

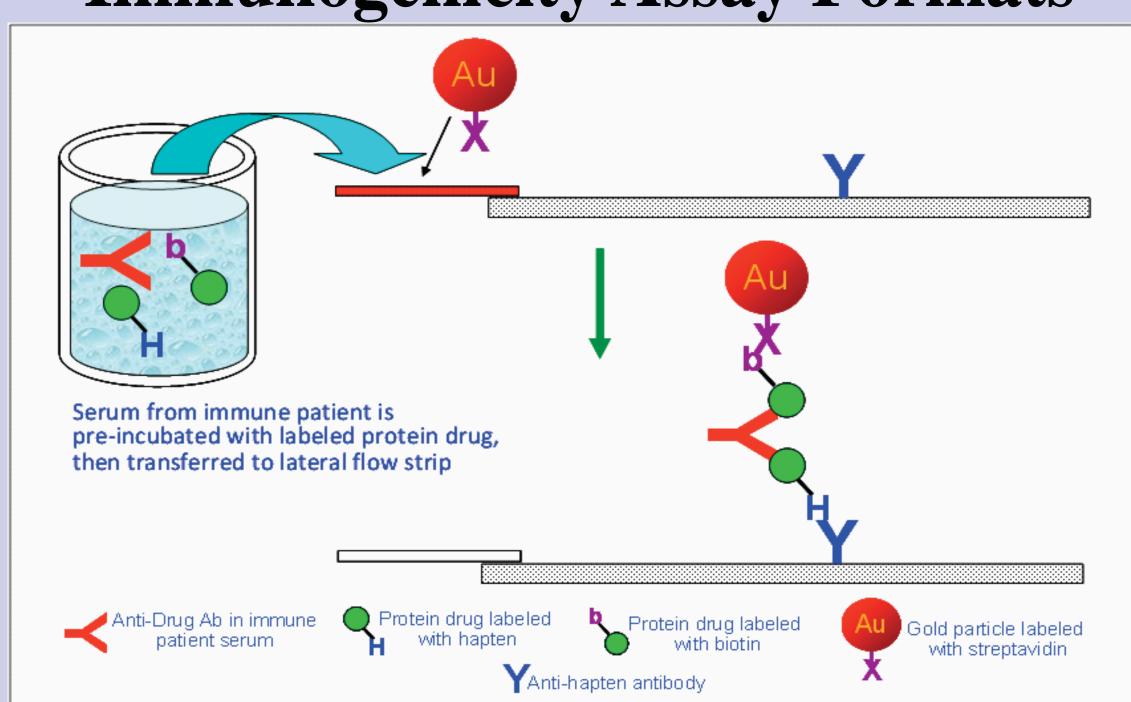
Comparison of the NIDS® Rapid Assay with ELISA Methods in Immunogenicity Testing of Two Biotherapeutics

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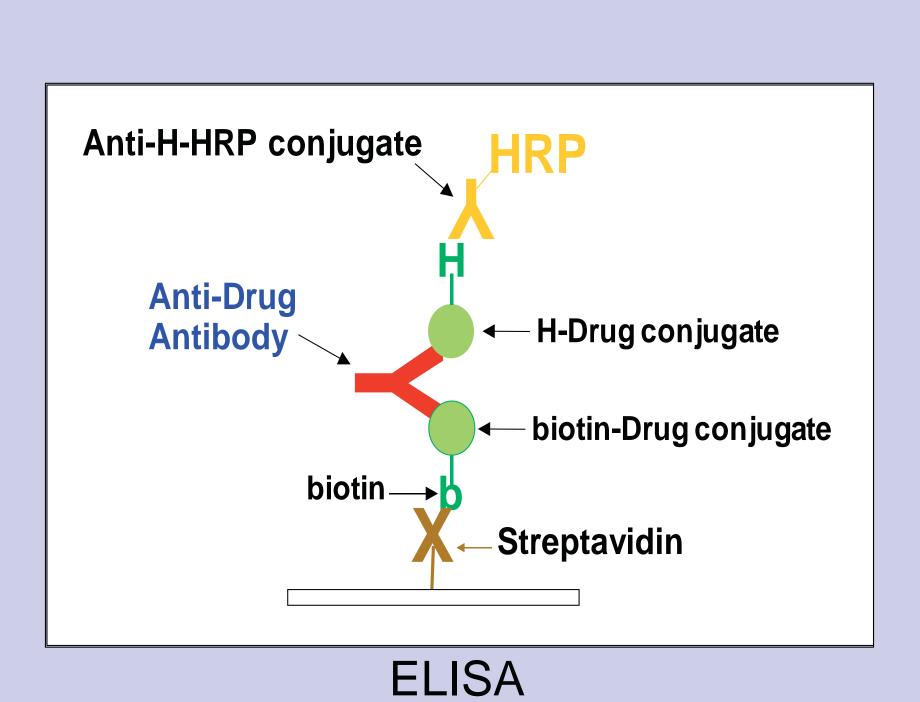
INTRODUCTION

