Development of an Acid Dissociation Bridging Elisa Method for Determination of Anti Bevacizumab Antibodies

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clinical ethical research

Objective: An acid dissociation bridging ELISA method for detecting antibodies against Bevacizumab has been developed and optimized.

Methodology:

Biotin labeled bevacizumab was pipetted into the micro plate wells previously coated with bevacizumab. Simultaneously, Diluted serum samples (diluted with 0.3 M acetic acid) were pipetted into the wells and the acid treated samples were then neutralized with 1 M Tris buffer, pH 9.5. The plate was then incubated overnight to allow formation of a bridging immune complex. HRP labeled streptavidin was then added to the washed microplate. Unbound streptavidin-HRP was removed and a chromogenic substrate added, resulting in development of the colored reaction product, which was proportional to the amount of ADA present in serum.

Analytical method summary:

Analyte: Anti-Bevacizumab monoclonal antibody

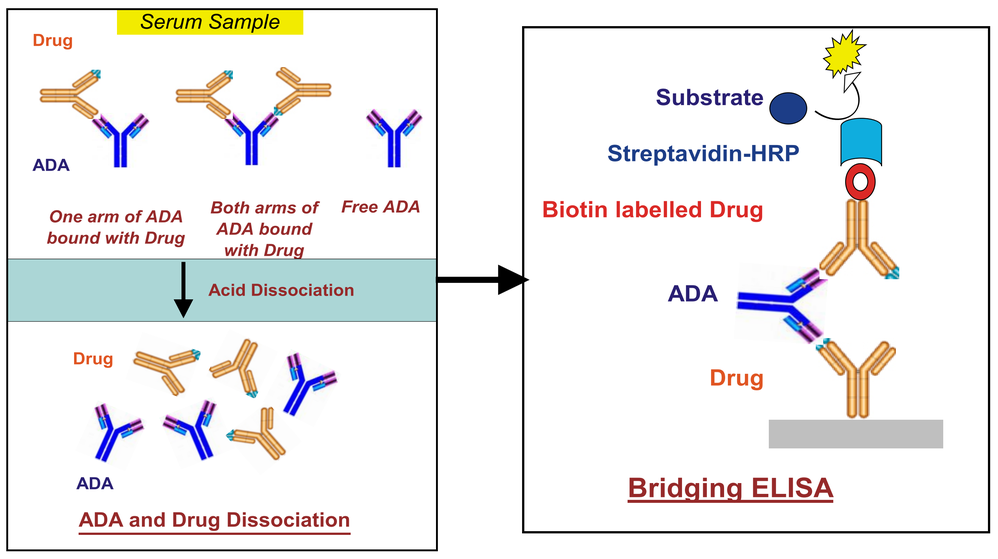
Method: Acid dissociation bridging ELISA

Quality controls (ng/ml): 5500 (HQC), 450 (LQC)

Assay volume: 50 ul

Sample preparation method: 1:10 Dilution with 0.3 M glacial acetic acid for acid dissociation of sample.

Matrix: Human Serum



Stability

Antibody Conc. (ng/mL)	Bench Top Stability at RT after 19 Hrs. (% bias)	Freeze & Thaw cycles (four) (% bias)
450.0 (LQC)	6.1	-4.8
5500 (HQC)	-0.5	2.9

Short term stability of anti-bevacizumab antibody in serum was established for 19 hours. Freeze (-20° c) and thaw (ambient temperature) stability of the antibody in serum was established for four freeze thaw cycles.

Precision

Antibody conc. (ng/mL)	Intra-assay precision (%CV)	Inter-assay precision (%CV)
450.0 (LQC)	10.0	11.6
5500 (HQC)	4.5	9.2
NQC	8.0	20.0

For ADA assay, intra and inter batch precision (%CV) of QC samples was 4.5-10% and 9.2-20%.

Selectivity (LQC):

Antibody conc. 450 ng/mL (LQC)	
Serum type / ID	% Bias
Lipemic	-12.663
551 *	-7.399
556 *	-9.856
739 *	-6.234
744 *	-11.095
746 *	-8.632

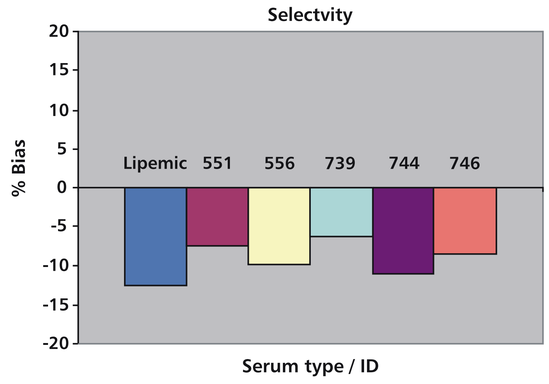


Fig: The selectivity was established for six different serum lots including lipemic for low positive control

Drug Tolerance:

Antibody conc. (ng/mL)	Drug conc. (ug/ml)	Mean response (OD)
5500	0	2.111
5500	1	0.809
5500	3	0.428
5500	5	0.293
5500	10	0.116
5500	20	0.05

Antibody conc. (ng/mL)	Drug conc. (ug/ml)	Mean response (OD)
450	0	0.176
450	1	0.082
450	3	0.048
450	5	0.035
450	10	0.021
450	20	0.015

