Supplementary Materials

Title: Evaluating the performance of two automated anti-drug antibodies assays for infliximab and adalimumab without acid dissociation

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Table of Contents for supplemental materials:

Supplementary Materials

Table S1: Assay imprecision near assay screening cut points.

Assay imprecision was evaluated based on authentic patient samples containing infliximab ADAs or adalimumab ADAs close to the screening cut points of IFX-ADA and ADL-ADA assays respectively.

IFX-ADA (RU)			ADL-ADA (RU)		
Patient sample	Average (N=3)	%CV	Patient sample	Average (N=3)	%CV
1	249	6.9	6	94	8.7
2	342	4.5	7	186	18.4
3	342	8.4	8	104	8.0
4	269	13.0	9	131	5.4
5	355	3.5	10	123	11.3

Supplementary Materials

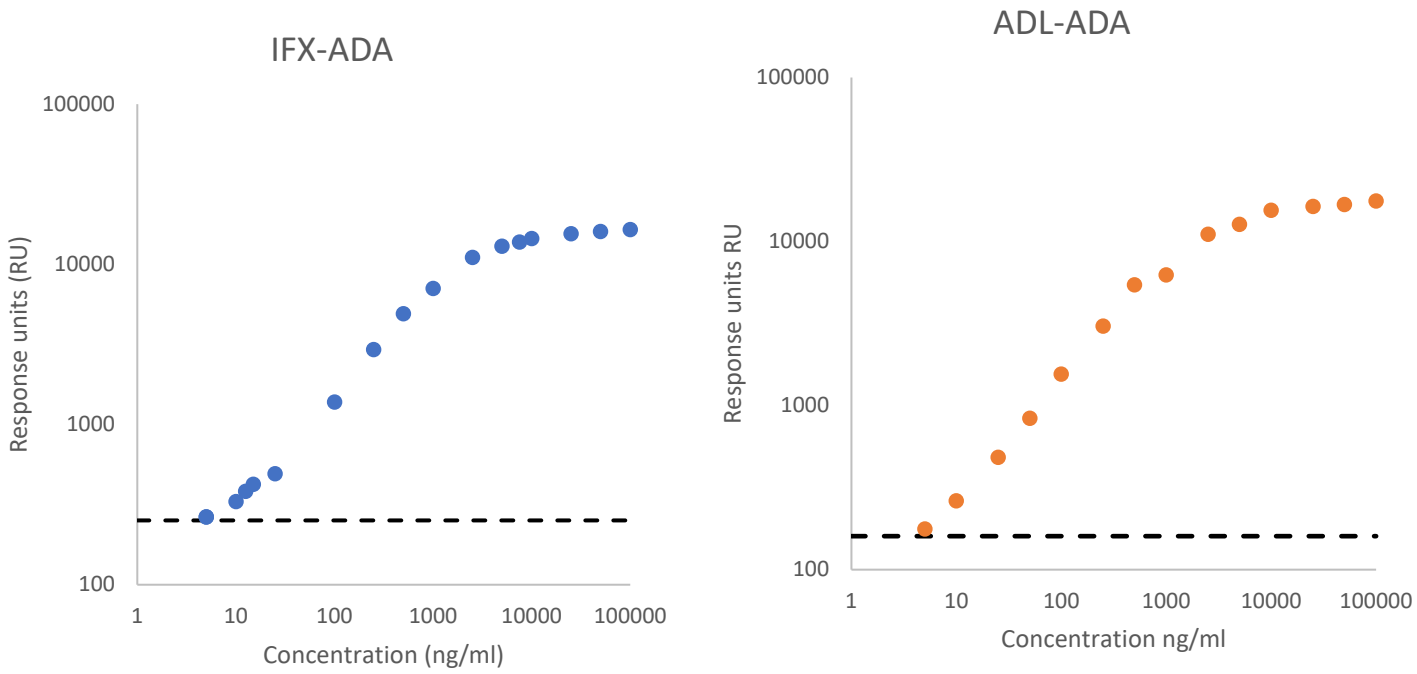
Table S2: Interference evaluation from 40 mg/dL conjugated bilirubin or free bilirubin, 3500 ng/mL biotin, 1000 mg/dL free hemoglobin, and 15 mg/mL intralipid.

IFX-ADA (RU)	Negative pool	Low pool	Mid pool	High pool
serum	82	668	4438	10698
bilirubin C	98	670	4115	10713
bilirubin F	84	725	4408	10917
biotin	18	85	535	1264
hemolysate	92	664	4304	10806
intralipid	92	716	4435	11250

ADL-ADA (RU)	Negative pool	Low pool	Mid pool	High pool
serum	50	214	264	7610
bilirubin C	65	181	275	7306
bilirubin F	64	176	267	7704
biotin	43	84	108	3687
hemolysate	108	198	296	7743
intralipid	59	176	283	7939

Supplementary Materials

Figure S1. Serial diluted positive control mAb measured by IFX-ADA and ADL-ADA assays to determine detection limit for each assay.



