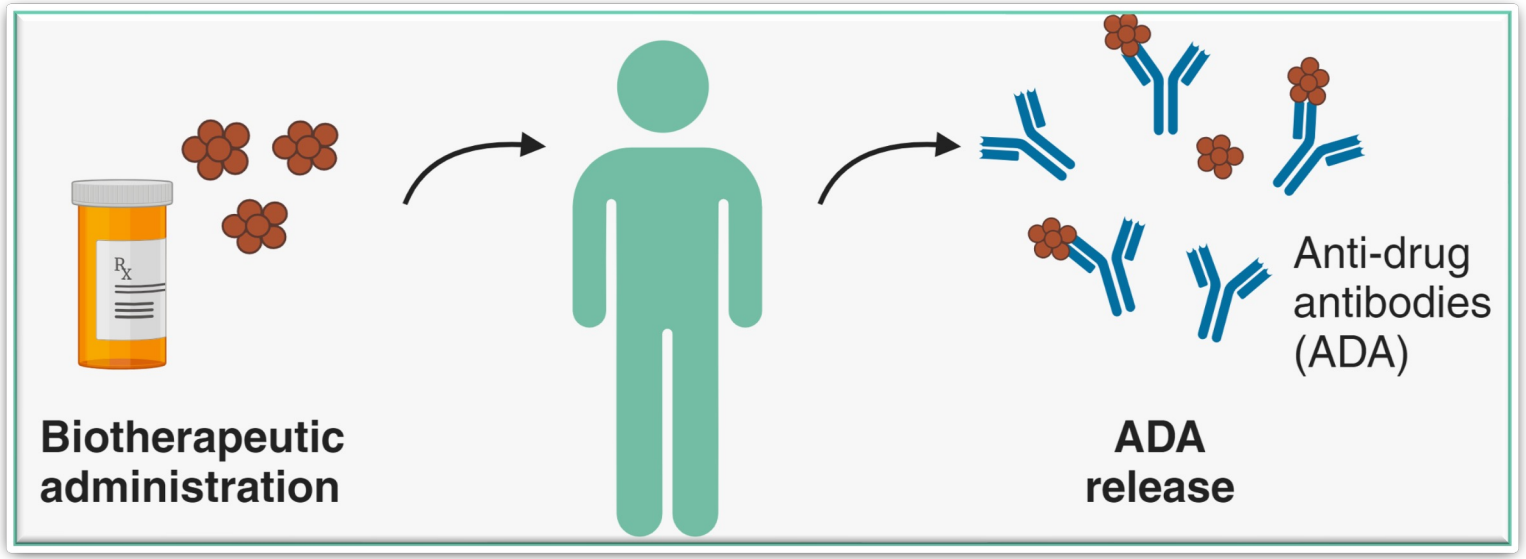
Development of a novel hybrid immunoaffinity-liquid chromatography mass spectrometry (IA-LCMS) approach to supplement ADA testing

Shivangi Awasthi

(16th November 2023)

Immunogenicity assessment by ADA measurements



LBA based bridging assay is the most common format, assessed in a tiered analysis

Positive control as surrogate for assay development and performance assessment

Cut-points are established based on signals observed with negative controls

Key challenges

- ADAs may bind to excess drug and not be detected leading to false negative
- Circulating soluble targets may be detected as false positive

ADA assessment using hybrid LCMS assay

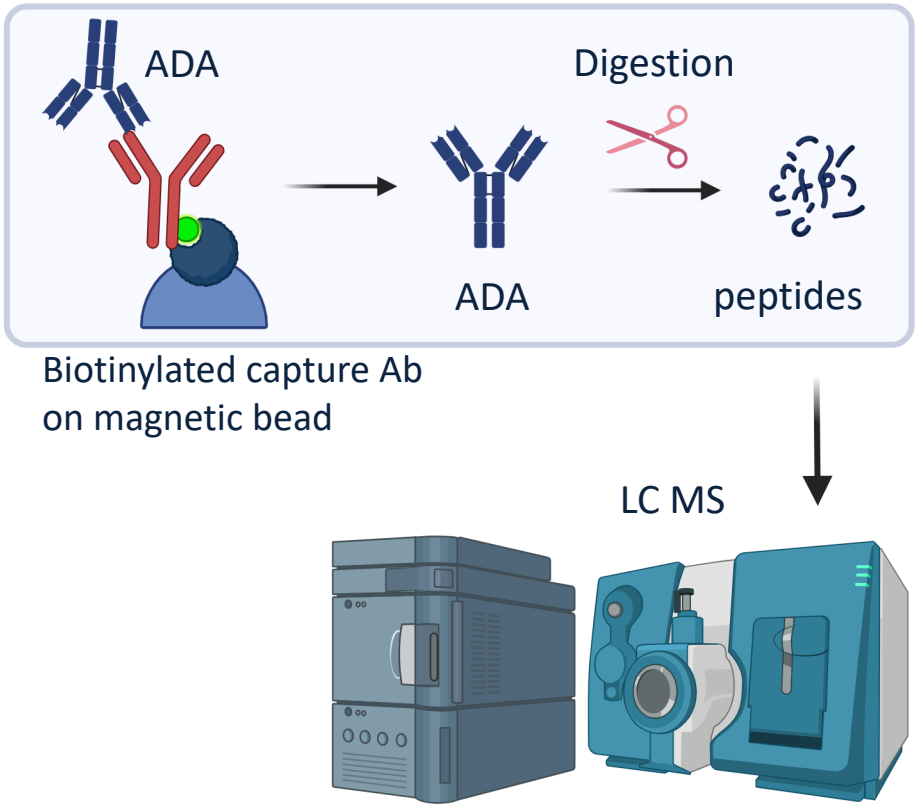
Biotinylated capture reagent to pull down ADA, followed by trypsin digestion and LCMS analysis of the proteotypic peptide

Advantages

- Capability to discern isotypes
- Reduced reagent demand
- Less vulnerable to drug interference
- Multiplexing capability for ADA isotyping in a single run

Limitations

- Complex workflow
- Less sensitive than LBA
- Lack of regulatory guidance
- Lack of correlation data with the standard LBA approach



Case study: Using LCMS for MK-A IgE ADA isotyping

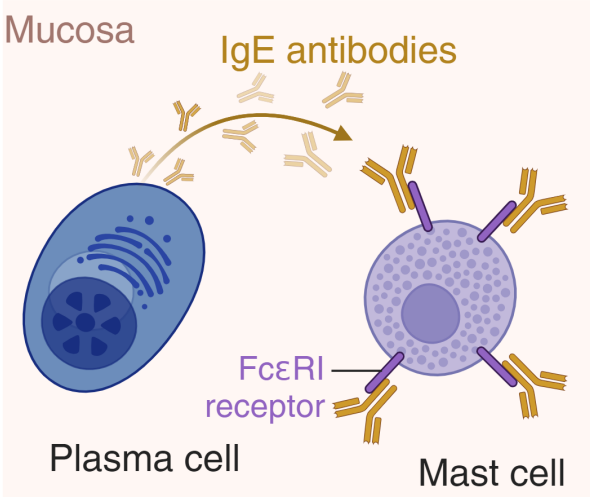
MK-A: a fully human IgG1 monoclonal antibody under clinical development

