W4285

Evaluation and Validation of a Commercial ELISA Kit for the Detection of Antibodies to Pegylated Therapeutic Drug (Pegfilgrastim) in Human Serum for Use in Support of Clinical Studies

J. Thompson and R. Islam
Celerion, Lincoln, NE USA

celerion
Applied Translational Medicine

Background

Biologic drugs commonly elicit immune responses, resulting in anti-drug antibodies. Formation of anti-drug antibodies can have a number of effects such as reduced efficacy, rapid clearance etc., on the therapeutic outcome of a biologic. A number of techniques have been developed to try to address the issue of immunogenicity from therapeutic proteins. One such technique involves pegylation of proteins. In general, pegylation of proteins prolongs the half-life in the circulatory system and reduces its immunogenicity. However, pegylation of therapeutic proteins poses bioanalytical challenges due to the presence of pre-existing anti-PEG antibodies.

Pegfilgrastim is a PEGylated form of filgrastim, a recombinant human granulocyte colony-stimulating factor (GCSF). The PEGylation of the molecule (with the n-terminus addition of 20 kDa polyethylene glycol) extends the half-life of the protein from 3-4 hr to 15-80 hr. The use of pegfilgrastim treatment serves to stimulate the bone marrow to produce more white blood cells (neutrophils) to help fight infection in patients undergoing chemotherapy.

Here we present a direct ELISA method based on a commercial kit that is capable of detecting anti-drug, anti-PEG and anti-protein IgG and IgM antibodies to a pegylated filgrastim. This method was rapidly developed by testing crucial parameters in a limited number of comprehensive experiments.

Methods

A commercial pegfilgrastim antibody ELISA kit from Somru BioScience was evaluated for the qualititative determination of anti-pegfilgrastim antibodies in human serum. The kit utilizes a direct ELISA design where the antigen, pegfilgrastim, is coated to the microtiter plate and anti-drug antibodies (ADA) are detected using a cocktail of anti-human IgG/IgM and anti-species antibodies with matching positive controls (Figure 1). Due to the multimeric nature of the drug, direct ELISA was found to be more appropriate for this assay, as opposed to the bridging approach. Assay parameters evaluated included screening cut point, confirmatory cut point, hook effect, lower limit of reliable detection (LLRD), drug tolerance, precision, and specificity.

Screening cut point was determined by using 32 individual normal human sera. Data distribution was investigated using the Shapiro-Wilk test for normality. Non-normality was found for all three runs. Accordingly, log transformation was applied and data distribution was rechecked and confirmed as normal. We calculated the median signal of the samples, and the median absolute deviation, which were used to establish an assay run specific cut point with a false positive rate of 5% (Figure 2).

All pre-existing antibodies detected during the screening assay were confirmed to be specific to PEG (Figure 3). Sensitivity was obtained by interpolating antibody concentration at the cut point from a curve (Figure 4). Assay precision and titration characteristics of the positive controls were evaluated using rabbit anti-pegfilgrastim antibodies at various levels.

Results

Assay screening and confirmation cut points for anti-filgrastim, anti-PEG and anti-pegfilgrastim were determined using robust statistical methods. All assays displayed acceptable precision (less than 20% CV). The statistical analysis supports assigning a unique floating cut point value for each plate. Sensitivity data obtained indicates that the assay detection limit is 93 ng/mL of equivalent rabbit anti-pegfilgrastim antibody. Selectivity in normal human serum, found to be acceptable with all spiked samples, is positive at 100 ng/mL and all unspiked samples shows response below assay cutpoint. The drug tolerance data shows that anti-drug antibodies could be detected in the presence of >10.0 mcg/mL of pegfilgrastim (Figure 5). No hook effect was observed for up to 10 mcg/mL of positive control (Figure 6).

Figure 1. Assay Design. Drug is coated on microtiter plate, followed by binding of serum antibodies, which are detected using HRP-conjugated secondary antibodies. Optical density is read on a spectrophotometer at a wavelength of 450 nm with a correction wavelength of 620 nm.

