Development and Validation of an Anti-Omalizumab Antibody Assay to Support Immunogenicity Assessments in Clinical Trials Using the MSD Platform in Asthma Patient Serum

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Background

- Omalizumab is a recombinant humanized IgG1 monoclonal antibody that specifically targets human immunoglobulin E (IgE).
- The direct action of Omalizumab involves binding to the C-epsilon-3 locus, domain at which IgE binds to Fc-epsilon-RI. This action lowers immunoglobulin levels and prevents them from interacting with their high-affinity receptor, mainly present on eosinophils, basophils and mast cells.

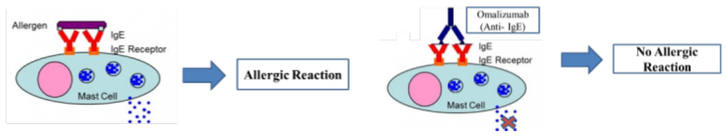
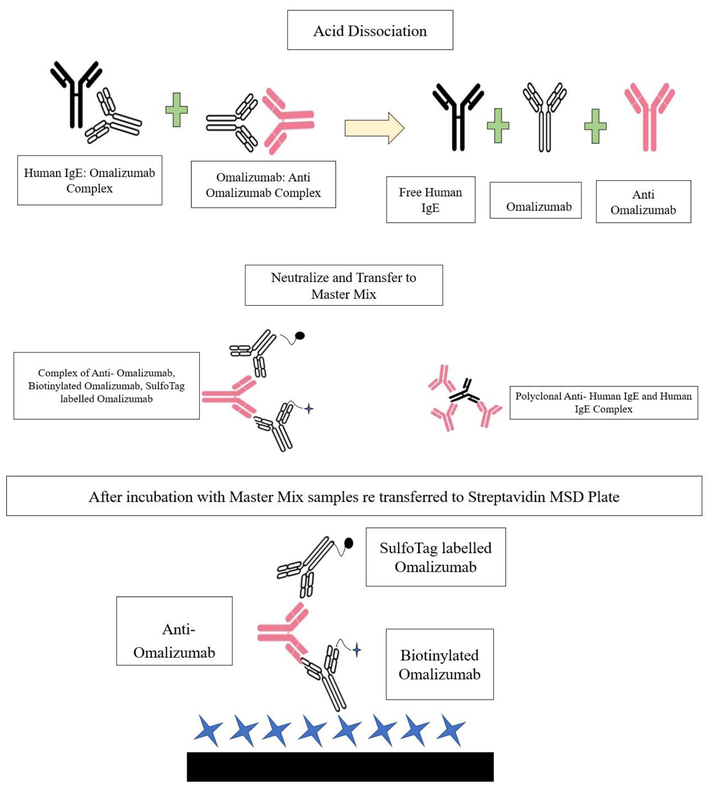