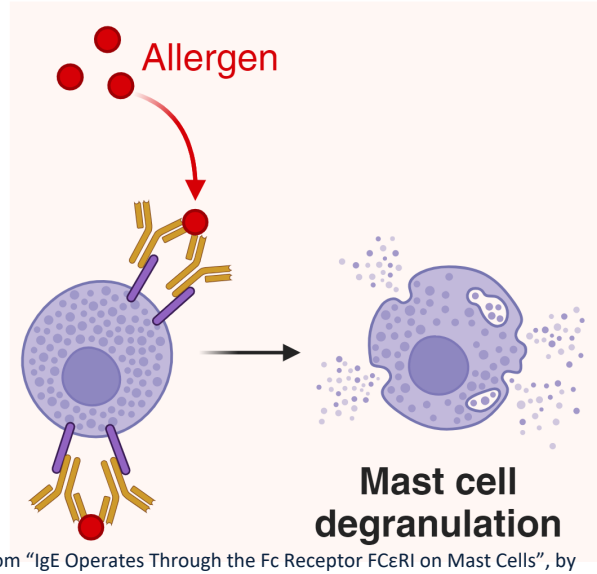
Regulatory request for collecting blood samples for potential evaluation of IgE in participants with Grade 3 or 4 anaphylaxis/hypersensitivity AESI

Drug specific ADA IgE isotype considered a risk factor for developing hypersensitivity, allergic reactions and anaphylaxis

Measurement of ADA by LBA - the gold standard

Utilization of LCMS for ADA measurement as an alternative approach to isotype MK-A specific ADA IgE





Adapted from "IgE Operates Through the Fc Receptor FCεRI on Mast Cells", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates

ADA assessment using LCMS – published literature

Detection of cynomolgus monkey anti-protein XYZ antibody using immunocapture-LC/MS

David Roos, Linzhi Chen*, Rajeev Vesapogu, Cheikh Kane, Jeffrey Duggan, Stephen Norris

Isotyping and Semi-Quantitation of Monkey Anti-Drug Antibodies by Immunocapture Liquid Chromatography-Mass Spectrometry

Xiaoxiao Huang,[1] Xiaobin Xu,[1,3] Michael A. Partridge,[2] Jihua Chen,[2] Ellen Koehler-Stec,[2] Giane Sumner,[2] Haibo Qiu,[1,3] Albert Torri,[2] and Ning Li[1]

Research Article

Development of Immunocapture-LC/MS Assay for ADA Isotyping and Semiquantitation

Lin-Zhi Chen, David Roos, and Elsy Philip

Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877, USA

REVIEW

Current Status of Anti-Drug Antibody Analysis Using Immunocapture-Liquid Chromatography/Mass Spectrometry

Linzhi Chen

Drug Metabolism and Pharmacokinetics, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT 06877, USA.

Perspectives on exploring hybrid LBA/LC–MS approach for clinical immunogenicity testing

Hao Jiang*,[1], Heather Myler[2], Jianing Zeng[1], Johanna Mora[1], Gerry Kolaitis[1] & Renuka Pillutla[1]

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Article

Optimization of a Quantitative Anti-Drug Antibodies against Infliximab Assay with the Liquid Chromatography-Tandem Mass Spectrometry: A Method Validation Study and Future Perspectives

Erin H. Smeijsters [1,*], Kim C. M. van der Elst [1], Amy Visch [1], Camiel Göbel [1], Floris C. Loeff [2], Theo Rispens [2], Alwin D. R. Huitema [1,3,4], Matthijs van Luin [1] and Mohsin El Amrani [1]

ADA LCMS workflow

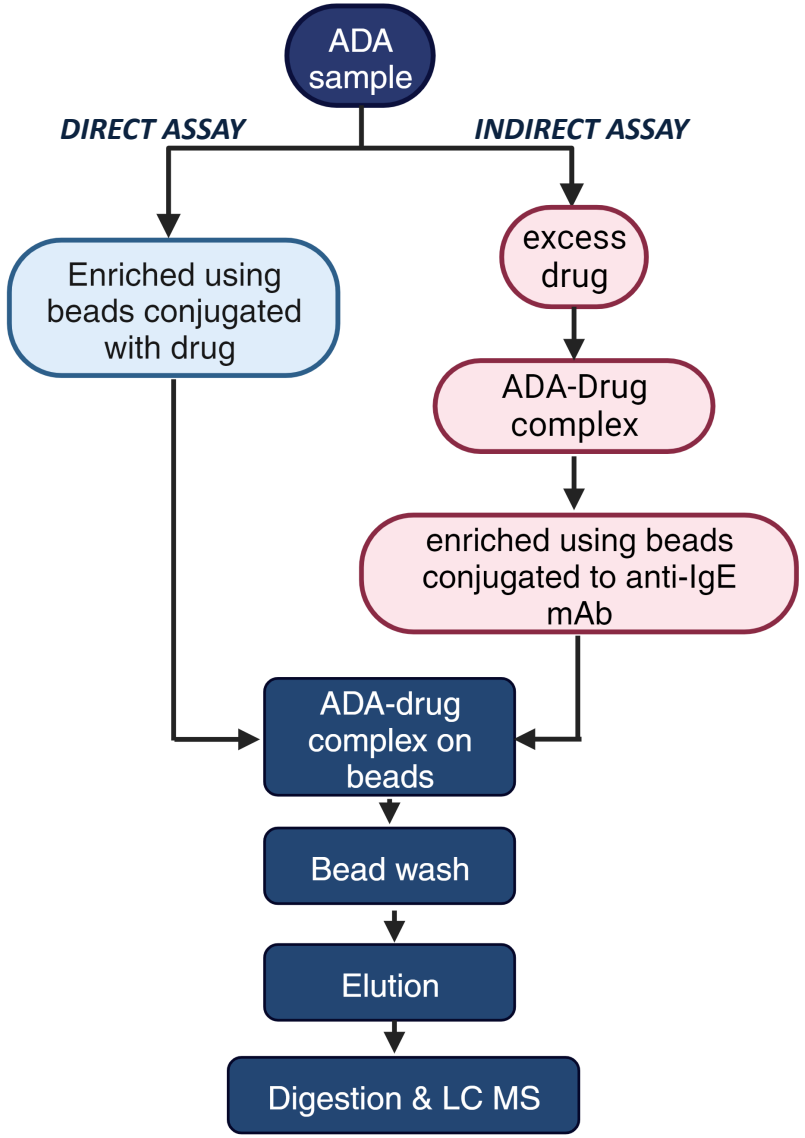
Surrogate peptide selection for ADA isotyping

