Overcoming IgE interference in PK and ADA assay of Omalizumab in the serum of asthma patients.

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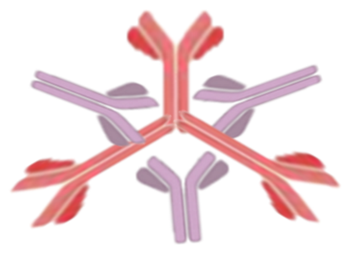
Introduction:

Accurate determination of Omalizumab concentration and detection of Anti-Omalizumab Antibodies (ADA) are crucial for assessing its therapeutic efficacy and safety. However, IgE interference poses challenges, especially at high serum concentrations.

These assays have the capability to precisely quantify Omalizumab levels and detect ADA with high specificity and target tolerance. Generally, serum IgE concentrations in asthma patients can range from 72-1680 ng/mL. In the developed assay, a target tolerance of IgE of more than 2400 ng/mL was achieved, thus this assay facilitates the quantification of total Omalizumab and highly specific ADA using MSD. By employing these advanced assays, researchers can obtain more reliable PK and ADA data from samples of asthma patients.



Human IgE : Omalizumab Hexamer Complex

Method Challenges	Mitigation Strategy
For PK:- High level of circulating endogenous IgE (drug target) forms complex with Omalizumab. which results into quantification of only free Omalizumab. instead of Total Omaliumab.	Acid dissociation of sample introduced which disrupt Omalizumab-IgE complex and Total Omalizumab can be quantified from the sample.
For ADA: Acid dissociation is required to achieve high drug tolerance in ADA assay. However acid dissociation also detaches Omalizumab: IgE complex. High level of circulating IgE (drug target) forms complex with Biotinylated and SulfoTag labelled Omalizumab in the master mix and gives "False Positive" result in ADA assay.	Polyclonal anti-human IgE antibody was added into master mix which binds with the free IgE from the serum and prohibits it from binding with Biotinylated and SulfoTag labelled Omalizumab and hence prevents "False Positive" result in ADA assay.

PK Method Summary

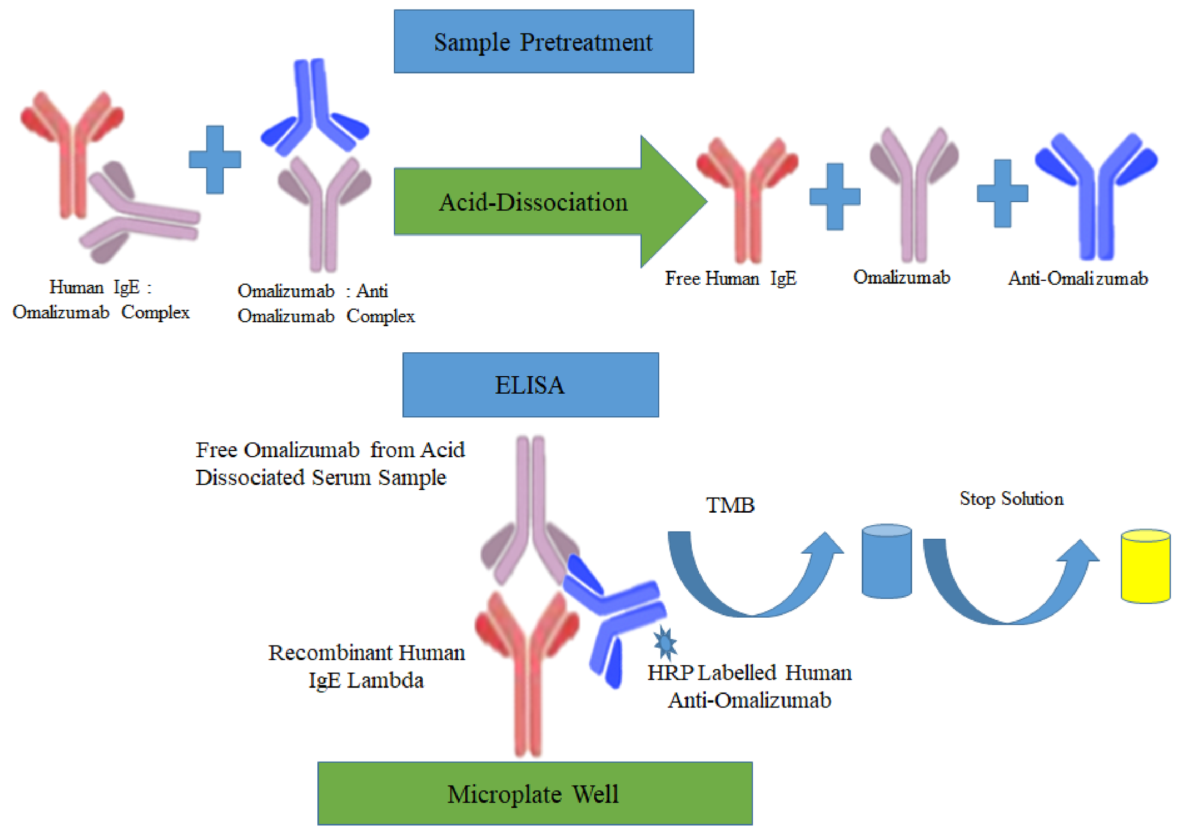
- Assay Format : Quantitative Sandwich Immunoassay
- Capture Antibody : Recombinant Human IgE Lambda
- Detection Antibody: Human Anti-Omalizumab (HRP)
- Sensitivity : 18.75 ng/mL
- Regression type : 5 Parameter Logistic (1/Y2 weighting)