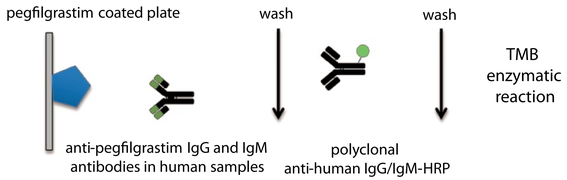


Figure 2. Screening Cut Point. A Shapiro-Wilk test was performed to identify outliers in initial screening of normal human serum samples for pegfilgrastim ADA. After outlier elimination, validation screening cut point (vCP) was determined on the remaining samples using a robust parametric approach.

	run 1	run 2	run 3	avg
median	0.084	0.076	0.069	
MAD	0.086	0.086	0.097	
vCP	0.227	0.218	0.229	0.224

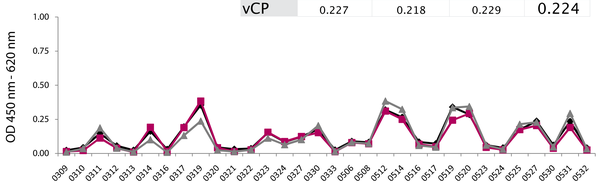


Figure 3. PEG Inhibition. To show that high OD values found in initial screening of some samples were PEG-specific, samples were incubated in the presence of PEG prior to addition to microtiter plates, resulting in a loss of signal.

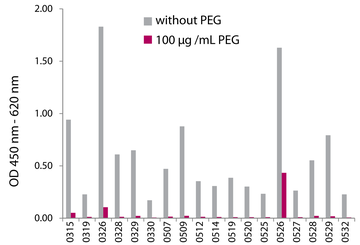


Figure 4. Lower Limit of Reliable Detection (LLRD).

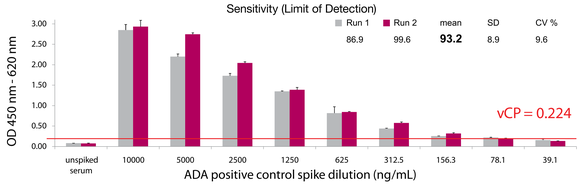


Figure 5. High Dose Hook Effect. Positive control ADA was spiked into pooled negative serum at various concentrations. OD values plotted against spike concentration are represented as a 4-parameter regression.

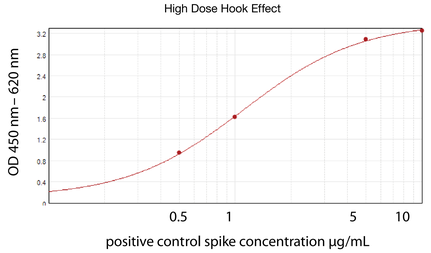
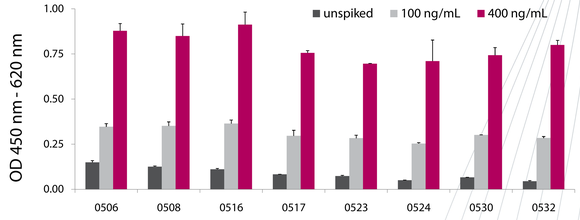


Figure 6. Spike and Recovery. Samples which screened negative for pegfilgrastim ADA were spiked with pegfilgrastim at either the LLRD (100 ng/mL) or 4 times the LLRD (400 ng/mL).



Conclusions and Future Work

A direct ELISA method was successfully optimized to detect anti-PEG, anti-filgrastim and anti-pegfilgrastim IgG and IgM antibodies. The assay shows acceptable sensitivity, precision and selectivity. Drug tolerance data indicates that the kit-based method is fit for the purpose of detecting antibodies to pegfilgrastim in human serum. The method can be validated as per industry best practices to support clinical studies.

References

1. Shankar G, “Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products”. J Pharm Biomed Analysis 48, 1267-1281 (2008)
2. FDA Guidance 2009 “Assay Development for Immunogenicity Testing of Therapeutic Proteins”.

Acknowledgement

The authors acknowledge the contribution of Somru BioScience Inc. for providing pegfilgrastim ELISA kits, positive controls and other critical reagents.

www.celerion.com