Dashed line indicates screening cut point for each assay. The positive control mAb binds specifically to human kappa light chain and is therefore diluted in rabbit serum in a serial dilution in 2x steps

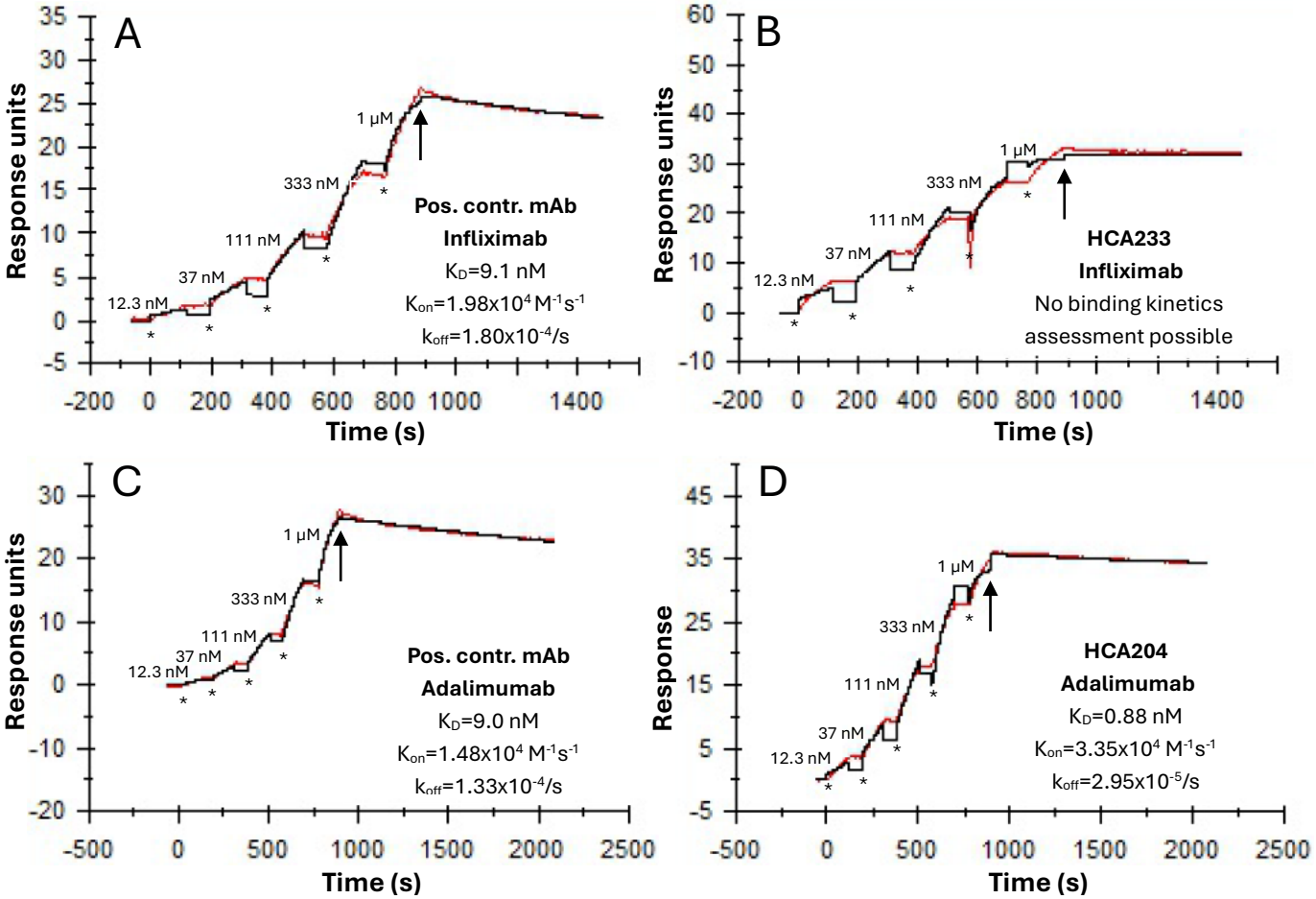


Figure S2. Surface plasmon resonance sensorgrams with antibody binding kinetics of Positive control mAb (A) and HCA233 (B) to immobilized infliximab, and Positive control mAb (C) and HCA204 (D) to immobilized adalimumab. The figure shows five cycles of increasing concentrations of injected mAb (indicated by asterisks) followed by a dissociation phase (indicated by arrow). The red line shows the antibody binding and dissociation and the black line indicate the 1:1 model fitting evaluated using the Single-Cycle Kinetics method. No binding affinity was derived for HCA233 due to its extremely low k_{off} rate (a dissociation time of up to 30 min was tested, data not shown).

Supplementary Materials

Table S3. Adalimumab concentrations in patient samples with discrepant and agreeable adalimumab ADA results.

