Poster# M8119

Pegfilgrastim Case Study: Bridging Immunoassay and Direct Drug Coating Immunoassay Fail to Detect All Anti-Drug Antibodies Against Pegylated Protein

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Purpose

Pegylation of therapeutic protein products has been widely used as PK enhancer for biotherapeutics. However, the immune responses to the polyethylene glycol (PEG) itself have been reported to cause loss of product efficacy and adverse safety consequences. Anti-PEG antibodies have also been found to be cross-reactive between pegylated products. It should also be noted that due to the ubiquitous presence of PEG in food products and cosmetics, it is expected that approximately 10-25% of the population will have preexisting antibodies. The detection and monitoring of ADAs against pegylated proteins is critical in understanding the safety and efficacy of pegylated biotherapeutics. The FDA and other global regulatory agencies require that for pegylated therapeutic protein products, the ADA assay should be able to detect both the anti-therapeutic protein antibodies and antibodies against the PEG moiety.

The most widely reported method for the detection of ADAs against pegylated protein is the bridging immunoassay. We demonstrate that the bridging immunoassay significantly underestimates anti-PEG backbone antibodies. The other method reported for the detection of ADAs against pegylated protein is the direct method where the drug is coated to the plate directly. We demonstrate that the direct coating of pegylated protein may mask the protein epitopes resulting in the under estimation of anti-protein specific antibodies.

Here we demonstrate that utilizing a semi-homogenous assay format is most suitable for the detection of both anti-PEG and anti-protein antibodies using pegfilgrastim as a case study.

Objective

The objective of the project is to evaluate various assay formats suitable for the reliable detection of antibodies against pegylated protein. Pegfilgrastim was used to illustrate the advantages and disadvantages of various assay formats routinely used for the detection of anti-drug antibodies.

Methods

Three method formats (bridging, direct and semi-homogenous) were used to investigate the detection of ADAs against anti-PEG and anti-protein antibodies. The bridging format involves the incubation of biotinylated pegfilgrastim and hapten-conjugated pegfilgrastim along with test samples. The immune complex is then captured on a streptavidin-coated plate and detected with HRP conjugated anti-hapten antibody.

The direct ELISA format involves coating a microtiter plate with pegfilgrastim. After coating and blocking steps, the samples containing ADAs are incubated on the plate. The captured ADAs are then detected with HRP-conjugated protein A/G or HRP conjugated anti-human IgG/IgM.

The semi-homogenous assay format involves incubating samples with biotin-pegfilgrastim, allowing the antibody binding with the pegfilgrastim molecule to occur in solution. The immune complex is then captured on a pre-blocked streptavidin coated plate. The bound complex is then detected with HRP-conjugated protein A/G.

For all the above described methods, the following positive controls were used to investigate the method performance: mouse anti-PEG (IgG and IgM) antibody, rabbit/mouse/goat anti-GCSF antibody and rabbit anti-pegfilgrastim antibody.

Results

The results demonstrate that bridging assay significantly underestimate the anti-PEG antibodies. It fails to detect the low level of both pre-existing antibodies and in-vitro prepared samples using anti-PEG antibody controls. While the sensitivity for PEG backbone IgM antibodies was 93 ng/mL, the IgG antibodies can only be detected above 800 ng/mL. An assay developed using IgM antibody as control, does not reflect the innate diversity of anti-PEG antibodies in human subjects and will most likely fail to detect low level of anti-PEG IgG antibodies.

The direct coating method significantly underestimates the anti-GCSF antibody. The sensitivity for the direct method was 2000 ng/ml at best using various anti-GCSF antibodies. We suspect that is due to the masking of GCSF epitope by PEG molecule on the ELISA plate.

The semi-homogenous assay format was tested using various both anti-PEG and anti-GCSF molecule. The assay was able to detect both antibodies in high sensitivity (less than 50 ng/mL). The assay was able to detect both anti-PEG backbone specific IgM and IgG in a comparable manner. Furthermore, when more than 300 normal human serum was screened for pre-existing antibodies, the semi-homogeneous assay has 97% sensitivity and 100% specificity. Direct assay has comparable results with 100% sensitivity and 94% specificity. In comparison, bridging assay has only 24% sensitivity and 100% specificity. Bridging assay failed to detect 76% of the positive anti-PEG antibody response.