✓In-silico prediction of tryptic peptides and MRM transitions

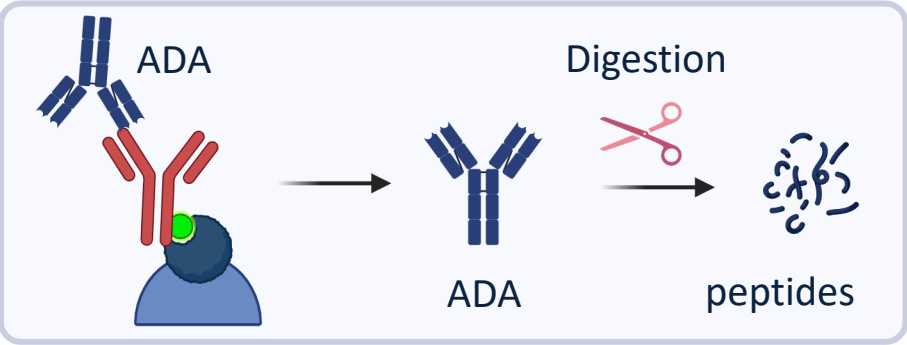
✓Candidate peptide sequence unique to isotype/subclasses in the conserved region and against background proteome

✓Final selection based on in-matrix assessment of S/N, interference and reproducibility

✓Avoid peptides with PTMs, variants or residues with stability issues



ADA IgE Isotyping by Direct LCMS Assay – testing positive controls



Biotinylated MK-A on magnetic bead

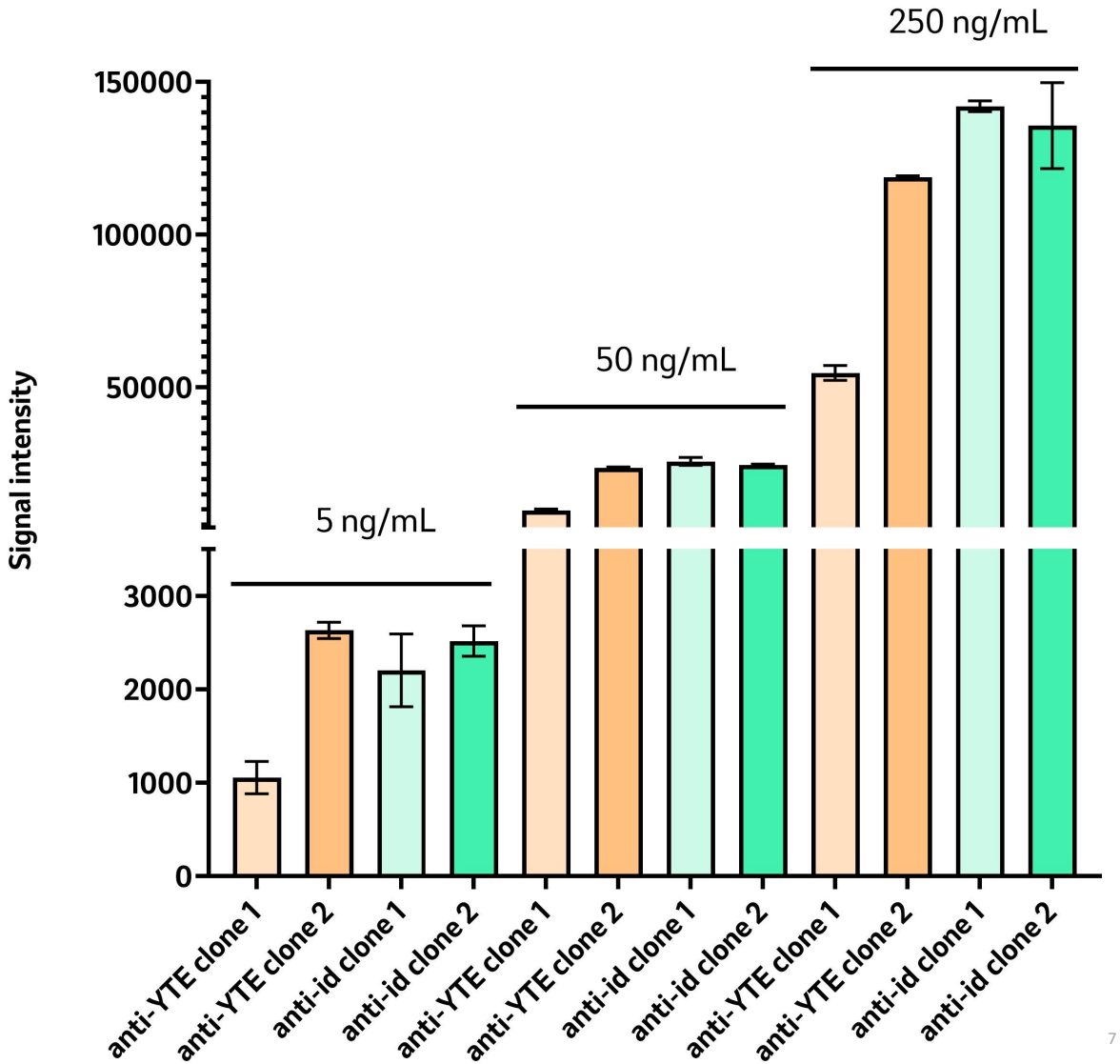
MK-A domains with potential higher IMG risk

- CDR
- YTE mutation

Recombinant drug specific PCs with human IgE backbone

- 2 anti-IDs
- 2 anti-YTE

LC-MS detection of surrogate peptides unique to human IgE and in the conserved region