ADL-ADA (RU)	ADL-ADA Qual Result	Clinical ELISA (AU/mL)	Clinical ELISA Qual Result	Adalimumab concentration (mcg/mL)
106	Neg	18.7	Pos	3.9
76	Neg	21.9	Pos	3.9
111	Neg	23	Pos	2.4
77	Neg	23.1	Pos	2.1
104	Neg	23.4	Pos	1.4
88	Neg	24.8	Pos	1.9
92	Neg	25	Pos	5.3
63	Neg	26	Pos	3.1
71	Neg	28.8	Pos	4.4
112	Neg	36.5	Pos	<0.8
113	Neg	42.7	Pos	6.1
100	Neg	49.8	Pos	2.2
103	Neg	64.4	Pos	4.5
109	Neg	65	Pos	<0.8
116	Neg	110.1	Pos	1.9
108	Neg	141.6	Pos	<0.8
79	Neg	142.4	Pos	<0.8
85	Neg	162.2	Pos	5.4
103	Neg	182.3	Pos	<0.8
113	Neg	189.4	Pos	<0.8
108	Neg	215.2	Pos	<0.8
139	Pos	15.2	Pos	1.7
125	Pos	18.2	Pos	1.1
145	Pos	38	Pos	2.4
121	Pos	62.9	Pos	1.1
149	Pos	64.3	Pos	4.7
416	Pos	70.5	Pos	0.9
120	Pos	115	Pos	1.8
143	Pos	115.8	Pos	<0.8
122	Pos	125	Pos	<0.8
147	Pos	125.6	Pos	6.9
162	Pos	139.9	Pos	0.9
152	Pos	157.9	Pos	0.8
141	Pos	170.5	Pos	2
138	Pos	182.4	Pos	1.9
118	Pos	185.4	Pos	6.6
129	Pos	186.1	Pos	6.2

178	Pos	193.4	Pos	3.7
151	Pos	211.8	Pos	<0.8
284	Pos	244.9	Pos	<0.8
134	Pos	251.2	Pos	1.2
178	Pos	255.7	Pos	2.2

Adalimumab concentrations in patient samples were measured as part of the clinical evaluation of adalimumab treatment responses, together with the adalimumab ADAs. The assay is a sandwich ELISA assay (Immundiagnostik AG) and has been validated to measure free adalimumab [1]. The analytical measurement range (AMR) of the assay is 0.80 to 45 mcg/mL.

Supplementary Materials

Table S4: Comparative analysis of adalimumab ADA results from clinical ELISA assay, ImmunoCAP ADL-ADA assay and functional RGA assay.

Clinical ELISA (AU/mL)	ADL-ADA (RU)	Functional RGA	Adalimumab Drug (mcg/mL)	RF IgM (IU/mL)
26	63	<1:20	3.9	1.20
28.8	71	<1:20	3.9	2.40
21.9	76	<1:20	2.4	1.40
<10	77	<1:20	4.5	3.10
<10	86	<1:20	1	1.40
24.8	88	<1:20	5.3	2.10
49.8	100	<1:20	4.4	2.60
64.4	103	<1:20	6.1	1.50
<10	104	<1:20	3.4	3.20
23.4	104	<1:20	2.2	1.20
18.7	106	<1:20	4.5	1.90
215.2	108	<1:20	1.9	4.80
65	109	<1:20	<0.8	2.70
23	111	<1:20	<0.8	1.20
42.7	113	<1:20	<0.8	2.00
110.1	116	<1:20	<0.8	1.40
185.4	118	<1:20	1.7	1.10
62.9	121	<1:20	2.4	1.00
350.9	136	<1:20	<0.8	0.80
182.4	138	1:95	<0.8	3.80
15.2	139	<1:20	6.9	1.10
>500	143	1:158	0.8	1.80
115.8	143	<1:20	1.9	1.00
38	145	<1:20	6.6	0.45
125.6	147	1:328	6.2	3.10
64.3	149	<1:20	3.7	3.10
157.9	152	1:23	<0.8	3.80
>500.0	175	1:69	<0.8	1.40
193.4	178	<1:20	4	1.60
>500	188	<1:20	1.0	1.90
>500.0	257	1:132	1.6	2.10
>500	864	1:146	<0.8	1.50
>500.0	2862	1:302	1.2	2.00
292.2	11864	>1:1500	<0.8	4.80

The AMR of the clinical ELISA for adalimumab ADAs is 10-500 AU/mL and the assay cutoff is 14 AU/mL. The screening cut point of the ImmunoCAP ADL-ADA assay is 117 RU. The functional RGA

assay utilizes reporter gene cells sensitive to TNF [1, 2]. The AMR of the functional RGA assay is between 1:20 to 1:1500 and the positive cutoff is at 1:20. The AMR for the adalimumab concentration assay is 0.80 to 45 mcg/mL. RF IgM was evaluated using EliA™ RF IgM assay (Thermo Fisher Scientific). Negative: <3.5 IU/mL; Equivocal: 3.5-5.0 IU/mL; Positive: >5.0 IU/mL.

Supplementary Materials

References:

1. Jain, D., et al., *Comparison of Two Clinical Laboratory Assays for Measuring Serum Adalimumab and Antibodies to Adalimumab.* J Appl Lab Med, 2023. **8**(6): p. 1054-1064.
2. Pavlov, I.Y., et al., *Clinical laboratory application of a reporter-gene assay for measurement of functional activity and neutralizing antibody response to infliximab.* Clin Chim Acta, 2016. **453**: p. 147-53.