Table 1: Evaluation of assay sensitivity and specificity using 300 human serum lots

	Direct		Semihomogenous		Bridging	
	Positive	Negative	Positive	Negative	Positive	Negative
Results- Positive	74	13	72	0	18	0
Results Negative	0	213	2	226	56	226
Sensitivity	100%		97%		24%	
Specificity	94%		100%		100%	

Figure 1: Signal to Noise ratio for 6 different antibodies at 100 ng/mL in three assay formats

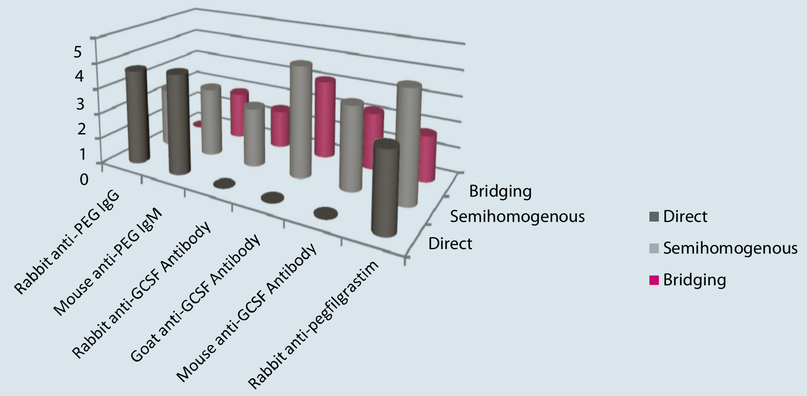


Figure 2: Example data using direct assay format

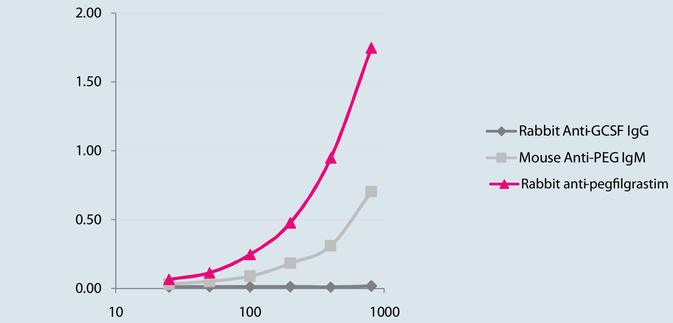


Figure 3: Example data using bridging assay format

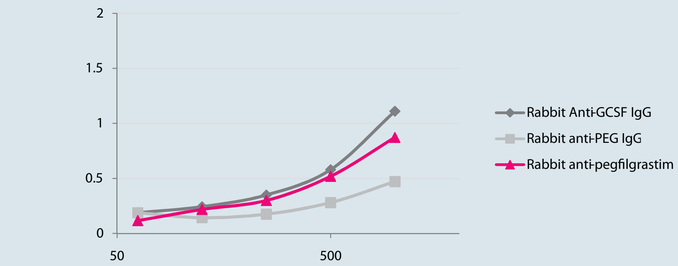
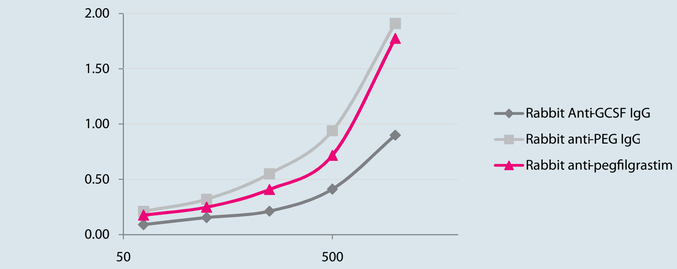


Figure 4: Example data using semihomogenous format



Conclusion

Based on the above data we concluded that the semi-homogenous assay format is most suitable for the detection of anti-PEG and anti-protein antibodies to support the clinical development of pegylated proteins. We have tested two additional pegylated proteins with 20kD PEG in addition to pegfilgrastim and achieved similar results. We plan to investigate additional pegylated proteins with varying sizes of proteins and PEG molecule in semi-homogeneous ADA assays to confirm the suitability of this assay.

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