Poster# T1230-03-019

Development and Validation of an ELISA Method for the Determination of Anti-Polyethylene Glycol (PEG) Antibodies in Human Serum

K. Xing, C. Sheldon, R. Islam
Celerion Inc.

aaps PharmSci 360
ADVANCING PHARMACEUTICAL SCIENCES, CAREERS, AND COMMUNITY

PURPOSE

Pegylation is a well-documented modification used to diminish the proteins' immunogenicity and has been widely used as PK enhancer for biotherapeutics. However, in contrast to the generally accepted assumption that polyethylene glycol (PEG) is non-immunogenic and non-antigenic, the immune responses to the PEG itself have been reported to cause loss of product efficacy and adverse safety consequences. Thus, screening and monitoring the anti-PEG antibodies are critical in understanding the safety and efficacy of the pegylated biotherapeutics. FDA and other global regulatory agencies require that for pegylated therapeutics, the ADA assay should be able to detect both the anti-therapeutic antibodies and antibodies against the PEG moiety. For this purpose, we developed and validated a direct ELISA method for the determination of anti-PEG antibodies in human serum.

Table 1. Challenges and Solutions

Challenges	Solutions
The presence of pre-existing anti-PEG antibodies in the normal human serum	Screening human serum for cut point assessment with positive samples being removed
The underestimation of anti-PEG IgG with bridging assay because of the presence of repeating epitopes in a single chain of PEG	Developing a direct ELISA to detect all anti-PEG antibodies
The underestimation of human anti-PEG IgM due to its weekly binding to protein A/G	Detecting all antibodies with anti-human (IgG + IgM + IgA) antibody
Lack of human anti-PEG antibodies for positive control	Utilizing both positive human serum and mouse anti-PEG antibodies for positive controls
Dual detection reagents	Utilizing the combined reagents optimized by the vendor by removing the cross-reactivity

OBJECTIVE

The objective of this project is to develop and to validate a direct ELISA method for the determination of anti-PEG antibodies in human serum by overcoming all challenges listed above.

METHODS

Serum anti-PEG antibodies are detected using a direct ELISA. Diluted serum samples were added to a microplate, which is coated with streptavidin followed by the binding with biotinylated PEG to streptavidin. The wells were washed to remove any unbound sample material and enzyme-labeled antibodies were added. Unbound enzyme-labeled antibodies were removed and a chromogenic substrate was added. The development of the colored reaction product was directly proportional to the amount of anti-PEG antibodies present in the sample. The microplate was then analyzed using a colorimetric plate reader. The method was thoroughly validated by determining the screening cut points and confirmatory cut points using 50 drug naïve normal human sera, calculating the assay sensitivity, and evaluating the matrix effect and free drug interference.

RESULTS

The anti-PEG antibodies detection immunoassay was successfully developed and validated. Assay screening cut points and confirmation cut points were determined using robust statistical methods. The assay displayed acceptable precision. The sensitivity of the screening assay and the confirmatory assay were 44.5 and 49.6 ng/mL, respectively. Selectivity in normal human serum found to be acceptable with 8 out of 10 spiked samples (at 128 ng/mL) showed response greater than rCP in screening assay and 9 of the same spiked samples showed % inhibition greater than iCP, while 1 out of 10 un-spiked samples detected as positive, suggesting the presence of pre-exiting anti-PEG antibodies. The drug tolerance data shows that anti-PEG antibodies at 128 ng/mL could be detected in the presence of 12.8 ng/mL of 20 kDa PEG. No hook effect was observed for up to 10 μg/mL of positive control.

Table 2. Validation Summary

Performance Characteristic	Results
Sensitivity	Screening: 44.5 ng/mL Confirmatory: 49.6 ng/mL
Cut Point Assessment	Screening: Correction Factor : 1.6728 (multiplicative) Confirmatory Cut Point: 34.9% inhibition
Precision	Intra-assay : 0.4 to 8.5 % CV Inter-assay : 18.0 to 42.8 % CV
Selectivity (Matrix Effect)	Interference by Matrix Components, Normal Human Serum: The degree of matrix variability was accceptable. For the screening assay, • 9 out of 10 unfortified samples scored negative; • 8 out of 10 low fortified samples scored positive; • 10 out of 10 low fortified + 50% samples scored positive; • 10 out of 10 high fortified samples scored positive. For the confirmatory assay, • 9 out of 10 unfortified samples scored negative; • 9 out of 10 low fortified samples scored positive; • 9 out of 10 low fortified + 50% samples scored positive; • 10 high fortified samples were confi rmed positive.
Target Tolerance	128 ng/mL of positive control anti-PEG antibodies can reliably be detected when less than 12.8 ng/mL PEG is present.