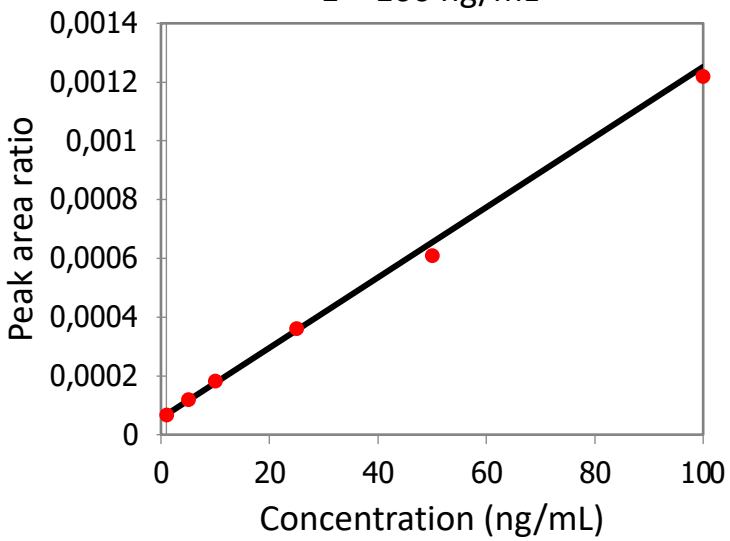


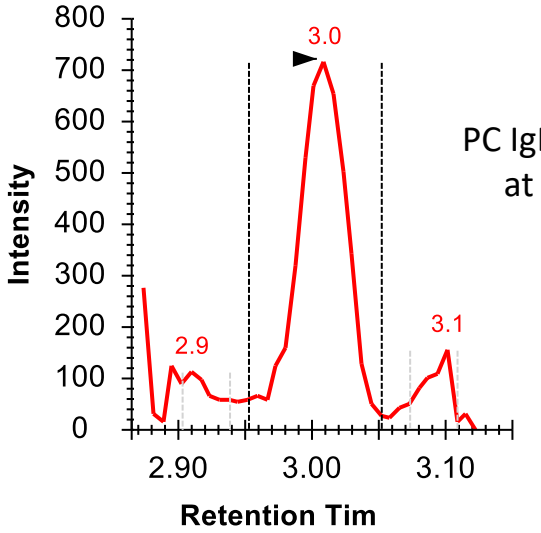
ADA IgE Isotyping by Direct LCMS Assay – anti-ID PC sensitivity assessment

The sensitive anti-ID PC chosen for sensitivity improvement

LLOQ: 1 ng/mL PC without drug, using the optimized LC MS assay

Curve shows linearity for PC concentrations 1 – 100 ng/mL

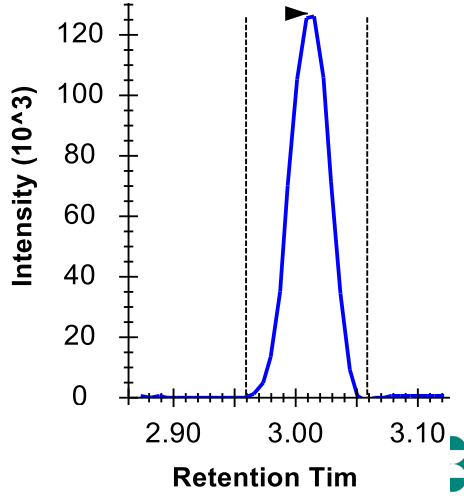




PC IgE response at 1 ng/mL

Curve showed good precision and accuracy

Expected Concentration	Mean experimental concentration	Standard Deviation	%CV	Accuracy
1	0.99	0.19	18.82	98.62
5	5.25	0.47	8.99	105.00
10	10.49	0.45	4.31	104.90
25	25.45	0.75	2.96	101.81
50	46.27	0.99	2.14	92.54
100	97.13	2.21	2.28	97.13



PC IgE SIL IS response

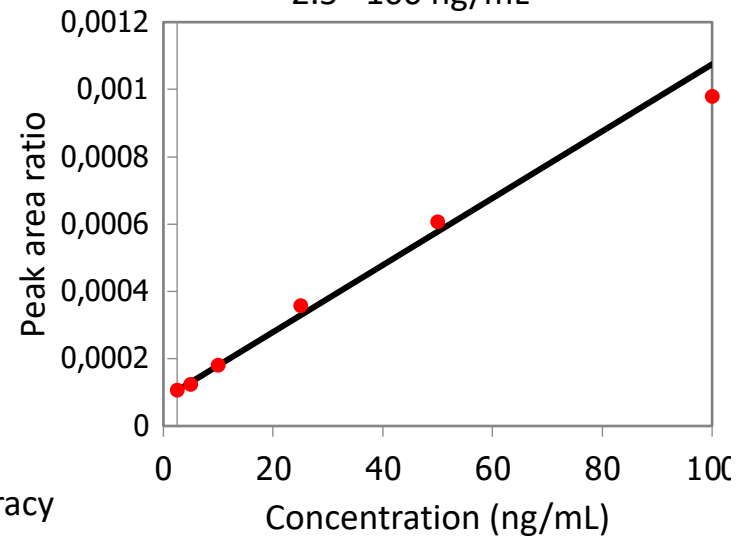
MSD

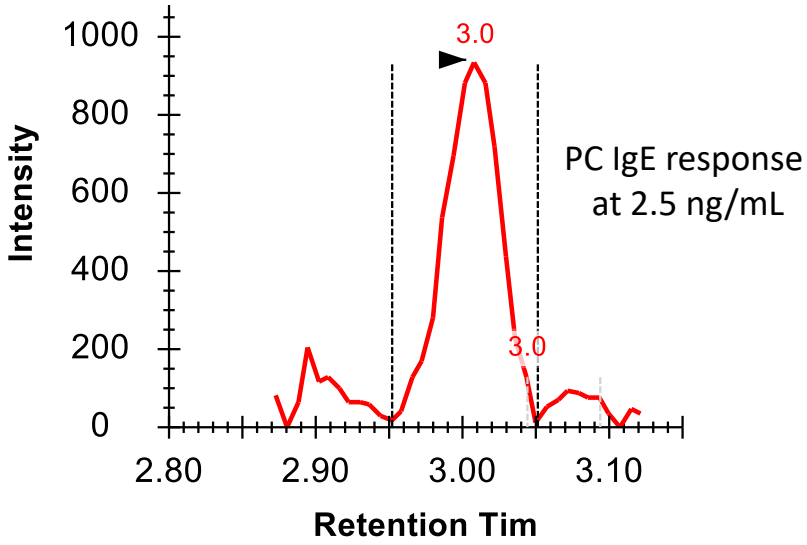
ADA IgE Isotyping by Direct LCMS Assay – anti-YTE PC sensitivity assessment

