Challenges associated with the immunogenicity assessment of pegylated products

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1 ABSTRACT

PEGylation is the process of covalent attachment of polyethylene glycol (PEG) polymer chains to another molecule, such as a therapeutic protein. The covalent attachment of PEG can reduce immunogenicty and antigenicity and can also prolong drug circulation time by reducing renal clearance. The presence of pre-existing PEG antibodies is known in some patients following exposure to a variety of products. These antibodies can potentially impact the PK profile of drugs and increase the risk of infusion reactions. Even if anti-PEG antibodies remain a concern, their presence is not necessarily associated with adverse events.

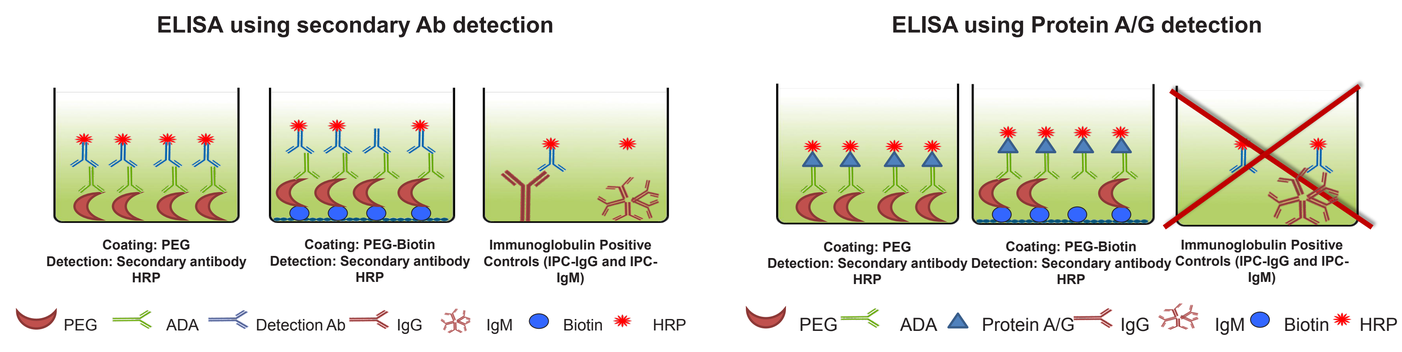
The diversity in size and physical properties of the PEG molecules makes it difficult to draw conclusions regarding the impact of PEGylation. When using anti-PEG assays, the observed incidence of antibodies may be influenced by several factors such as assay sensitivity. Therefore, in some instances, anti-PEG Abs may be present but not detected.

In this study, a direct ELISA format, in which the anti-PEG antibodies are captured with plate-bound PEG was developed. The detection reagents were by necessity isotype specific and required several rounds of optimization. Their performance was evaluated based on several parameters including sensitivity, dynamic range, and signal produced from IgM and IgG positive controls. The assay MRD was selected using a comparative approach allowing the generation of a provisional cut point. The evaluation of several additional parameters such as selectivity and drug tolerance were also evaluated. The direct ELISA format developed to detect anti-PEG antibodies proved to be reproducible, sensitive and drug tolerant.

2 ASSAY DESIGN

Due to the repetitive nature of the PEG, anti-PEG IgG antibodies bind to the repeating epitopes of a single chain of PEG resulting in the absence of bridging. However, the bridging may be possible for the IgM antibodies but the assay will be limited to the detection of anti-PEG IgM antibodies.

The assay preferred format selected was the Direct ELISA detected using species specific anti-isotype HRP antibodies or protein A/G HRP.



3 CASE STUDY RESULTS

- An Immunogenicity method was developed to detect antibodies to PEG induced by several pegylated compounds containing different PEG molecular weights (550 Da and 2000 Da)
- 2 PEG molecular weights were used in assay development to mimic the PEG linked to the different drug compounds
- The goal was to develop 1 single method that could be used with various pegylated compounds.

