NIDS® Double Antigen Bridging Immunogenicity ELISA for the Detection of Anti-PEG Antibodies

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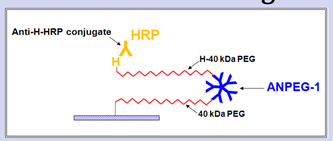
Introduction

PEGylation of biotherapeutics has been widely used to reduce their immunogenicity and enhance their stability and efficacy *in vivo*. However, PEG moieties can themselves elicit antibodies in patients. Furthermore, a small but significant percentage of the naive population may have pre-existing anti-PEG antibodies. A double antigen bridging immunogenicity (IM) ELISA for the detection of antibodies to PEG in typical polymer size ranges used in biotherapeutics has been successfully developed.

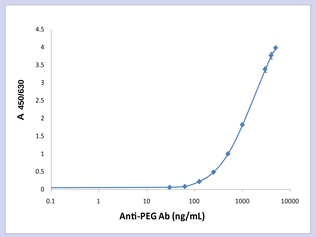
Principle of the PEG Immunogenicity ELISA



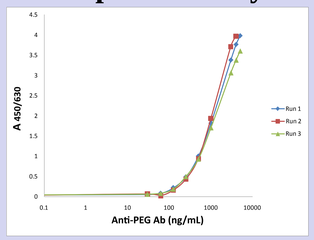
ANPEG-1 is a mouse monoclonal IgM anti-PEG antibody used as the positive control in the PEG IM ELISA.

Dose Response Curve

Human Serum Calibrators



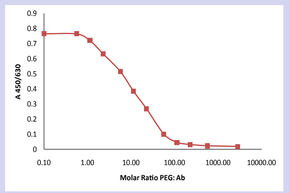
Reproducibility



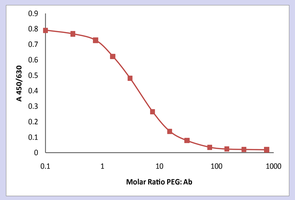
Recovery of QC Controls obtained in 3 separate assays performed by 2 operators

QC Control ng/mL Ab	Run 1 ng/mL(recovery)	Run 2 ng/mL (recovery)	Run 3 ng/mL (recovery)
200	189.9 (94.9%)	195.9 (98.0%)	193.3 (96.7%)
1000	901.2 (90.1%)	934.8 (93.5%)	939.2 (93.9%)

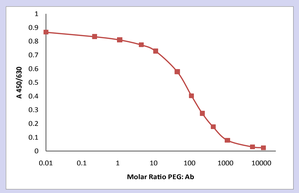
Depletion with Various PEG Polymers



Depletion of 1000 ng/mL anti-PEG Ab with 40 kDa PEG in neat pooled human serum



Depletion of 1000 ng/mL anti-PEG Ab with 30 kDa PEG in neat pooled human serum



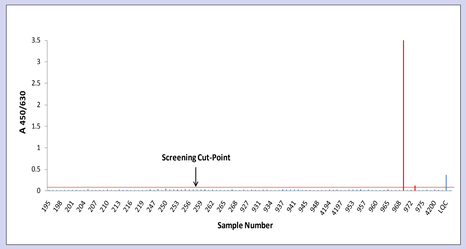
Depletion of 1000 ng/mL anti-PEG Ab with 20 kDa PEG in neat pooled human serum

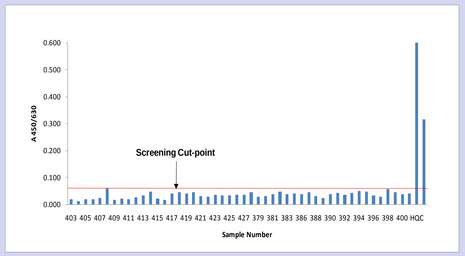
SAMPLE MATRIX EFFECTS
Recovery of ANPEG-1 Positive Control Antibody in 4 Random Naive Human Sera