ADA Method Summary

- Assay Format : Bridging ECL using MSD
- Drug tolerance : 40.00 µg/mL in the presence of 100 ng/mL of PC
- Regression type : 4 Parameter Logistic for sensitivity
- Specificity : Specificity was evaluated in 1000 µg/mL Human IgG in screening and confirmatory

PK Methods

For the PK assay, plates coated with recombinant human IgE Lambda, as the capture molecule for Omalizumab were used. Samples are first treated with 600 mM glacial acetic acid to dissociate any bound Omalizumab-IgE & Omalizumab-ADA complexes. After incubation, samples are neutralized using 1M Tris buffer and immediately transferred to the coated plate which is already blocked and washed. Subsequently, detection is performed using highly specific HRP-conjugated anti-Omalizumab antibodies. Upon addition of the TMB substrate, color develops, and the reaction is stopped. Finally, the absorbance of each well is measured, providing a quantitative assessment of Omalizumab concentration.

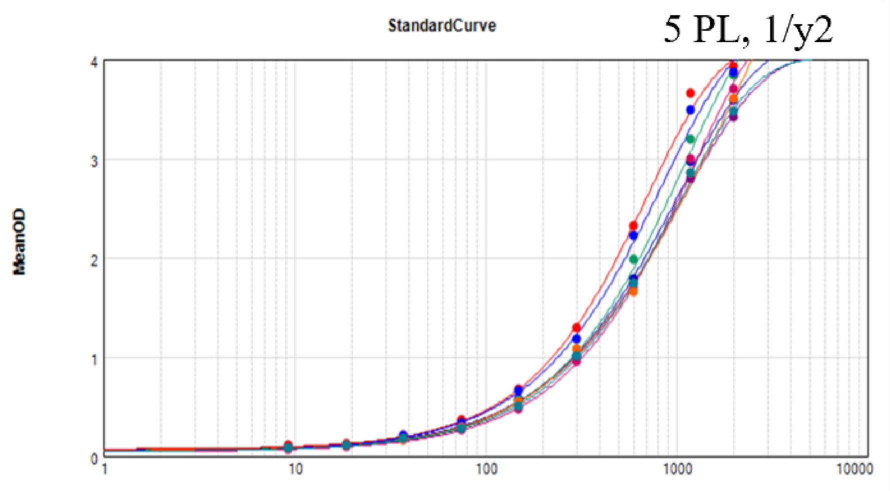


Result:

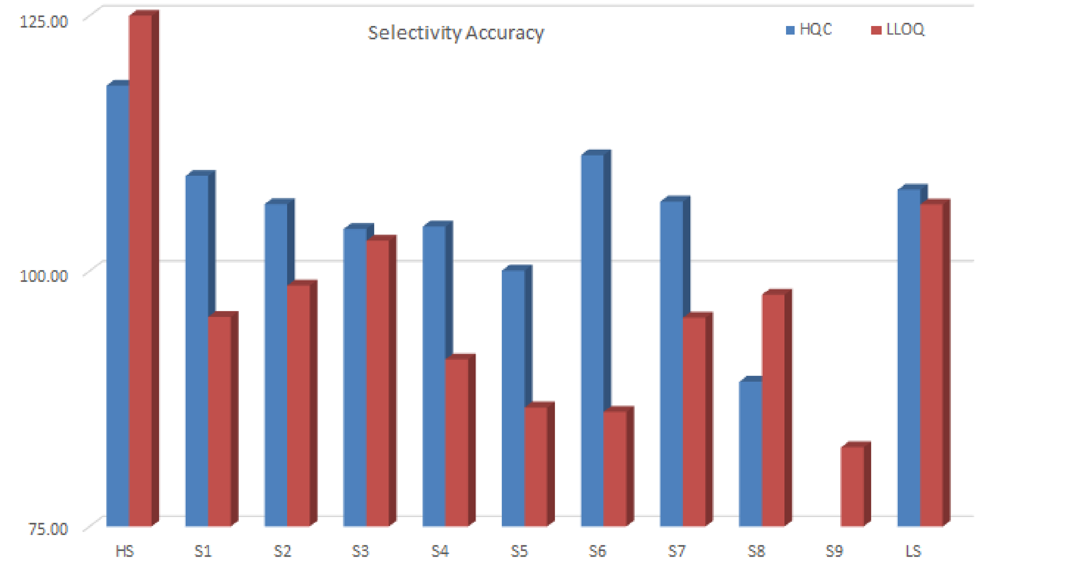
Standards			
Sample ID	Nomimal Conc. ng/mL	n=6 Accuracy (%)	% CV
ST1 (AP)	2000	93.32	4.9
ST2	1200	107.53	3.5
ST3	600	100.28	3.1
ST4	300	98.15	3
ST5	150	99.32	2.5
ST6	75	98.76	3.2
ST7	37.5	108.69	2.1
ST8	18.75	92.53	7.8
ST9 (AP)	9.375	104.51	8.4

Target Tolerance		
Human IgE Conc[n]	LLOQ (18.75) ng/mL	%Bias
4800 ng/mL	16.211	-13.54
	17.201	-8.26
2400 ng/mL	21.759	16.05
	17.642	-5.91
1200 ng/mL	15.99	-14.72
	14.164	-24.46
600 ng/mL	16.122	-14.02
	14.142	-24.58

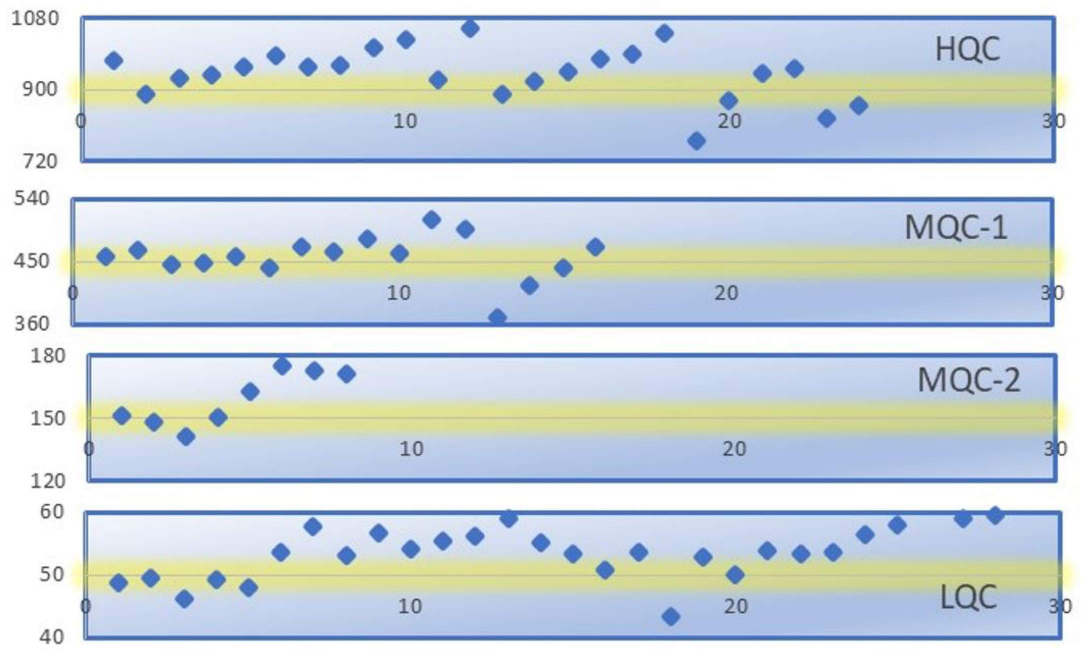
Intra and Inter assay precision (%CV) of QCs sample was 1.3 to 7.5 % & 6.1 to 7.7 % & Accuracy (%bias) of QCs sample was -1.48 to 16.81 % & 3.88 to 7.87 %.