The sensitive anti-YTE PC chosen for sensitivity improvement

LLOQ: 2.5 ng/mL PC without drug using the optimized LC MS assay

Curve shows linearity for PC concentrations 2.5– 100 ng/mL

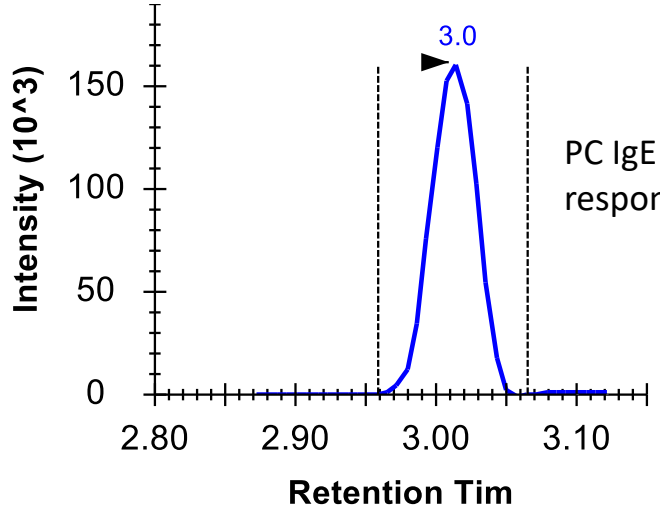




PC IgE response at 2.5 ng/mL

Curve showed good precision and accuracy

Expected Concentration	Mean experimental concentration	Standard Deviation	%CV	Accuracy
2.5	2.63	0.59	20.60	105.27
5	4.36	0.43	9.95	87.12
10	9.99	0.31	3.13	99.93
25	27.85	1.42	5.10	111.41
50	52.88	3.96	7.48	105.77
100	90.51	0.13	0.14	90.51



PC IgE SIL IS response

ADA IgE Isotyping by Direct LCMS Assay - drug tolerance

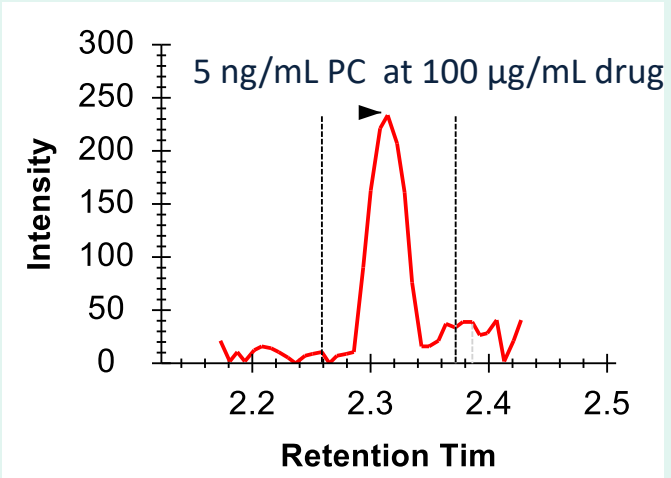
PCs incubated with 100 and 300 µg/mL drug for an hour and frozen

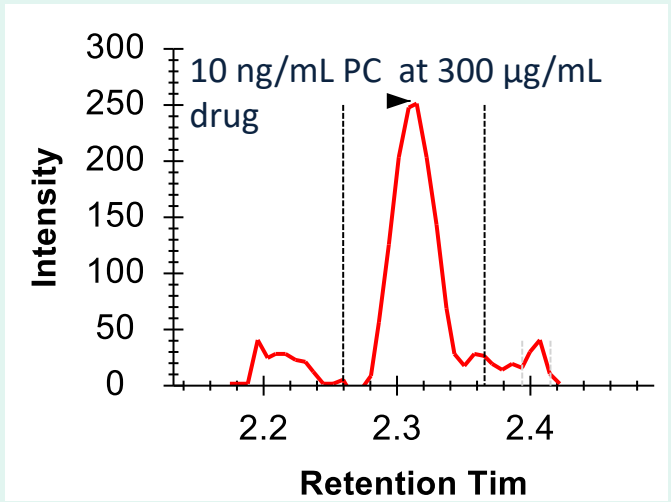
Drug tolerance:

5 ng/mL PC at 100 µg/mL and

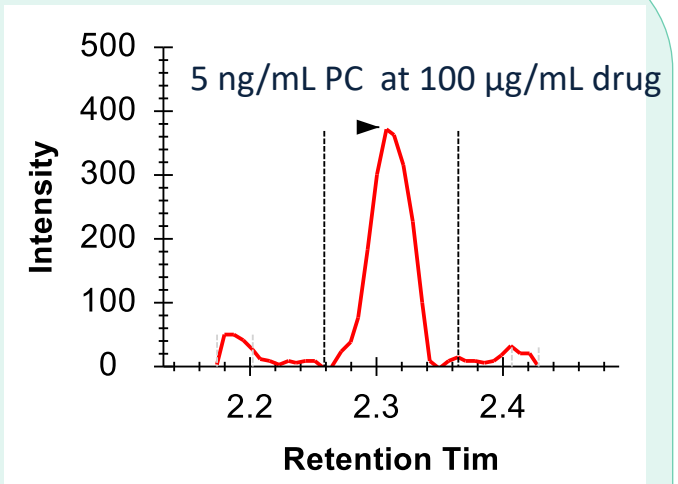
10 ng/mL PC at 300 µg/mL

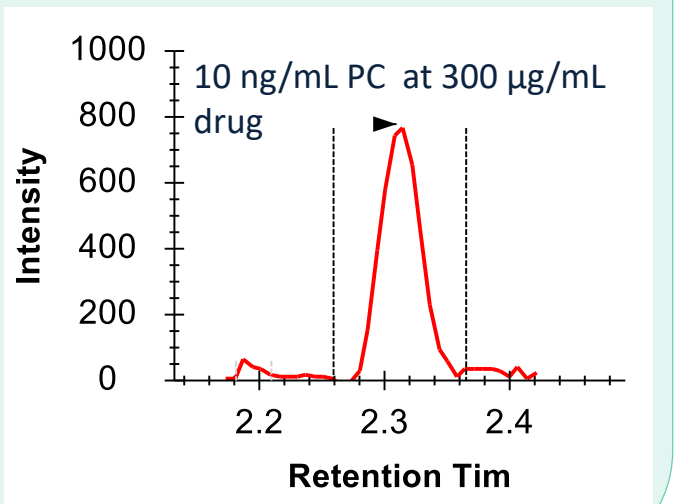
Anti-ID PC



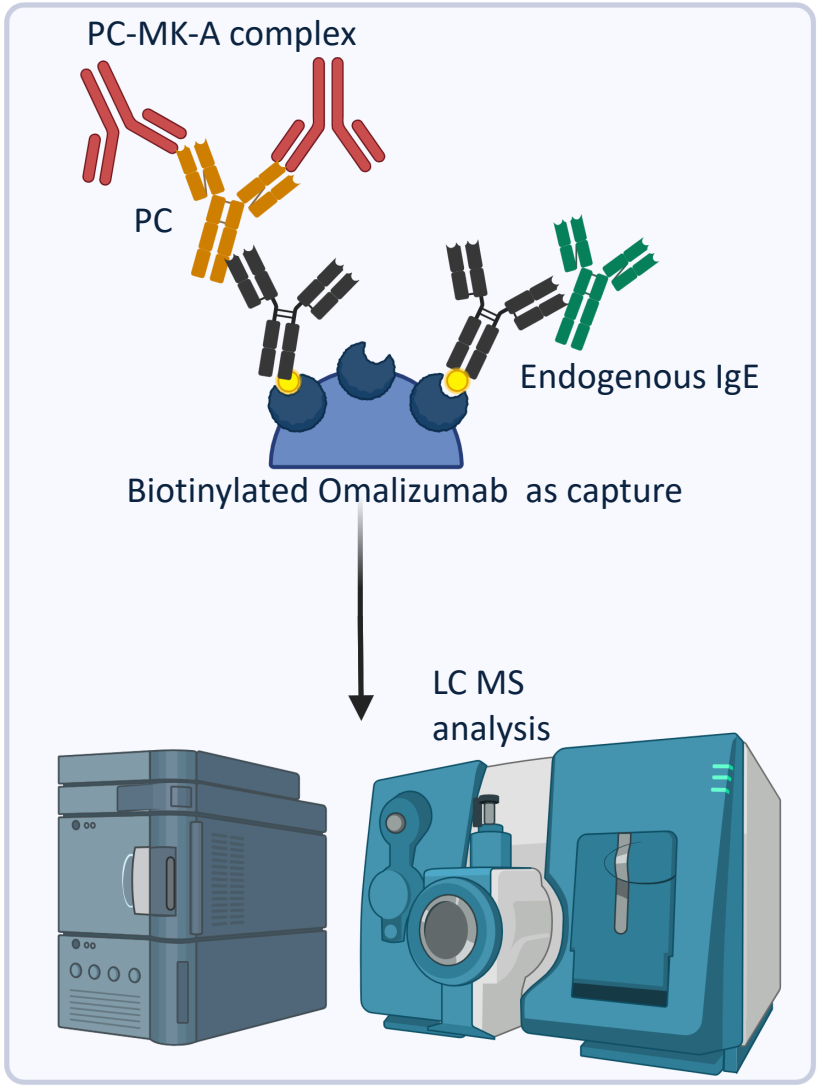


Anti-YTE PC





ADA IgE Isotyping by Indirect LCMS Assay



Measured analyte: MK-A signature peptide

PC: anti-YTE and anti-ID

Add excess drug to bind ADA and form immune complex

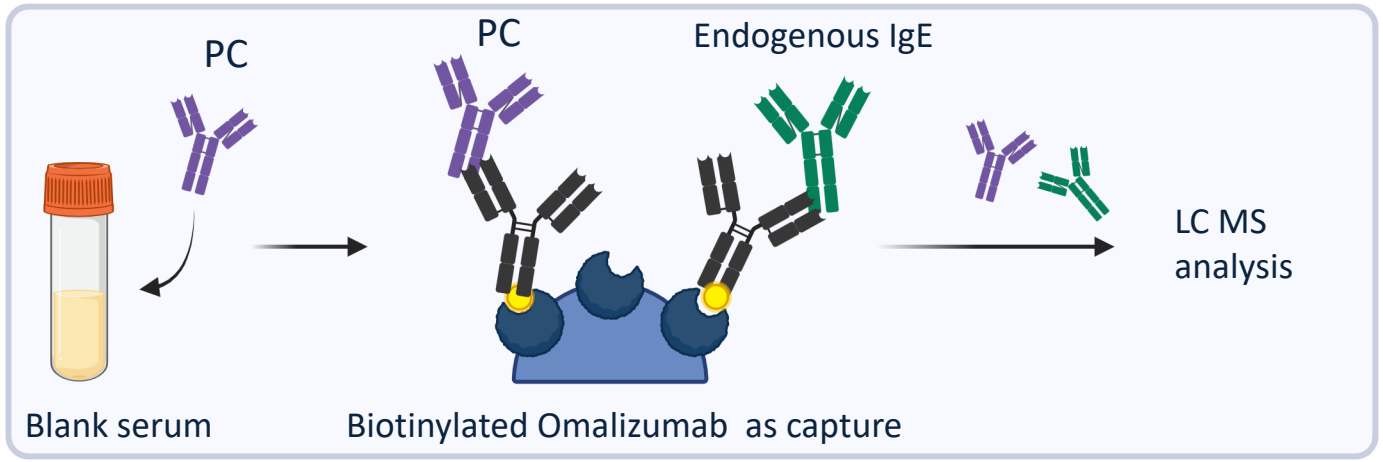
Anti-IgE capture of PC-MK-A complex by biotinylated omalizumab

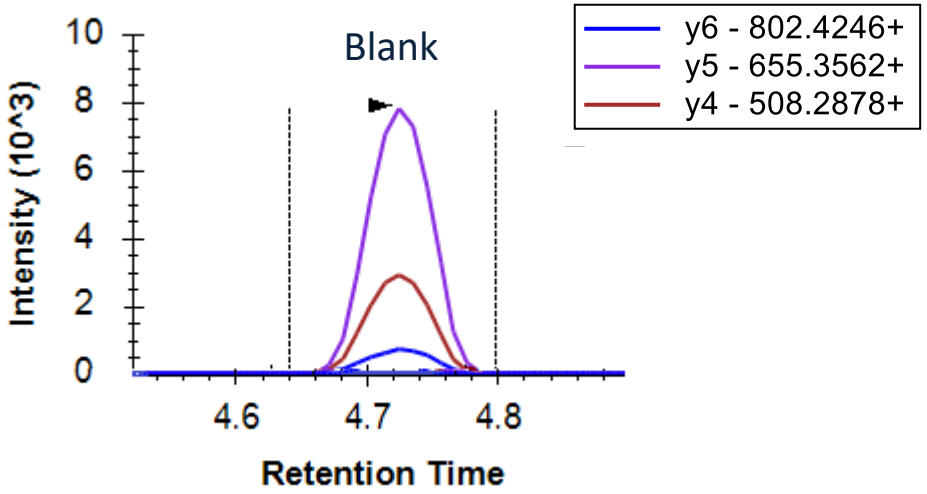
Omalizumab: a recombinant humanized mAb against IgE; binds selectively to Fc fragment on the heavy chain

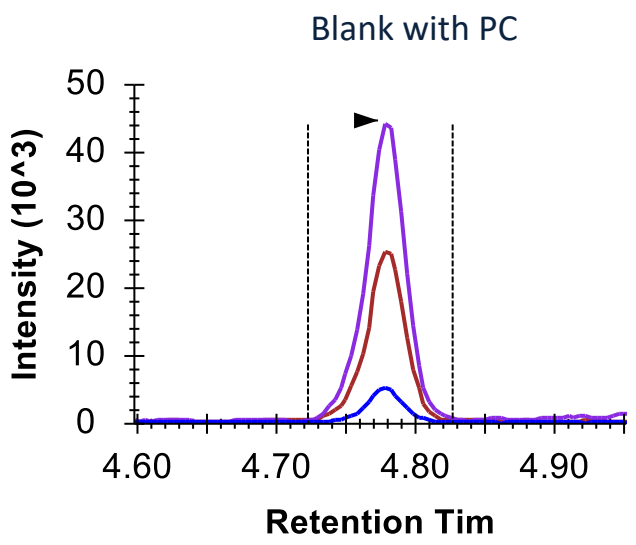
LCMS detection of surrogate peptides unique to MK-A

