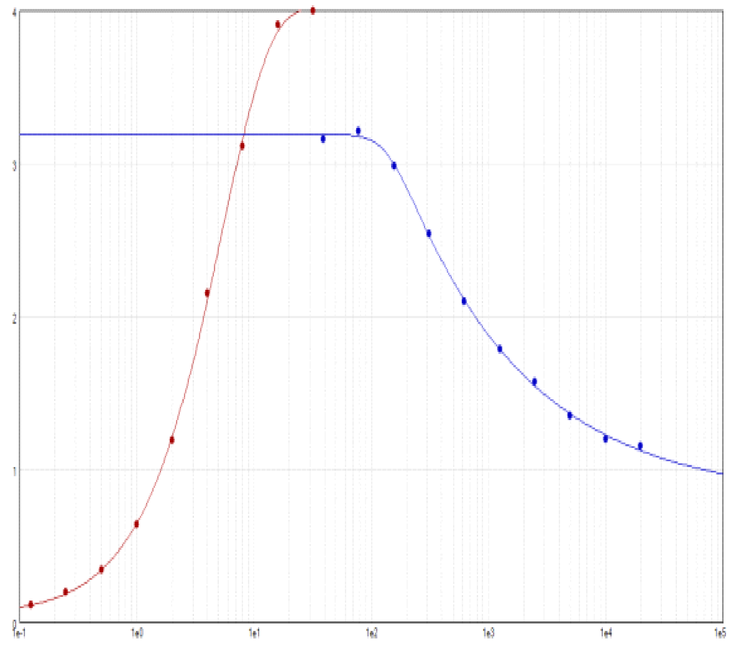


Figure 8: Representative drug response curve and sensitivity curve. Sensitivity curve interpolets at Ec70 value on DRC.
Drug response curve ● Sensitivity curve ●

Novel Approach: Development of NAb assay that is both highly sensitive and drug tolerant is difficult due to presence of high amount of circulating drug in the sample which results into false negative result. In our approach we used affinity purification of samples using Dynabeads along with use of biotinylated version of drug to detect neutralizing antibody which helped to obtain exceptional sensitivity of 166 ng/mL with 6 µg/mL of drug tolerance.

Conclusion: The development of this highly sensitive and tolerant assay is a significant advancement in the detection of NAbs against Omalizumab. By overcoming the challenges of drug and target interference, this assay provides a reliable tool for assessing the immunogenicity of therapeutic proteins, ultimately contributing to better patient safety.