Poster#
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Evaluation of an ELISA method using novel amplification reagent and its comparison with traditional colorimetric ELISA assay for the measurement of Aflibercept in biological matrices

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INTRODUCTION

Aflibercept is a recombinant fusion protein consisting of vascular endothelial growth factor (VEGF)-binding portions from the extracellular domains of human VEGF receptors 1 and 2 fused to the Fc portion of the human IgG1 immunoglobulin.

Aflibercept (Eylea®) is used for the treatment of wet macular degeneration and is administered as an intravitreal injection, that is, into the eye. Ziv-aflibercept (Zaltrap®) is used for cancer treatment, is given intravenously in combination with the other cancer drugs 5-fluorouracil and irinotecan and the adjuvant folinic acid. In addition aflibercept was also indicated for the treatment of patients with visual impairment due to diabetic macular edema.

Systemic exposure to aflibercept is relatively low. In addition there are some novel delivery mechanisms that can deliver low amount of aflibercept in a consistent manner. This requires a highly sensitive method. In addition, some of the patients in clinical trial may be in other anti-VEGF therapy requiring highly specific bioanalytical method that can measure aflibercept without interference from other anti-VEGF therapy.

Here we report a sensitive, robust ELISA method using routine colorimetric detection for the measurement of aflibercept in plasma using highly specific antibody. In addition we also developed a method using unique amplification reagent and were able to improve sensitivity by 3 fold compare to colorimetric method.

METHODS

The method is based on the ELISA kit developed by Somru Bioscience for the measurement of aflibercept in human plasma. Briefly, rhVEGF is coated to the ELISA plate, after blocking the plate samples containing aflibercept is added. Aflibercept is captured by rhVEGF and a Goat anti-aflibercept antibody is used for detection (Figure 1). Goat polyclonal antibody was generated against a unique portion of the aflibercept so that the detection antibody does not bind to other anti-VEGF therapeutic molecules.

For high sensitivity method we utilized quantum dot fluorescent beads. The beads contain hundreds of quantum dots which offer high luminescence with great long-term colloidal stability. Anti-goat antibodies were conjugated to the surface of the beads (Figure 2). This allows us to amplify signal significantly and improve sensitivity without much optimization.

RESULTS

The results from qualification experiments for colorimetric ELISA indicate that the assay is "validatable" and meets FDA Bioanalytical Guidance for pharmacokinetic assays and industry best practices. The accuracy and precision were within ± 20% (25% at LLOQ). The selectivity was evaluated in 9 normal plasma. The recovery at 0.8 ng/mL was within ± 25% for 8 out of 9 matrices. The range of 0.8-25,000 pg/mL was found to be sufficient to measure clinical samples to support pharmacokinetic of the assay. rhVEGF was found not to interfere with the assay up to at 10,000 pg/mL which are well above the normal physiological concentrations of VEGF.

The high sensitivity method was able to improve sensitivity by approximately 3 fold (0.25 ng/mL). The assay ranges from 0.25-20 ng/mL. The selectivity as evaluated in 10 AMD patient plasma including hemolyzed and lipaemic plasma. The recovery was within 25% for all lots.

CONCLUSION

We have developed a sensitive and highly specific method for the measurement of aflibercept in biological matrices that is free from interference from target ligand (hVEGF) and other anti-VEGF therapy. We also demonstrated utility of novel plug-n-play signal amplifier that can improve sensitivity by 3 fold. The assay is found to be suitable for the measurement of aflibercept and its biosimilar in clinical samples to support biosimilar development.

Table 1: Colorimetric ELISA - Accuracy and Precision

Batch	LLOQ QC	ULOQ QC	QC A	QC B	QC C
			Concentration (ng/mL)		
	0.8	2	8	16	25
1	*	1.37	6.45	11.44	17.95
	0.332a	1.46	6.28	10.99	18.08
	0.175a	1.44	5.87	10.97	18.08
	0.44	1.57	7.80	14.33	21.67
	0.73	1.74	7.85	14.11	21.52
	0.60	1.62	6.83	13.72	20.53
Intra-Batch Mean	0.59	1.53	6.85	12.59	19.64
Intra-Batch SD	0.15	0.14	0.82	1.62	1.80
Intra-Batch % CV	25	9	12	13	9
Intra-Batch % Bias	-26	-23	-14	-21	-21
n	3	3	3	3	3
2	0.75	1.92	7.28	12.48	21.20
	0.77	1.90	6.95	12.82	21.65
	0.81	1.91	7.75	12.66	21.71
	0.95	2.22	7.83	13.33	27.28
	1.09	2.31	8.20	16.60	24.35
	1.11	2.63	9.08	14.49	24.66
Intra-Batch Mean	0.92	2.15	7.85	13.73	23.48
Intra-Batch SD	0.16	0.29	0.75	1.58	2.38
Intra-Batch % CV	17	14	10	12	10
Intra-Batch % Bias	14	7	-2	-14	-6
n	3	3	3	3	3
3	0.70	1.73	7.76	14.01	22.65
	0.46	1.82	7.01	14.56	22.43
	0.79	1.87	7.75	14.00	23.38
	0.86	2.64	9.27	16.88	24.64
	0.80	2.22	8.51	17.47	24.99
	0.89	3.21	9.25	18.36	25.61
Intra-Batch Mean	0.75	2.25	8.26	15.88	23.95
Intra-Batch SD	0.16	0.58	0.91	1.92	1.32
Intra-Batch % CV	21	26	11	12	5
Intra-Batch % Bias	-6	12	3	-1	-4
n	3	3	3	3	3
Inter-Batch Mean	0.75	1.98	7.65	14.07	22.35
Inter-Batch SD	0.16	0.39	0.73	1.67	2.36
Inter-Batch % CV	22	20	9	12	11
Inter-Batch % Bias	-6	-1	-4	-12	-11
n	3	3	3	3	3