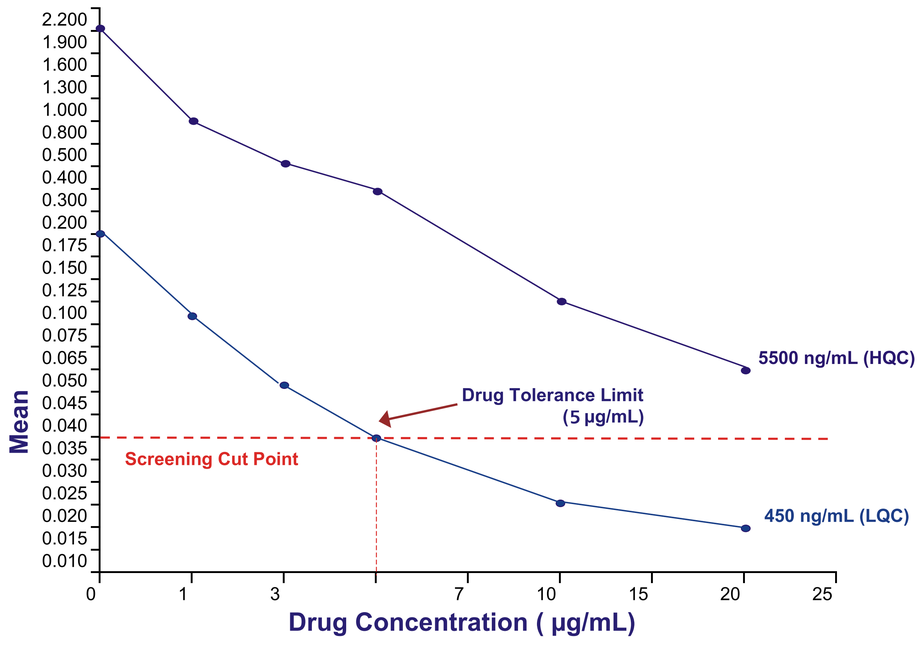


Fig: The drug tolerance limit was determined to be 5 ug/mL for this method since at this drug concentration, the low positive control (LQC) produced a response that is above screening cut point (SCP). On the other hand, HQC generated response that remains above SCP even at a drug concentration as high as 20 ug/mL.

Sensitivity:

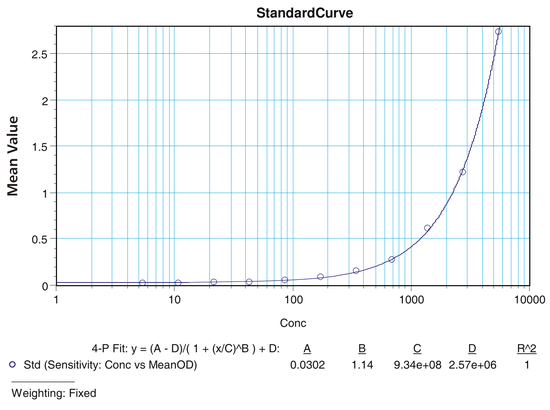


Fig: Representative dilution curve of the positive control anti Bevacizumab antibody. Dilution curves were fitted by the 4 – PL curve fitting method and the corresponding concentration at the screening cut point value were interpolated.

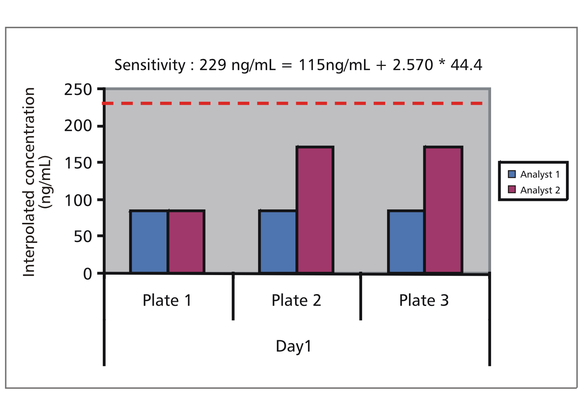


Fig: Interpolated antibody concentration at the screening cut point compared to sensitivity calculated for a 95% consistency level (Sensitivity = Mean interpolated concentration at cut point + t0.05, df * SD)

Titration Assay:

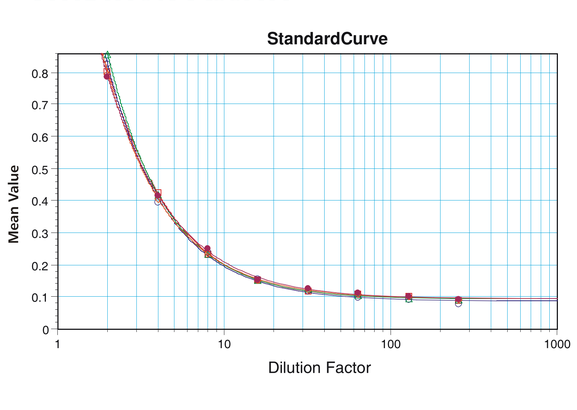


Fig: Representative titration assay curve of five mock ADA positive samples.

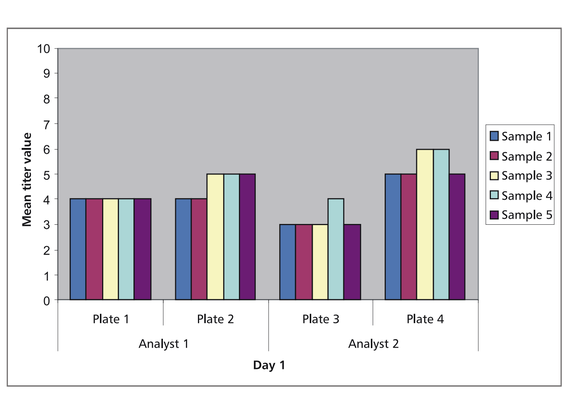


Fig: Mean titer value of five mock positive ADA samples analyzed across four assay plates by two analysts on a single day. Titer is expressed as log of dilution factor.

Conclusion: A robust, sensitive and specific elisa based method has been developed and optimized for detection and quasi-quantitative estimation of Anti-Bevacizumab antibodies in human serum samples.

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