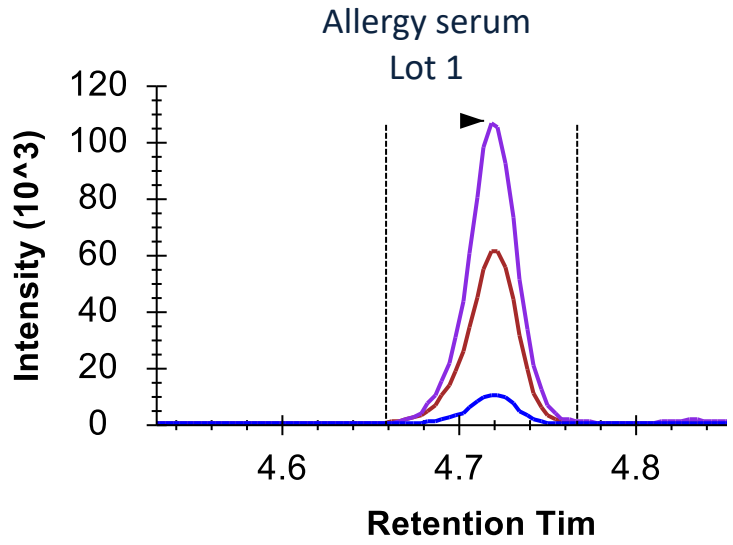
Expected to be resistant to drug interference

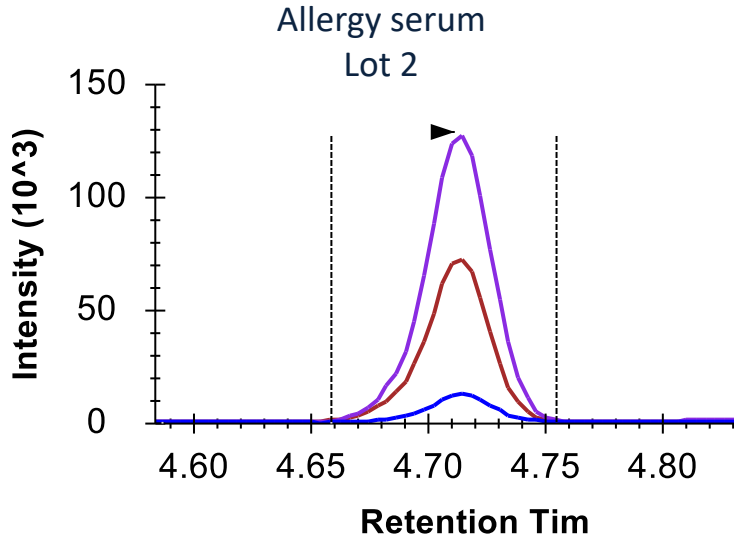
Omalizumab detects IgE in blank serum, PC spiked serum and in allergy serum