Selection of streptavidin assay plate

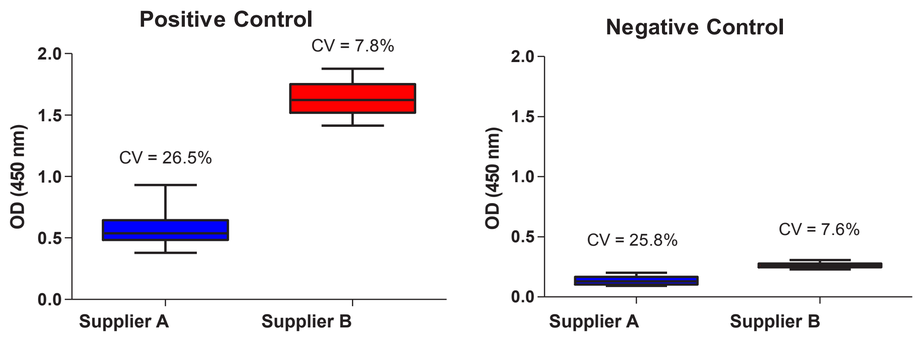


Figure 1: Difference in signal was observed between plates from different suppliers. Better signal with plates from supplier B and lower variability

Optimization of coating conditions

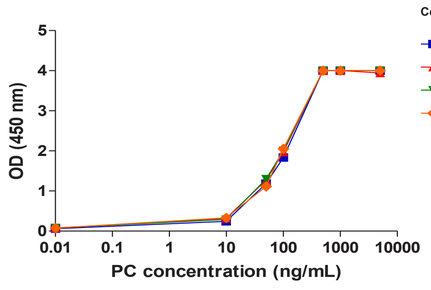


Figure 2: Similar signals with all concentrations of PEG tested at all PC concentrations. 1 µg/mL was selected as the optimal coating concentration

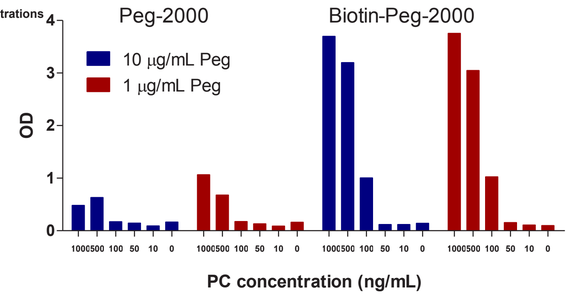


Figure 3: Biotinylated Peg was selected as optimal coating reagent since a stronger signal was observed and it was better binding/coating to the plate

Optimization of detection conditions

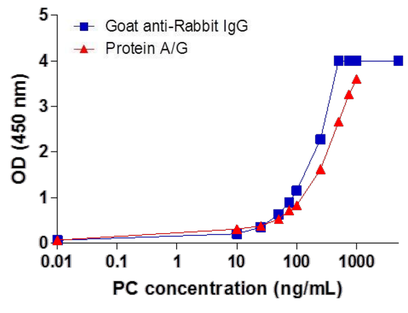


Figure 4: A slightly better detection was obtained with the rabbit PC when using goat anti-Rabbit IgG. However, good signal obtained with both detection reagents

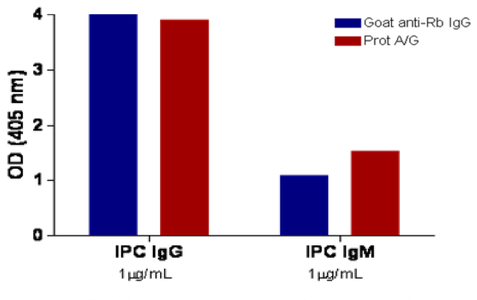


Figure 5: PC wells were coated with human IgG or IgM. Goat anti-human IgG+IgM and Protein A/G both detects Human IgG. Surprisingly Prot A/G detects IgM better than the goat anti-human IgG+IgM. As human IgG and IgM were better detected with the Prot A/G, this was selected as detection reagent

