Overcoming Target Interference in Canine Plasma During Development of an ADA Assay to Support a Biotherapeutic for Treatment of Canine Chronic Kidney Disease

Lisa Dewey[1], Carla Mejia[1], Amanda Hays[1] Bernard Hummel[2]
[1]BioAgilytix Labs, Durham, NC, USA
[2]Zoetis, Kalamazoo, MI, USA

BioAgilytix

zoetis

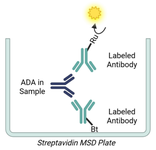
PURPOSE

Canine chronic kidney disease (CKD) is a progressive and irreversible condition characterized by the gradual loss of kidney function over time. CKD is generally associated with fibrosis and inflammation leading to tubulointerstitial and segmental kidney fibrosis, ultimately resulting in the slow loss of nephron function in the kidney. A biotherapeutic was developed to treat CKD in canines using a monoclonal antibody that targets a signaling molecule implicated in the progression of chronic kidney disease in canines. An anti-drug antibody (ADA) assay was developed to detect anti-drug antibodies in canine plasma to support drug development studies. The assay required optimization to mitigate target interference (≤10 ng/mL) and drug tolerance.

METHODS

ADA Assay Set up – Bridging Format

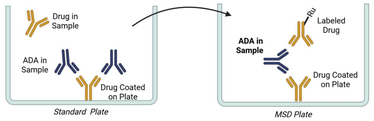
The bridging ADA assay was developed by labeling the monoclonal antibody drug with biotin and ruthenium. To evaluate interference, a competitive antibody was added to the neutralization step of the bridging assay to reduce target interference in matrix.



Streptavidin MSD Plate

ADA Assay Set up – ACE Format

The ADA assay to detect anti-drug antibodies to this monoclonal antibody biotherapeutic in canine serum was developed with an affinity capture elution (ACE) format using drug to capture the ADA with samples diluted to a 1:60 minimum required dilution (MRD) overnight. The following day the ADAs were eluted and added directly to a standard MSD plate and detected with ruthenylated drug.



Standard Plate *MSD Plate*

Images created with Biorender

RESULTS

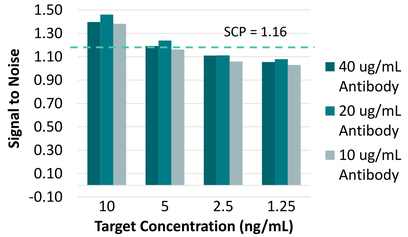
1) Target Interference Evaluation with Competitive Antibodies in Master Mix

Target interference was assessed with samples spiked with target protein. Samples were diluted to MRD20 in assay buffer with and without two different competitive antibodies that were added to the screening and confirmatory master mix. The preliminary assay cut point was determined to be 1.16 (screening) and 27.1% (confirmatory).

	10 µg/mL Competitive Antibody #1						10 µg/mL Competitive Antibody #2					
	Screening			*Confirmatory*			*Screening*			*Confirmatory*		
Target (ng/mL)	Mean Lum	%CV	S/N	Mean Lum	%CV	%Inh	Mean Lum	%CV	S/N	Mean Lum	%CV	%Inh
10.0	86	0.8	1.37	57	2.5	33.7	102	0.7	1.59	59	3.6	42.2
5.00	73	2.9	1.16	55	0.0	24.7	84	0.8	1.31	57	2.5	32.1
2.50	67	4.2	1.06	52	2.7	22.4	72	1.0	1.13	54	0.0	25.0
1.25	64	2.2	1.02	52	1.4	18.8	66	2.1	1.03	53	4.0	19.7
0.625	66	1.1	1.05	57	1.3	13.6	67	1.1	1.05	53	2.7	20.9
0.313	65	2.2	1.03	56	0.0	13.8	66	2.1	1.03	54	2.6	18.2
0	63	3.5	1.00	52	3.4	17.5	64	3.2	1.00	52	5.9	18.8

2) Target Interference Evaluation with Varying Concentrations of Competitive Antibody #2 in Master Mix

Target interference is similar across multiple competing antibody concentrations. Target tolerance is observed up to 2.5 ng/mL in screening assay. 10 µg/mL of competitive antibody was chosen for target interference mitigation in the assay.

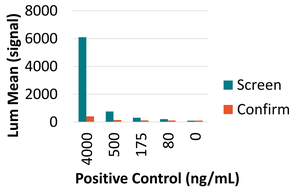


3) Sensitivity Evaluation Comparison

Sensitivity was assessed using the bridging master mix method at MRD20 with competitive antibody and using an ACE method at MRD60 without a competitive antibody. The screening and confirmatory assay sensitivity improved in the ACE method to 12.5 and 25 ng/mL respectively compared to 50 and 100 ng/mL in the bridging master mix assay.