Table 3. Intra-Assay Precision of Controls

	NegC 0 ng/mL	HPC 1600 ng/mL			LPC 128 ng/mL			HSPC N/AP		
	Replicate	Mean	Replicate	Mean	% Inhibition	Replicate	Mean	% Inhibition	Replicate	Mean	% Inhibition
	0.1250	2.0195	0.2114	89.5	0.3020	0.1441	52.3	0.6697	0.1351	79.8
	0.1077	1.9279	0.2073	89.2	0.2814	0.1254	55.4	0.5609	0.1137	79.7
	0.1117	1.7729	0.1799	89.9	0.2556	0.1344	47.4	0.5506	0.1245	77.4
	0.1190	1.8280	0.1885	89.7	0.2499	0.1413	43.5	0.5790	0.1222	78.9
	0.1155	1.7995	0.1765	90.2	0.2715	0.1298	52.2	0.5677	0.1157	79.6
	0.1079	1.7481	0.1857	89.4	0.2519	0.1279	49.2	0.5465	0.1154	78.9
Mean	0.1144	1.8493	0.1915	89.6	0.2687	0.1338	50.0	0.5790	0.1211	79.1
SD	0.0068	0.1041	0.0145	0.3	0.0204	0.0075	4.2	0.0459	0.0080	0.9
% CV	5.9	5.6	7.6	0.4	7.6	5.6	8.5	7.9	6.6	1.2

Table 4. Matrix Effect

Uninhibited Samples

		Unspiked		Low Spike 128 ng/mL		Low Spike + 50% 192 ng/mL		HPC Spike 1600 ng/mL	
Batch #	Lot #	Mean Response OD	Negative or positive	Mean Response OD	Negative or positive	Mean Response OD	Negative or positive	Mean Response OD	Negative or positive
33	1	0.1562	Negative	0.3226	Positive	0.3825	Positive	2.1032	Positive
	2	0.0945	Negative	0.2651	Negative	0.3722	Positive	1.9969	Positive
34	3	0.0772	Negative	0.2163	Negative	0.2831	Positive	1.6091	Positive
	4	0.1716	Negative	0.4085	Positive	0.4544	Positive	2.1291	Positive
	5	0.1626	Negative	0.2977	Positive	0.3658	Positive	1.5793	Positive
	6	0.1445	Negative	0.3398	Positive	0.4750	Positive	1.9656	Positive
35	7	0.1122	Negative	0.2298	Positive	0.2859	Positive	1.5679	Positive
	8	0.1174	Negative	0.3021	Positive	0.3706	Positive	1.4666	Positive
	9	0.0930	Negative	0.2258	Positive	0.2753	Positive	1.6393	Positive
	10	0.1517	Positive	0.2693	Positive	0.3015	Positive	1.4423	Positive
33	CMP	0.1431	Negative	0.3819	Positive	0.4778	Positive	2.0325	Positive
33	Buffer	0.0163		0.2355		0.3148		2.1045	
Acceptance Range:									
rCP, #33		0.2668							
rCP, #34		0.2333							
rCP, #35		0.1510							

Inhibited Samples

		Unspiked			Low Spike 128 ng/mL			Low Spike + 50% 192 ng/mL			HPC Spike 1600 ng/mL		
Batch #	Lot #	Mean Response OD	% Inhibition	Accepted or not	Mean Response OD	% Inhibition	Accepted or not	Mean Response OD	% Inhibition	Accepted or not	Mean Response OD	% Inhibition	Accepted or not
33	1	0.1528	2.1	Negative	0.1518	53.0	Positive	0.1568	59.0	Positive	0.2517	88.0	Positive
	2	0.0952	-0.7	Negative	0.1115	57.9	Positive	0.1049	71.8	Positive	0.2012	89.9	Positive
34	3	0.0795	-3.0	Negative	0.0909	58.0	Positive	0.1064	62.4	Positive	0.2410	85.0	Positive
	4	0.1497	12.8	Negative	0.2051	49.8	Positive	0.2045	55.0	Positive	0.3519	83.5	Positive
	5	0.1475	9.3	Negative	0.1446	51.4	Positive	0.1539	57.9	Positive	0.3131	80.2	Positive
	6	0.1224	15.3	Negative	0.1477	56.5	Positive	0.1546	67.4	Positive	0.2752	86.0	Positive
35	7	0.1081	3.6	Negative	0.1459	36.5	Positive	0.1655	42.1	Positive	0.6260	60.1	Positive
	8	0.0871	25.9	Negative	0.1781	41.0	Positive	0.2107	43.1	Positive	0.6116	58.3	Positive
	9	0.0835	10.2	Negative	0.1493	33.9	Negative	0.1802	34.5	Negative	0.6886	58.0	Positive
	10	0.0947	37.6	Positive	0.1511	43.9	Positive	0.1724	42.8	Positive	0.4374	69.7	Positive
33	CMP	0.1269	11.4	Negative	0.1522	60.2	Positive	0.1629	65.9	Positive	0.2273	88.8	Positive
33	Buffer	0.0179	-9.5		0.0364	84.5		0.0487	84.7		0.2458	88.3	
Acceptance Range													
iCP			34.9										

CONCLUSION

- A direct ELISA method has been successfully developed and validated by overcoming the challenges to detect both anti-PEG IgG and IgM antibodies in human serum.
- The assay shows acceptable sensitivity, precision, selectivity and drug tolerance.
- This assay can be validated as per industry best practices to support clinical studies for pegylated biotherapeutics.

ACKNOWLEDGEMENT

The authors acknowledge the contribution of Somru BioScience Inc. for providing critical reagents and technical supports.

celerion
Translating Science to Medicine