Rapid lateral flow immunogenicity assays for the detection of anti-drug antibodies (ADAs) to two biotherapeutic proteins, designated Antibody 1 and Antibody 2, were developed using ANP's Nano-Intelligent Detection System (NIDS®) and compared to their ELISA counterparts. For the rapid assays, biotin- and haptenlabeled drug conjugates were incubated with the patient serum sample to allow ADA to form a bridge complex with each drug conjugate. The reaction mixture was then added to a test strip with an anti-hapten capture zone which binds the bridge complex. The bridge complexed biotinylated drug was then reacted with streptavidin-labeled gold particles in situ. The signal developed at the capture zone, which was directly proportional to ADA in the sample, was then quantitatively measured with a handheld reader. The counterpart ELISAs were run using the same reagents. Dose response, specificity/free drug depletion, and screening cut-point assays were performed for both methods.

Immunogenicity Assay Formats

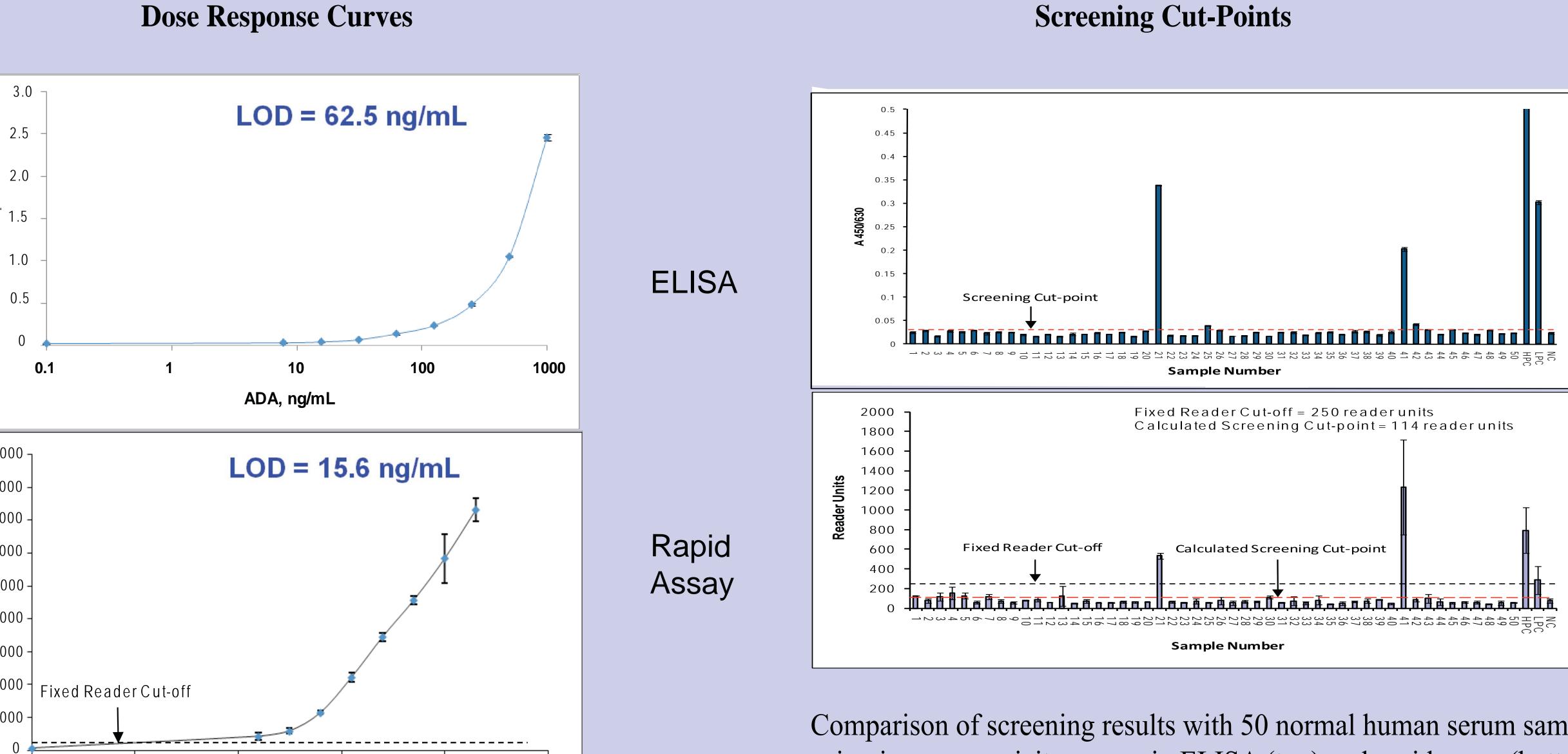


Rapid Assay