Sensitivity Assessment

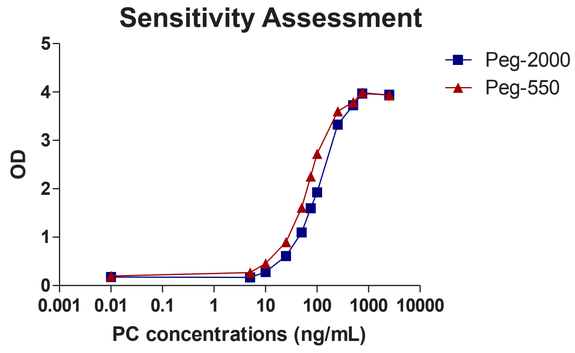


Figure 6: Similar sensitivity with both Peg biotinylated molecules

Binding of PC to PEGs of different length

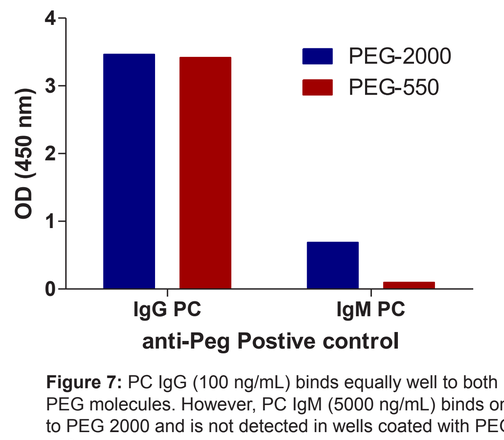


Figure 7: PC IgG (100 ng/mL) binds equally well to both PEG molecules. However, PC IgM (5000 ng/mL) binds only to PEG 2000 and is not detected in wells coated with PEG 550

Drug tolerance

Drug (ng/mL)	Positive Controls + Drug A				Positive Controls + Drug B				Positive Controls + Drug C			
	IgG PC: 250 ng/ml		IgG PC: 50 ng/ml		IgG PC: 250 ng/ml		IgG PC: 50 ng/ml		IgG PC: 250 ng/ml		IgG PC: 50 ng/ml	
	A_{450nm}	% Inhib	A_{450nm}	% Inhib	A_{450nm}	% Inhib	A_{450nm}	% Inhib	A_{450nm}	% Inhib	A_{450nm}	% Inhib
50,000	0.084	97.6	0.066	94.9	0.100	97.0	0.054	94.8	0.071	97.6	0.060	91.7
5,000	0.085	97.5	0.053	95.9	0.093	97.2	0.060	94.3	0.147	95.0	0.058	91.9
2,500	0.081	97.7	0.058	95.5	0.081	97.5	0.062	94.0	0.160	94.6	0.080	88.9
500	0.093	97.3	0.057	95.6	0.086	97.4	0.057	94.6	**0.279**	**90.6**	0.083	88.5
250	0.104	97.0	0.057	95.6	0.165	95.0	0.058	94.4	0.476	83.9	0.095	86.7
125	**0.420**	**87.9**	0.075	94.2	**0.384**	**88.4**	0.095	90.9	0.585	80.2	0.096	86.7
75	0.710	79.5	0.099	92.4	0.763	77.0	**0.140**	**86.6**	1.564	47.0	**0.163**	**77.3**
0	3.466	N/Ap	**1.306**	**N/Ap**	3.314	N/Ap	1.044	N/Ap	2.952	N/Ap	0.719	N/Ap

- **Table 1:** The drug tolerance for each compound was as follows:
- **Drug A**: 125 ng/mL tolerated at PC 250 ng/mL; no tolerance at PC 50 ng/mL
- **Drug B**: 125 ng/mL tolerated at PC 250 ng/mL; 75 ng/mL tolerated at PC 50 ng/mL
- **Drug C**: 500 ng/mL tolerated at PC 250 ng/mL; 75 ng/mL tolerated at PC 50 ng/mL
- Preliminary cut-point: **0.176**

Additional parameters tested prior to assay validation

Pre-validation parameters
Determination of preliminary CP and CCP
Intra and inter precision
Precision of titers
Prozone
Selectivity and specificity

4 CONCLUSION

- Screening and confirmation should be done using the whole PEGylated drug (CCP to be determined for each particular drug)
- Further characterization may be done by competition with the whole PEG molecule or the construct minus PEG
- Anti-PEG IgM may not be detected when smaller MW PEG is used for coating. Therefore, in our case, it was decided to only move forward with the PEG 2000
- CCP and drug tolerance need to be evaluated with each individual compound
- Anti-PEG antibody data are highly dependent on the selected assay format and the selection of critical reagents
- Standardization of the anti-PEG assays and the development of positive control antibodies are needed to have a good understanding of the real anti-Peg antibody incidence in the population