	Bridging Assay				ACE Method			
PC Conc (ng/mL)	Mean Lum	CV (%)	S/N	%Inh	Mean Lum	CV (%)	S/N	%Inh
400	154	2.3	2.66	56.7	705	2.3	6.65	80.7
200	109	3.3	1.88	42.4	445	1.9	4.20	72.9
100	87	6.5	1.5	34.5	303	4.2	2.86	63.4
50	74	5.7	1.28	27.0	218	0.0	2.06	50.0
25	62	5.7	1.07	5.7	159	9.4	1.50	31.9
12.5	62	1.1	1.07	2.4	136	5.2	1.28	18.4
6.25	59	1.2	1.02	2.6	120	1.2	1.13	6.7
0	65	0	1.12	16.9	111	1.9	1.05	6.8
Plate NC	58				106			

4) ACE Format Assessment with Competitive Antibody in Neutralization Step



	Screening			Confirmatory		
PC (ng/mL)	Lum Mean	%CV	S/N	Lum Mean	%Inh	%CV
4000	6100	4.2	61.0	407	93.3	0.7
500	761	11.3	7.61	147	80.7	1.4
175	309	5.3	3.09	118	61.8	3.0
80	214	1.7	2.14	115	46.3	0.6
0	100	5.4	1.00	106	-6.00	4.6

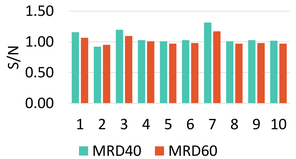
	Screen			Confirm		
Target (ng/mL)	Lum Mean	%CV	S/N	Lum Mean	%CV	%Inh
40.0	144	0.5	**1.44***	111	0.6	**22.9***
20.0	109	0.7	1.09	105	6.7	3.7
10.0	110	2.6	1.10	106	4.0	3.6
5.0	109	0.7	1.09	107	3.3	1.8
2.5	109	3.3	1.09	108	3.9	0.9
0	107	2.0	1.07	106	4.0	0.9

***Bold**: Target Interference observed > SCP (1.24) and > CCP (40.6%)**

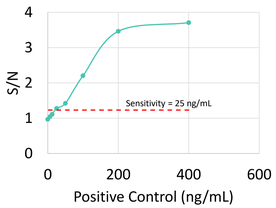
5) Target Interference and MRD Assessment with ACE Method

Ten canine samples were assessed at MRD40 and MRD60. Target interference was also assessed at both MRDs. MRD60 reduced target interference compared to MRD40.

	MRD40		MRD80	
Target (ng/mL)	S/N	%Inh	S/N	%Inh
20	1.51	32.5	1.21	26.0
10	1.21	20.3	1.03	14.8
5	1.11	11.5	0.924	7.2
2.5	1.04	9.4	0.990	9.6
1.25	1.05	9.3	0.990	10.6



6) ACE Assay with Sufficiency Sensitivity and Target Tolerance



PC (ng/mL)	Screen	%CV	S/N	Confirm	%CV	%Inh
4000 HPC	4144	11.1	34.8	305	10.4	92.6
500 MPC	620	9.5	5.21	157	1.4	74.7
75 cLPC	188	3.0	1.58	114	6.2	39.4
35 sLPC	159	11.6	1.34	121	5.3	23.9
0 NC	119	6.5	1.0	113	2.9	5.0

	Screening			Confirmatory		
Target (ng/mL)	Mean Lum	%CV	S/N	Mean Lum	%CV	%Inh
20.0	139	2.6	1.17	115	1.2	17.3
15.0	133	1.6	1.12	123	4.0	7.5
10.0	127	2.8	1.07	117	0.6	7.9
7.50	125	0.6	1.05	120	1.8	4.0
5.00	126	2.8	1.06	108	2.6	14.3
2.50	131	2.2	1.10	108	6.5	17.6
1.25	125	0.6	1.05	110	8.4	12.0
0	119	0.0	1.00	105	5.4	11.8

CONCLUSIONS

- This ADA assay was optimized in canine matrix to mitigate target interference and drug tolerance.
- Ultimately, a competitive antibody was not required in this method to overcome target interference.
- The final assay was developed as an ACE method that met the criteria needed for target and drug tolerance in the assay. The assay was then validated to support detection of ADAs in a canine study.