Handheld Reader and Rapid Assay Test Ticket

Antibody 1 ELISA and Rapid Immunogenicity Assays



Drug Depletion Assay in neat human serum

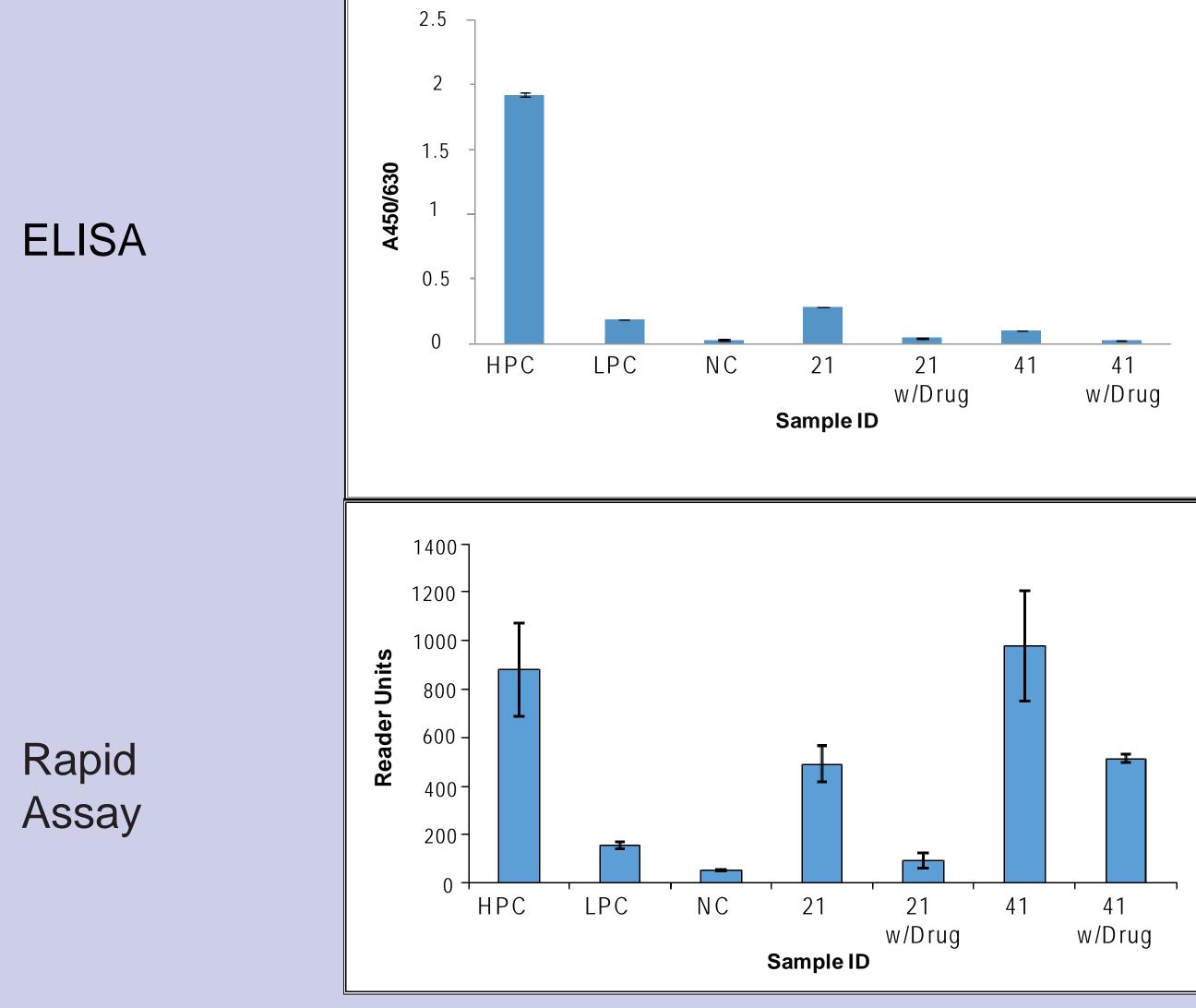
Molar Ratio Drug: ADA

Fixed Reader Cut-off

800.0

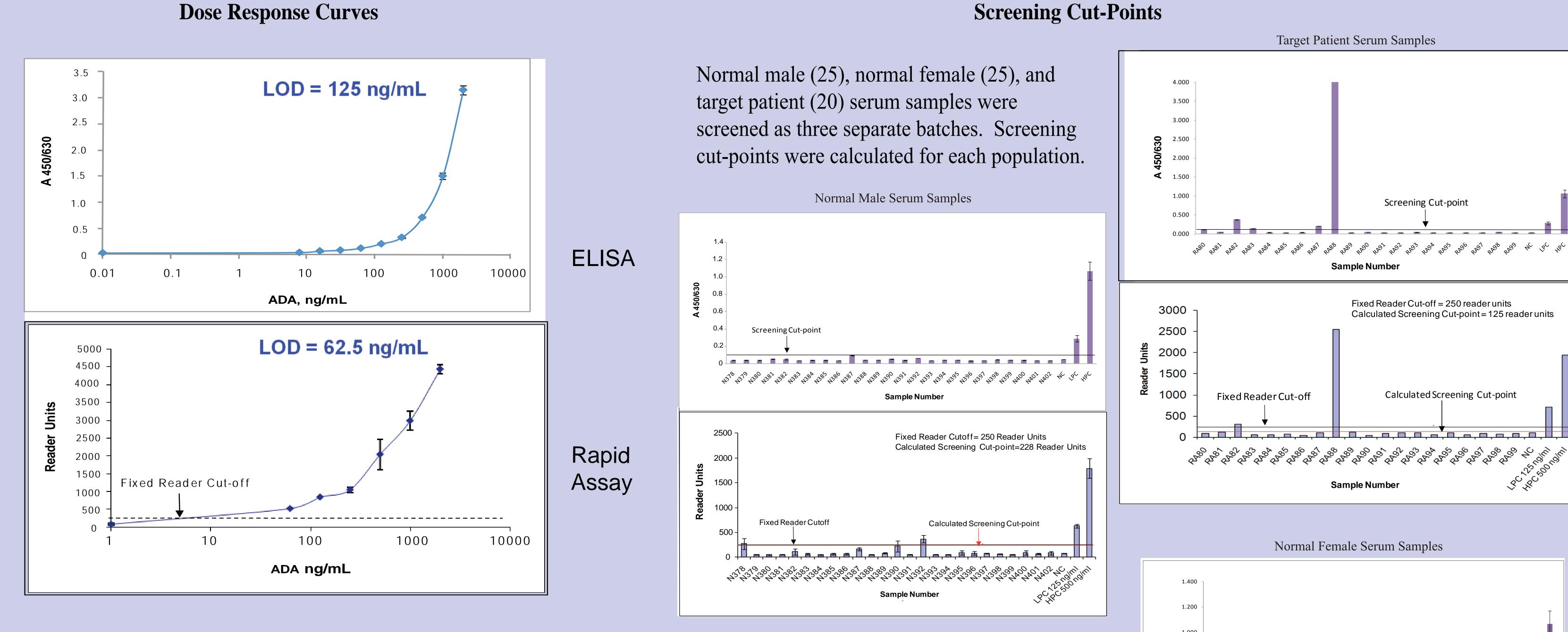
Comparison of screening results with 50 normal human serum samples using immunogenicity assays in ELISA (top) and rapid assay (bottom) formats for the detection of ADA to Antibody 1. Samples 21 and 41 were identified as positive samples by both methods.