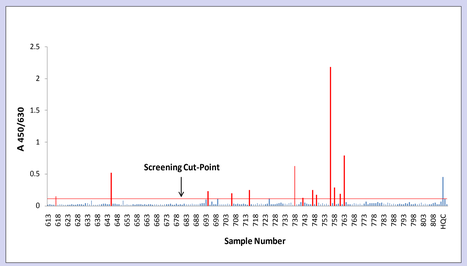
Sample number	200 ng/mL spike ng/mL(recovery)	500 ng/mL spike ng/mL (recovery)
1	162.4 (81.2%)	420.1 (84.0%)
2	172.2(86.1%)	552.4 (110.5%)
3	170.5 (85.3%)	499.7 (99.9%)
4	189.1 (94.6%)	486.3 (97.3%)

Screening Cut-Points

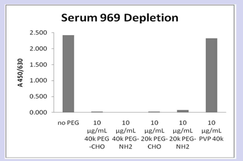
Normal human sera (n=350) were screened with the ELISA in 3 batches. A floating cut-point was calculated for each batch. Fifteen positive samples were found.

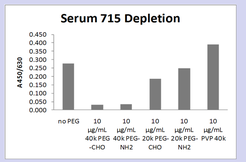


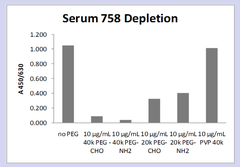


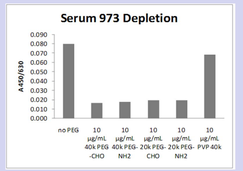


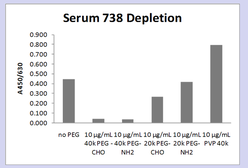
Depletion of 15 Positive Sera with various PEG polymers and PVP

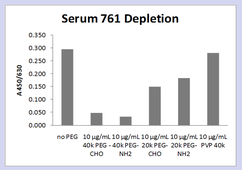


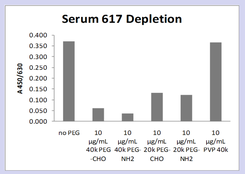


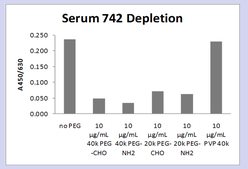


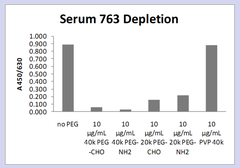


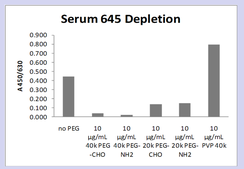


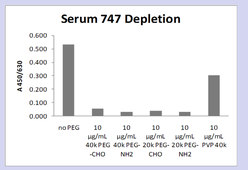


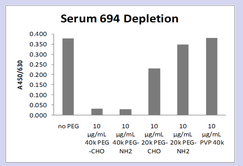


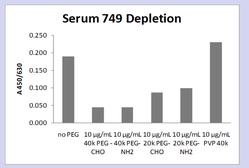


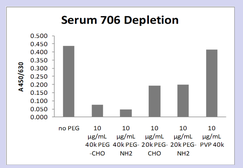


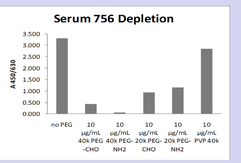












SUMMARY

1. The PEG IM ELISA can detect antibodies to PEG of various sizes and different linking groups (aldehyde or amine).
2. Of 350 human sera screened, 15 positive samples were found and confirmed as true positives in depletion assays with diverse antibody specificities to different PEG sizes and functional groups.
3. The antibody binding in the positive samples was not depleted by the same level of a similar polymer, polyvinyl pyrrolidone (PVP), indicating PEG-specific antibody responses.
4. When all confirmed positives were removed from the screening cut-point calculations, new cutoffs classified 17 out of 335 samples as false positives for a total false positive rate of 5.1 %.