Specificity with 2000 ng/mL Human IgG				
ULOQ 1200		LLOQ 18.75		Blank NA
ng/mL	%Bias	ng/mL	%Bias	ng/mL
1233.285	2.77	16.47	-12.15	BLQ
1246.624	3.89	23.02	22.79	BLQ



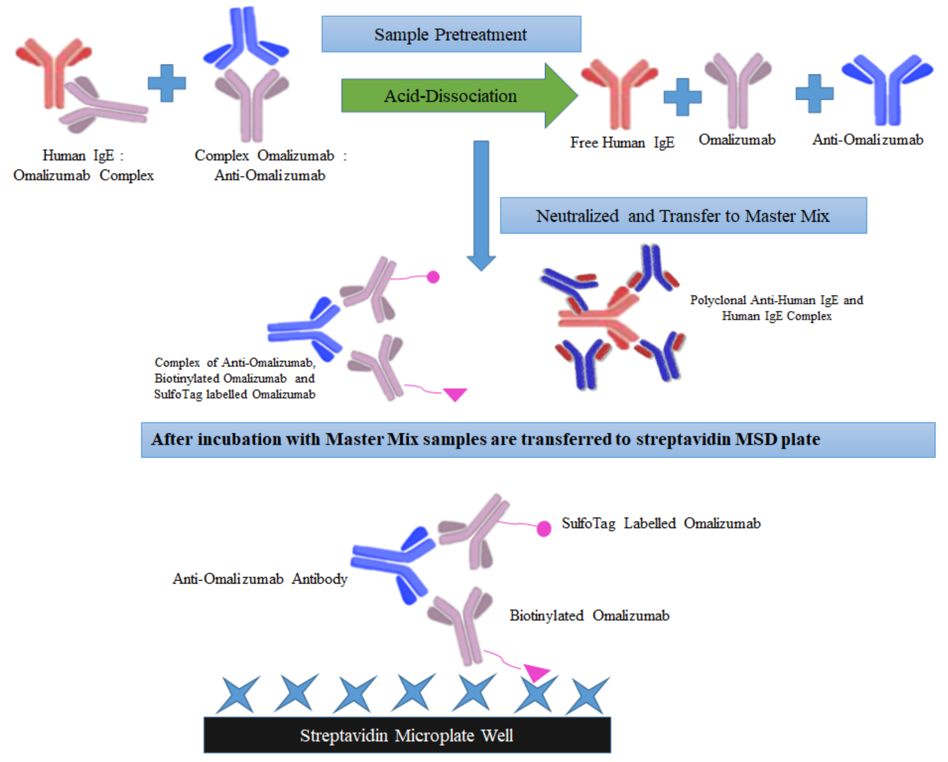
08 out of 09 individual lots and lipemic and haemolysed, it was observed that they did not interfere with high igE serum matrix.