Evaluation of Positive Samples with the Drug Depletion Assay

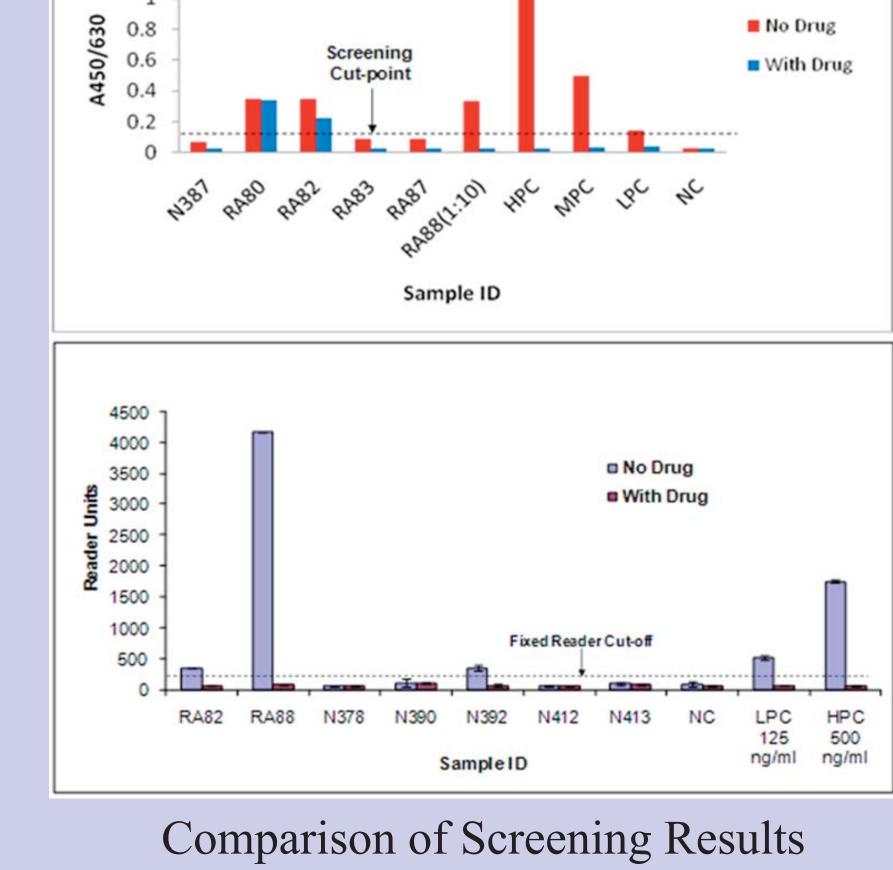


Comparison of drug depletion assay results with 2 normal human serum samples found positive using immunogenicity assays in ELISA (top) and rapid assay (bottom) formats for the detection of ADA to Antibody 1. The ELISA assay used 10 µg/mL Antibody 1, while the rapid assay used 5 µg/mL of Antibody 1. HPC=high positive control, LPC=low positive control, NC=negative control.