Figure: Omalizumab Mechanism of Action

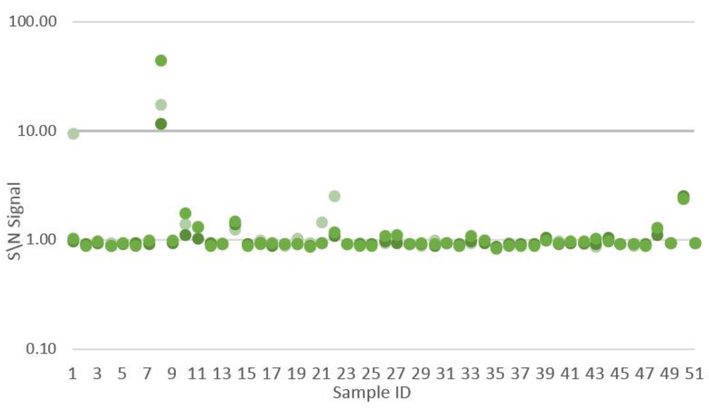
Assay Principle



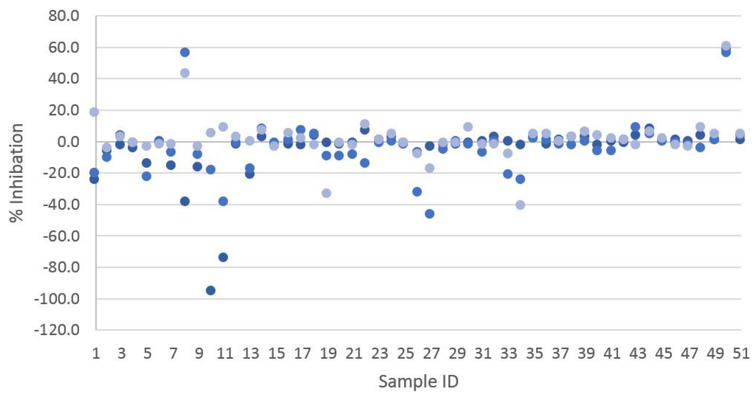
Method Development

- During method development biotin and SULFO-TAG labelling with Omalizumab was performed on different challenge ratios. Interference of IgE was checked by adding different concentration of recombinant Human IgE in human serum.
- Optimization of different concentration of Biotinylated Omalizumab, SULFO-TAG labelled Omalizumab and Goat Anti-Human IgE used for preparation of Master Mix.
- Optimization of Acid and base ratio, Blocker, assay diluent, incubation time, rpm and MRD.

Method Validation Cut Point



Screening Cut point

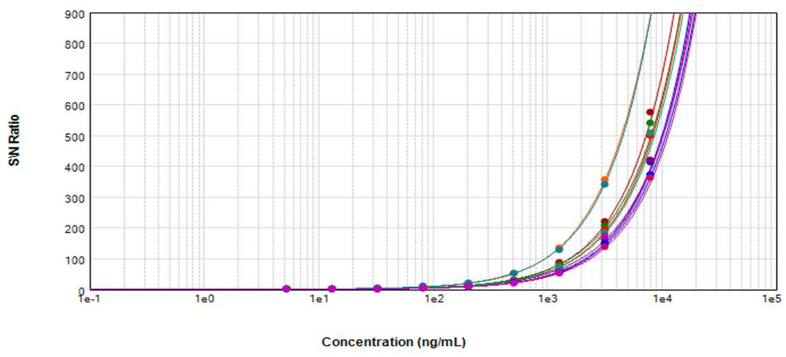


Confirmatory Cut point

Correction Factor for screening: 1.0921
Correction factor for (% Inhibition PC): 10.847

Sensitivity

To determine the lowest concentration at which a positive control antibody preparation consistently produces a positive result readout equal to the cut point determination. The surrogate positive control was serially diluted on different days by different analysts. Each of these dilution curves was fitted by a 4PL regression model to interpolate the concentration corresponding to the plate specific cut point.



Precision

Screening Precision

QCs	HPC	MPC	LPC	NC
Intra Run CV%	7.4 to 11.7 %	5.7 to 10.8 %	2.5 to 6.5 %	1.5 to 34.1 %
Inter Run CV%	14.1 %	11.3 %	8.3 %	16.4 %

Confirmatory Precision

QCs	HPC	MPC	LPC
Intra Run CV%	0.0 %	0.0 to 0.1 %	1.5 to 3.4%
Inter Run CV%	0.0 %	0.1 %	3.7 %

Selectivity

Samples	HPC	LPC	Blank
	S/N Ratio	S/N Ratio	S/N Ratio
1	776.06	2.86	1.03
2	755.23	2.58	0.96
3	748.46	2.62	1.01
4	790.70	2.68	0.92
5	774.08	2.89	0.99
6	782.39	2.79	0.99
7	766.31	2.90	1.23
8	840.00	2.65	0.94
9	764.79	2.79	0.99
10	749.41	2.69	0.90

The HPC spiked selectivity samples has acceptable results. The LPC spiked selectivity samples were above the plate specific cut point and the blank individual samples were below the plate specific cut point.

