# Evaluation and Optimization of a Commercial ELISA Kit for the Detection of Antibodies to Polyethylene Glycol (PEG) in Human Serum for Use in Support of Clinical Studies for PEGylated Therapeutic Drug

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## Introduction

PEGylation, the covalent attachment of polyethylene glycol to a drug or therapeutic protein, can do the following:

- Extend a drug's half-life by increasing its hydrodynamic size and reducing renal clearance.
- Provide water solubility to hydrophobic drugs and proteins.

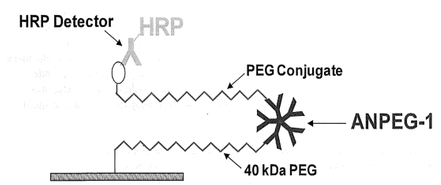
It has long been believed that PEGylation can reduce a drug's immunogenicity by masking its immunogenic epitopes and disguising it from the host's immune system. However, it has been recently found that PEGylated proteins can elicit antibody formation against PEG. In humans, PEG antibodies can be produced and may limit therapeutic efficacy and/or reduce tolerance of PEG-therapeutic drug in patients.

## Purpose

To adapt a commercial ELISA kit as fit for the purpose of detecting antibodies to PEGylated-Therapeutic Drugs (PEG-TD) in human serum (NHS).

## Methodology

### Double Antigen Bridging Assay




A conventional double antigen bridging ELISA format was used. Anti-PEG antibodies in human serum pool were incubated with 40 kDa PEG pre-coated on the plate and with a hapten-labeled PEG conjugate to form bridging complexes between immobilized PEG and the PEG conjugate. After removal of unbound material, a monoclonal mouse anti-hapten HRP conjugate was added which reacted with the immobilized bridging complexes. After incubation and washing, a TMB substrate was added to the plate to produce a colored product in proportion to the amount of anti-PEG antibodies in the samples.

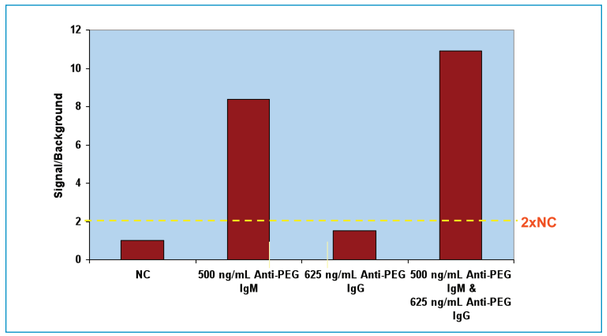



**Figure 1. Evaluation of assay feasibility for the detection of anti-PEG IgM and IgG antibodies using a commercial ELISA kit.** Mouse anti-PEG monoclonal IgM and rabbit anti-PEG monoclonal IgG antibodies were tested with the anti-PEG commercial ELISA kit. The mouse anti-PEG monoclonal IgM antibody showed a response within anticipated parameters. However, the rabbit anti-PEG IgG failed to generate a response above background level. However, an increase in response was observed in the anti-PEG IgM positive controls by the addition of IgG anti-PEG antibody. The data showed that this commercial ELISA kit could detect anti-PEG IgM antibodies or IgM&IgG mixtures, but not anti-PEG IgG antibodies alone.

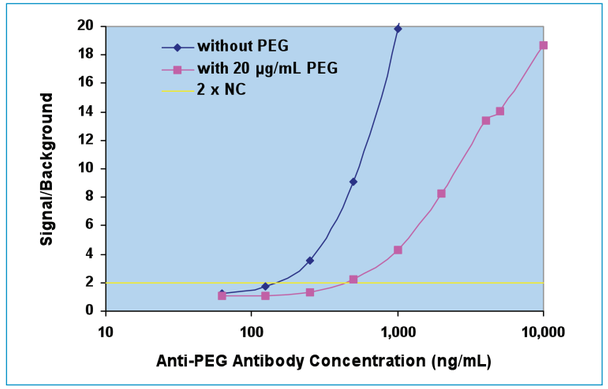



**Figure 2. Assessment of assay sensitivity in the absence or presence of 20 µg/mL PEG.** The assessment of assay sensitivity relies almost exclusively on the characteristics of the positive Ab used, such as affinity, isotype and subclass, purity, etc. During the conduct of this method validation, mouse anti-PEG monoclonal IgM antibody was used to assess the assay sensitivity in the absence and presence of PEG. When the samples contained no PEG, anti-PEG IgM antibody spiked at a concentration of 200 ng/mL in neat serum was the lowest concentration that generated a response two times higher than background. When the samples contained 20 µg/mL PEG, anti-PEG IgM antibody spiked at a concentration of 500 ng/mL in neat serum was the lowest concentration that generated a response two times higher than background. The results showed that the assay sensitivity was reduced with the presence of PEG.