Target Tolerance					
Human IgE Conc[n]	ULOQ (1200.0) ng/mL	%Bias	LLOQ (18.75) ng/mL	%Bias	Blank ng/mL
4800 ng/mL	1189.962	-0.84	17.066	-8.98	BLQ
2400 ng/mL	1100.302	-8.31	16.514	-11.93	BLQ

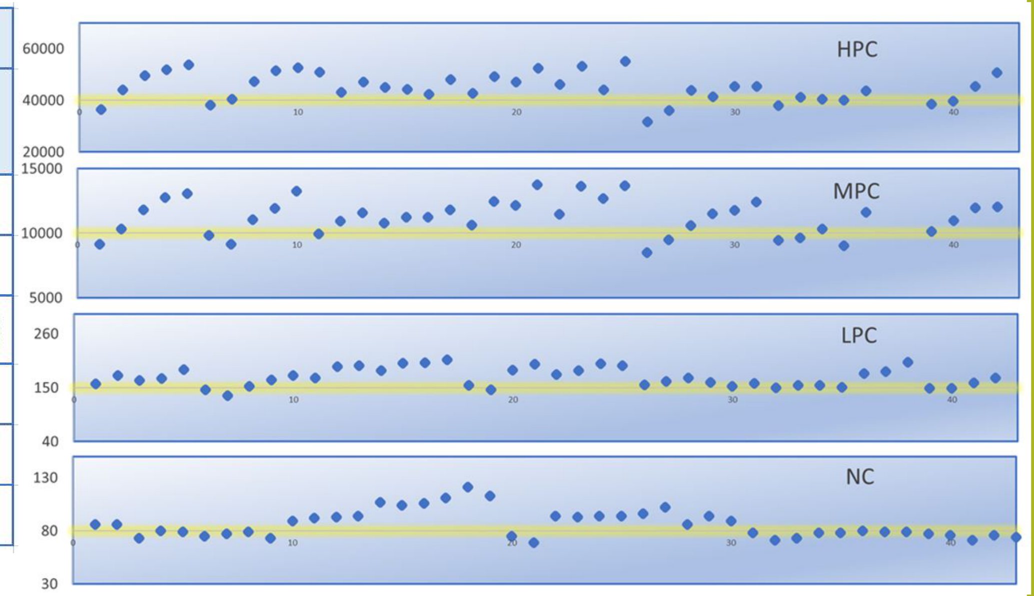
ADA Methods

In the ADA assay for the detection of ADA against Omalizumab, samples containing ADA are subjected to acid dissociation to disrupt any pre-existing Omalizumab-ADA complexes and Omalizumab-IgE complexes. After incubation, acid dissociated samples are added to a mixture comprising biotinylated: Sulfo-Omalizumab and anti-human IgE antibodies and allowed to incubate to form the bridge. Anti-IgE antibody present in the mixture binds with the endogenous IgE present in the sample and prevents binding of IgE with biotinylated: Sulfo-Omalizumab and thus the terminate generation of non-specific signal. Biotinylated: Sulfo-Omalizumab specifically binds to anti Omalizumab antibody, while anti-human IgE captures any IgE molecules within the sample. After bridging incubation samples are added to the streptavidin coated plate. This is followed by addition of read buffer. Finally, the MSD plate is immediately read using a MSD instrument, enabling the detection of ADA.