Antibody 2 ELISA and Rapid Immunogenicity Assays







RA88 Positive Positive

Comparison of Assay Formats

Calculated Screening Cutpoint= 172 Reader Units

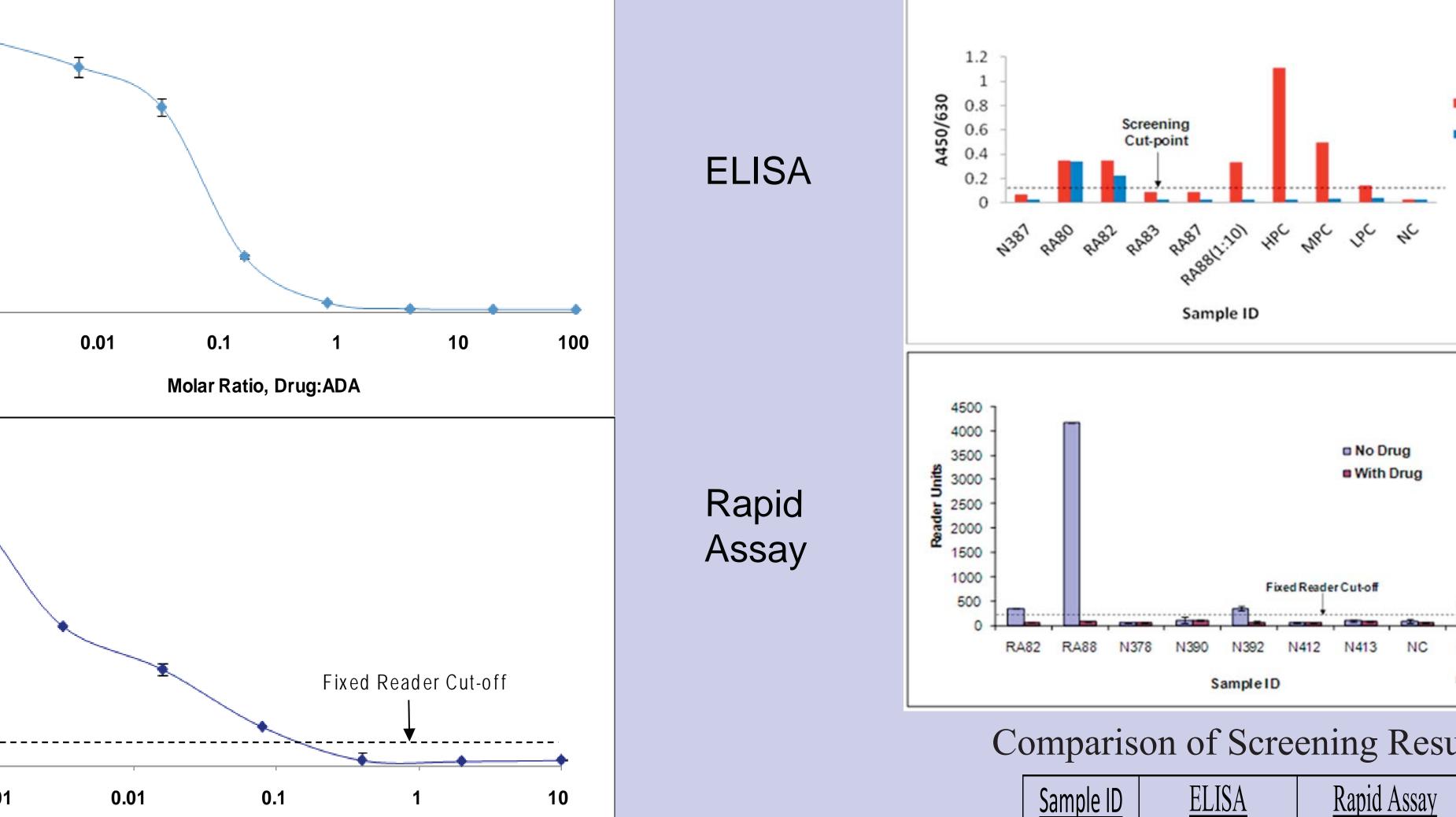
Rapid

ELISA

Assay

ELISA	
	Assay
62.5	15.6
4.2	6.3
125	62.5
5.9	6
	62.5 4.2





Molar Ratio, Drug:ADA