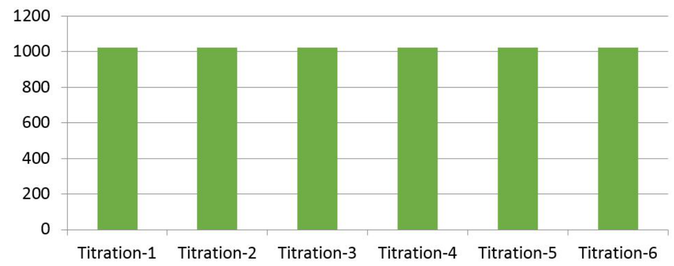
Specificity

HPC	LPC
S/N Ratio	S/N Ratio
Specificity with Human IgG (1000.000 µg/mL) for Screening	
781.04	2.72
800.08	2.65
814.11	2.69
Specificity with Human IgG (34.483 µg/mL) for Confirmatory	
740.11	2.67
687.76	2.61
774.56	2.64

Drug Tolerance

MPC		PC (500.000ng/mL)		PC (100.000ng/mL)		LPC	
Drug Con. (µg/mL)	S/N ratio	Drug Con. (µg/mL)	S/N ratio	Drug Con. (µg/mL)	S/N ratio	Drug Con. (µg/mL)	S/N ratio
350.00	1.11	200.00	1.04	150.00	0.96	60.00	1.18
300.00	1.11	150.00	1.24	100.00	1.07	50.00	1.04
250.00	1.14	100.00	1.63	75.00	1.21	40.00	1.11
200.00	1.29	75.00	2.61	50.00	1.64	30.00	1.26
100.00	3.23	0.00	46.13	25.00	3.08	20.00	1.43
50.00	13.89			0.00	12.25	0.000	2.99
0.00	209.84						
350.00 µg/mL		150.00 µg/mL		75.00 µg/mL		40.00 µg/mL	

Titration



Single Assay Suitability

Precision Comparability

QCs	HPC	MPC	LPC	NC
Intra-Run (CV%)	4.2 to 6.7 %	1.9 to 7.4 %	1.4 to 4.7 %	1.3 %
Inter-Run (CV%)	8.1 %	7.5 %	5.5 %	1.5 %

Comparability

Positive control	Test	Reference
MPC	300.00 µg/mL	300.00 µg/mL
PC (100.00 ng/mL)	100.00 µg/mL	100.00 µg/mL
LPC	50.00 µg/mL	50.00 µg/mL

Assay Summary

Assay Parameter	Result
Assay matrix	Asthma patient serum
Sample analysis Platform	Electro-Chemiluminescent Immune Assay (ECLIA)
Minimum Required Dilution	10-Fold (At Acid Dissociation), 1.25 Fold (At Neutralization) and 3 Fold (At Bridging)
Correction Factor for Screening Assay	1.092 (Multiplicative)
Confirmatory Cut Point	10.847 %
Sensitivity Value	8.12 ng/mL
Regression Type for Sensitivity Curves	4 Parameter Logistic (4PL), Fixed
Titration Assay	All the samples of titer dilution provided repeatable results within two (02) fold dilution variation.
Selectivity	All selectivity samples [Nine (09) lots of normal human serums, One (01) Lipemic and one (01) hemolysed lot] passed
Target Tolerance	IgE (4800.00 ng/mL)
Robustness	Blocking time, Sample Incubation, Bridging time, Microplate washing, Plate reading time
Stability, Freeze/Thaw cycle	6 Freeze thaw cycles
Stability, Bench top (Ambient Temperature)	26 Hours
Stability, -20 °C	Stable at -20 °C

Conclusion: An assay which is precise, sensitive and specific for detection of Anti-drug antibodies against Omalizumab in asthma patient serum was successfully developed and validated using MSD.