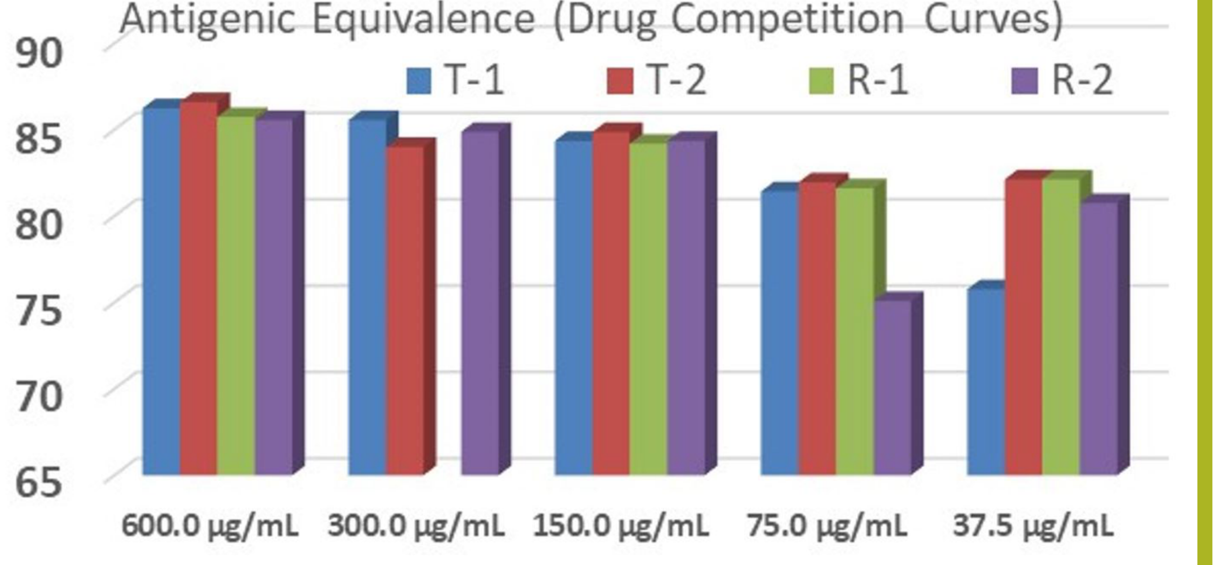


Result:

Interference 4800 ng/mL IgE RLU Signal			
QCs	HPC (8000 ng/mL)	NC	S/N
Mastermix w/o Anti Human IgE:			
NA	62766	8787	7.14
Mastermix with different conc. of Anti Human IgE			
4800 ng/mL	30982	107	289.55
2400 ng/mL	34809	523	66.56
1200 ng/mL	41129	5429	7.58



Target Tolerance			
Human IgE	HPC S/N	LPC S/N	NC S/N
4800 ng/mL	576.56	2.15	1.12
	574.84	2.20	1.13
	587.40	2.23	
2400 ng/mL	586.61	2.05	1.00
	587.55	2.07	1.03
	587.37	2.08	
1200 ng/mL	583.87	2.01	1.01
	582.69	2.07	1.01
	599.44	2.09	



Sensitivity data of Anti Omalizumab				
Conc. ng/mL	Signal	Signal	Signal	Signal
20000.00	1905.66	1125.71	1656.95	1366.30
8000.00	648.36	415.27	527.18	422.55
3200.00	277.03	176.37	216.07	201.84
1280.00	114.96	68.92	80.03	76.48
512.00	41.10	24.18	30.77	22.04
204.80	15.82	10.52	13.27	11.30
81.920	6.39	4.89	5.35	5.17
32.768	3.21	2.64	2.62	2.48
13.107	1.91	1.63	1.57	1.58
5.243	1.38	1.30	1.19	1.22
2.097	1.16	1.08	1.09	1.06
0.839	1.08	0.99	1.03	1.00
Sensitivity	1.99	3.21	3.77	3.79

Inhibitory Comparability Drug Tolerance					
Drug Conc. (µg/mL)	2000.0 ng/mL T1_RLU	2000.0 ng/mL R1_RLU	Drug Conc. (µg/mL)	LPC T1_RLU	LPC R1_RLU
400	1.08	1.06	60	1.09	1.14
200	1.43	1.36	30	1.23	1.35
100	4.52	4.25	15	1.55	1.69
0	189.09	206.06	0	2.4	2.53
DT µg/mL	200	200	DT µg/mL	30	30

Comparability: For both Test and Reference Drug tolerance is obtained at 200.00 µg/mL at 2000.00 ng/mL and for LPC 30.00 µg/mL

Conclusion

The development of a highly sensitive (LLOQ- 18.75 ng/mL) assay and demonstrated the ability to tolerate higher target tolerance levels than anticipated in patient serum samples. Our approach incorporated a highly specific HRP-Conjugated for detection, enhancing specificity and minimized non-specific quantification in the assay. The ADA assay of Omalizumab is critical for advancing our understanding of its therapeutic profile. This advanced assay offers a reliable means to overcome drug target interference, enabling precise quantification of Omalizumab levels and accurate detection of ADA.