* - Out of range
a- excluded from calculation

Table 2: Colorimetric ELISA - Matrix Effect

Serum Lot#	Un-spiked	Concentration 2 ng/ml	% Deviation	Concentration 16 ng/ml	% Deviation
1	BLQ	1.82	-9	16.24	2
2	BLQ	1.67	-17	14.72	-8
3	BLQ	1.76	-12	13.57	-15
4	BLQ	1.89	-5	14.81	-7
5	BLQ	1.58	-21	14.47	-10
6	BLQ	1.62	-19	13.37	-16
7	BLQ	1.84	-8	16.65	4
8	BLQ	1.78	-11	15.12	-6
9	BLQ	5.251*	163	15.19	-5
	Mean	1.74		14.90	
	%CV	6.41		7.26	
	%Theoretical	87.24		93.15	
	n	8		9	

* Excluded from calculation
BLQ= Below the limit of Quantitation

Table 3: QD ELISA - Accuracy and Precision

Batch	LLOQ QC	ULOQ QC	QC A	QC B	QC C
			Concentration (ng/mL)		
	0.25	0.75	6	16	20
	0.25	0.75	6	16	20
1	0.22	0.72	6.15	13.23	18.88
	0.19	0.74	7.45	14.02	17.11
	0.20	0.95	6.95	14.98	19.45
Intra-Batch Mean	0.20	0.80	6.85	14.08	18.48
Intra-Batch SD	0.02	0.13	0.66	0.88	1.22
Intra-Batch % CV	8	16	10	6	7
Intra-Batch % Bias	-19	7	14	-12	-8
n	3	3	3	3	3
2	0.23	0.89	7.11	12.88	16.98
	0.27	0.70	7.01	15.87	24.78*
	0.29	0.73	6.08	15.87	18.05
Intra-Batch Mean	0.26	0.77	6.73	14.87	17.52
Intra-Batch SD	0.03	0.10	0.57	1.73	0.76
Intra-Batch % CV	12	13	8	12	4
Intra-Batch % Bias	5	3	12	-7	-12
n	3	3	3	3	3
3	0.27	0.55	6.66	14.78	20.65
	0.29	0.72	6.01	14.02	19.43
	0.25	0.69	6.11	14.61	19.70
Intra-Batch Mean	0.27	0.65	6.26	14.47	19.93
Intra-Batch SD	0.02	0.09	0.35	0.40	0.64
Intra-Batch % CV	7	14	6	3	3
Intra-Batch % Bias	8	-13	4	-10	0
n	3	3	3	3	3
Inter-Batch Mean	0.25	0.74	6.61	14.47	18.64
Inter-Batch SD	0.04	0.08	0.31	0.40	1.21
Inter-Batch % CV	15	11	5	3	7
Inter-Batch % Bias	-2	-1	10	-10	-7
	3	3	3	3	3

Table 4: QD ELISA – Matrix Effect

Serum Lot#	Un-spiked	Concentration 0.25 ng/ml	% Deviation
AMD 1	BLQ	0.28	12
AMD 2	BLQ	0.23	8
AMD 3	BLQ	0.26	4
AMD 4	BLQ	0.25	0
AMD 5	BLQ	0.31	24
AMD 6	BLQ	0.22	12
AMD 7	BLQ	0.24	4
AMD 8	BLQ	0.28	-12
AMD 9	BLQ	0.26	-4
AMD 10	BLQ	0.28	12
Hemolyzed 1	BLQ	0.29	16
Hemolyzed 2	BLQ	0.26	3
Lipaemic 1	BLQ	0.26	5
Lipaemic 2	BLQ	0.22	-10
	Mean	0.26	
	%CV	8.22	
	%Theoretical	13.24	
	n	8	

BLQ= Below the limit of Quantitation

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