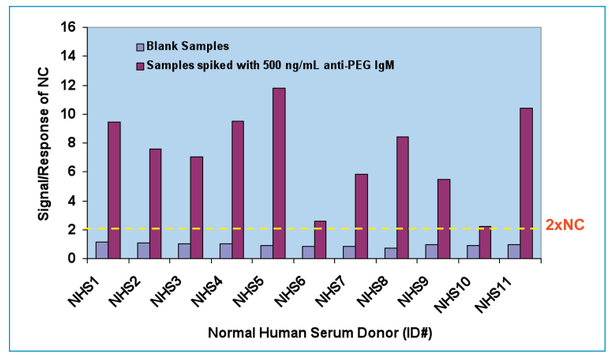



**Figure 3. Evaluation of method selectivity in normal human serum.** Total of 11 individual normal human serum samples unspiked and spiked with 500 ng/mL anti-PEG IgM antibodies were used to assess matrix interference. The spiked and unspiked samples from each donor were tested on the same plate. Eleven unspiked serum samples (100%) yielded results less than the two times of NC response. All (100%) individual spiked samples tested in screening assay yielded results greater than the two times of NC response. These results confirmed that the matrix effect in human serum samples was insignificant.

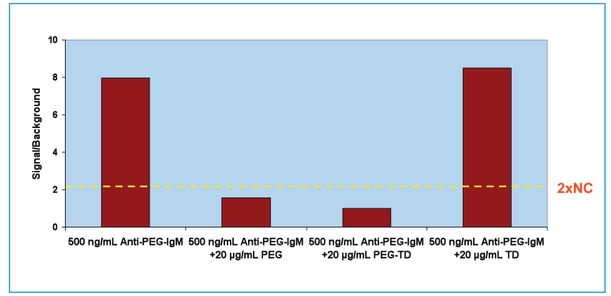



**Figure 4. Assessment of assay specificity.** Assay specificity was evaluated by incubating 500 ng/mL anti-PEG IgM with 20 µg/mL PEG, PEGylated Therapeutic Drug (PEG-TD), and Therapeutic Drug (TD). The mean signal/background value for anti-PEG IgM was 7.96. The mean signal/background values of an antibody in the presence of 20 µg/mL PEG and PEG-TD were 1.57 and 1.00, respectively, which were significantly less than the mean signal/background value for anti-PEG IgM. However, the mean signal/background values of an antibody in the presence of 20 µg/mL TD were 8.51, which were no significant change compared to the mean signal/background value for anti-PEG IgM alone. These results showed that the anti-PEG antibody response was inhibited by PEG and PEG-TD, but not TD alone, which confirmed that the antibody binding response observed in this assay can be conclusively attributed to antibodies against PEG.

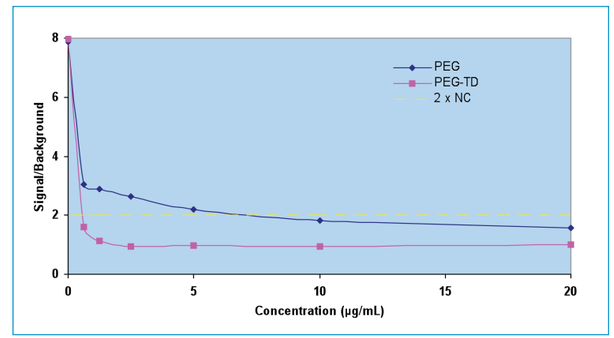



**Figure 5. Evaluation of assay drug tolerance.** The drug tolerance was evaluated using the 500 ng/mL anti-PEG IgM antibody with the PEG or PEG-TD added in two-fold concentration increments that ranged from 20.0 to 0.625 µg/mL. Data showed that 500 ng/mL of anti-PEG antibodies could be detected in the presence of approximately 5 µg/mL of PEG and 625 ng/mL PEG-TD.

**Table 1. Summary of Inter- and Intra-Assay Precision**

| | Intra-assay (n=3 in 3 assays) | | Inter-assay (n=9) |
|---|---|---|---|
| Sample | %CVp | %CV Range | % $CV_t$ |
| NC | 5.3 | 3.9 to 6.3 | 16.0 |
| LPC | 4.6 | 1.0 to 7.6 | 13.4 |
| MPC | 5.7 | 3.3 to 6.5 | 13.1 |
| HPC | 3.9 | 3.2 to 4.4 | 17.7 |

Three levels of positive control samples were prepared in normal human serum at concentrations of 2000, 500 and 250 ng/mL and tested in three independent assays performed over two days by two analysts. The intra- and inter-assay precision observed was within 18%.

## Conclusions

- The commercial ELISA kit is able to detect anti-PEG IgM antibodies, but not anti-PEG-IgG.
- The assay, as adapted, shows acceptable sensitivity, precision and selectivity. It indicates that the kit is fit for the purpose of detecting IgM antibodies to PEG-TD in human serum.
- Drug tolerance data indicates that the assay has a good tolerance to free PEG, but not PEG-TD in human serum.

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