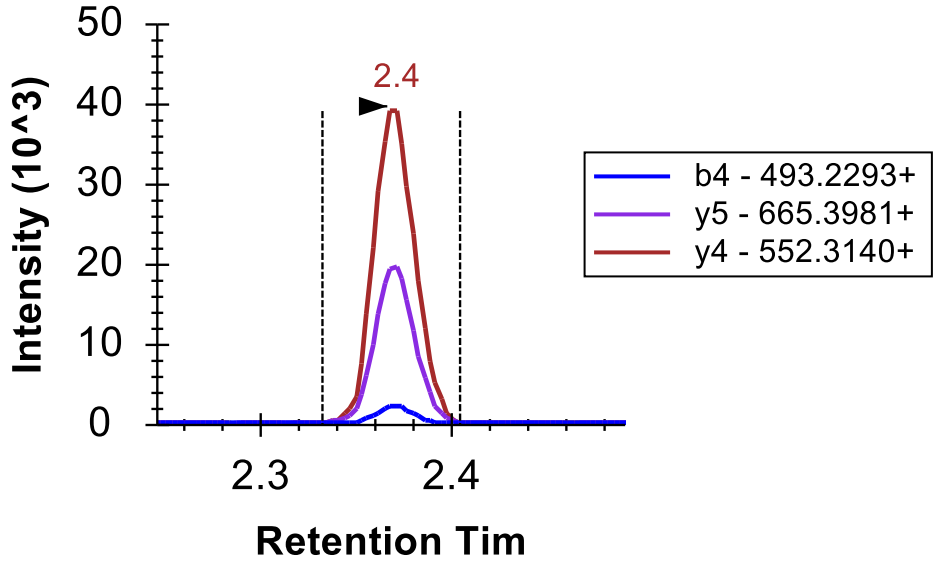


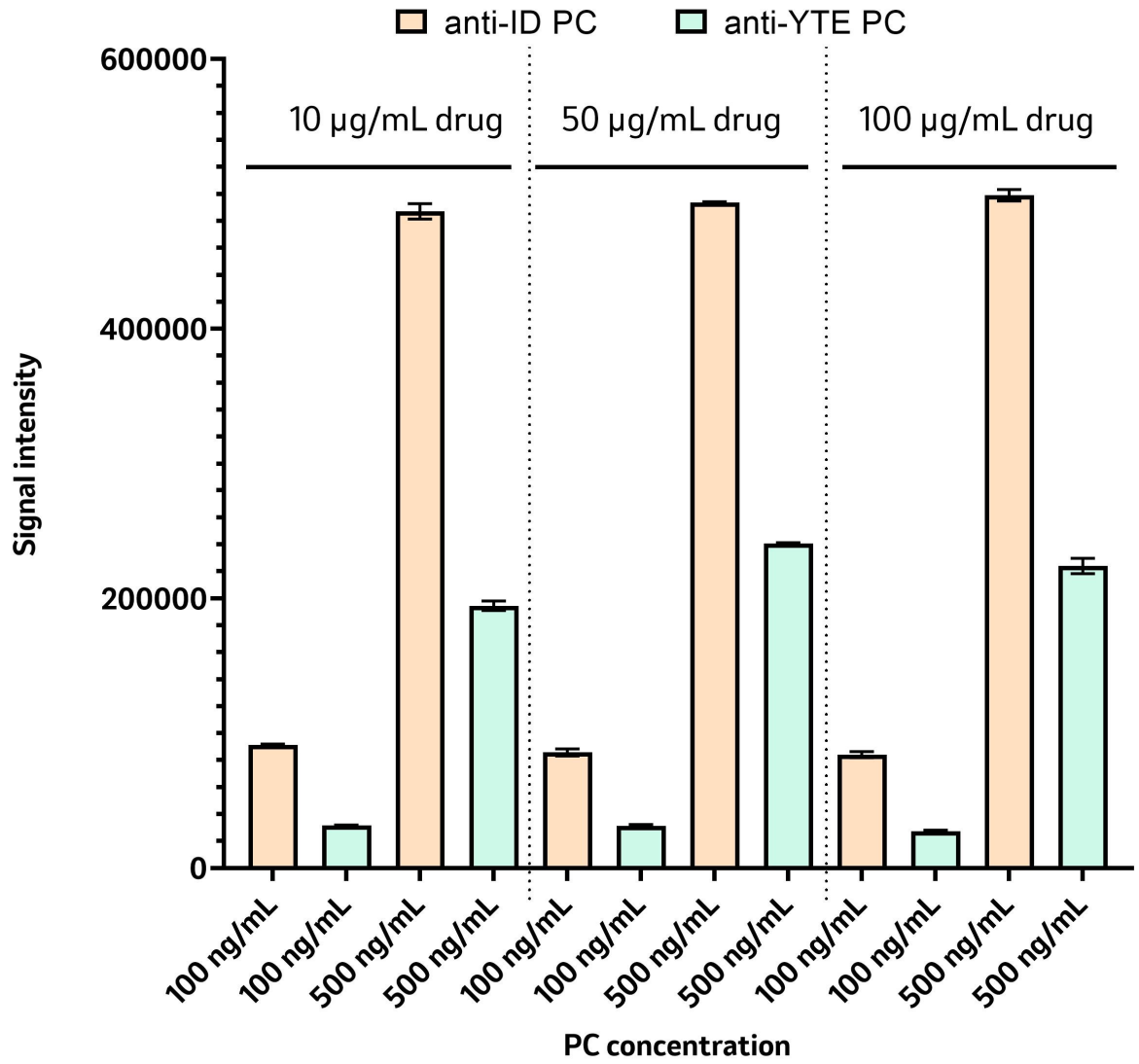
ADA IgE Isotyping by Indirect LCMS Assay - testing positive controls in increasing drug concentration

Tested PCs one of each kind, anti-ID and anti-YTE in presence of excess drug

Anti-ID PC has better response compared to anti-YTE. (SPR testing is ongoing)

Representative chromatogram for anti-ID PC, drug response





ADA IgE Isotyping by Indirect LCMS Assay – testing response linearity

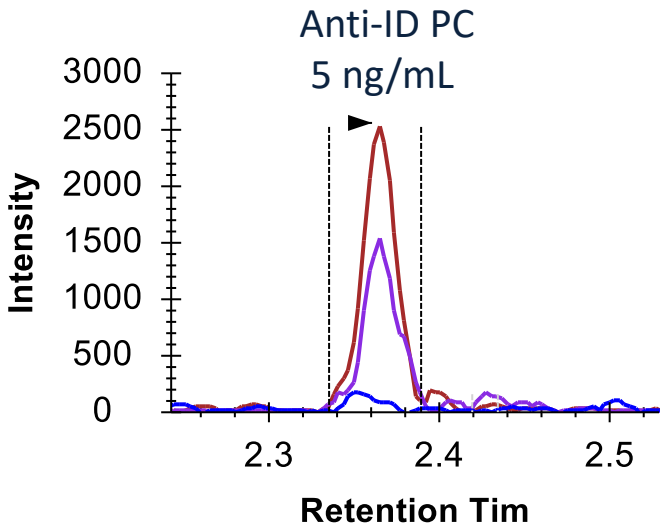
Tested a curve range for PCs to check the linearity of response

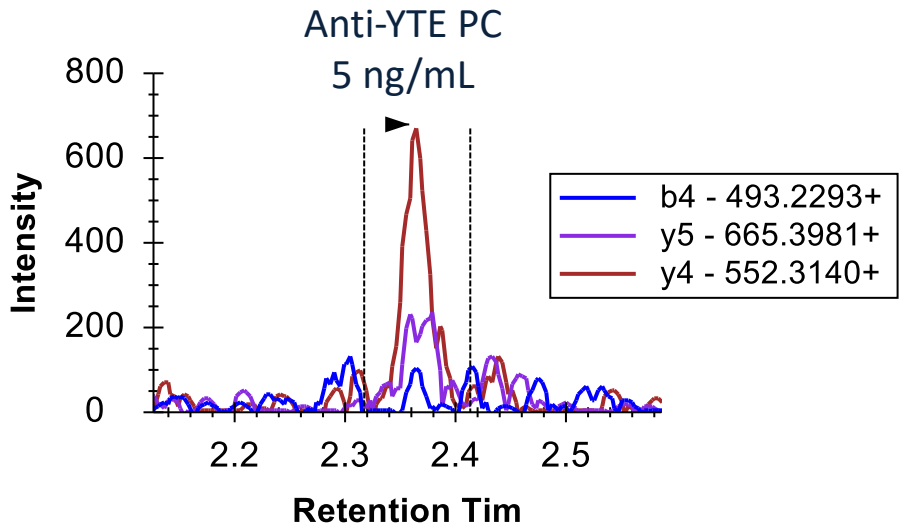
Linear response observed over the range tested

Drug tolerant LLOQ: 5 ng/mL PC at 300 µg/mL MK-A

PC (5 -500 ng/mL) incubated with 300µg/mL drug in human serum

Expected Concentration	Mean	Standard Deviation	%CV	Accuracy
5	5.11	1.05	20.52	102.14
10	9.63	0.86	8.97	96.26
50	45.19	5.88	13.00	90.38
100	114.70	7.54	6.57	114.70
250	238.77	18.68	7.82	95.51
500	501.99	9.21	1.83	100.40





Anti-ID PC has better response compared to anti-YTE

Hypersensitivity grades and levels of allergen/drug specific IgE

FDA 2019 guidance “Immunogenicity Testing of Therapeutic Protein Products - Developing and Validating Assays for Anti-Drug Antibody Detection”
FDA recommended assay sensitivity - high pg/mL to low ng/mL range

Rating of specific IgE level (kUA/L)	Grade/Class	
Absent or undetectable (< 0.35)	0	
Low (0.35–0.69)	I	transient flushing or rash a fever of less than 38°C (100.4°F)
Moderate (0.70–3.49)	II	rash or flushing, urticaria, and dyspnea with or without a fever of more than 38°C;
High (3.50–17.49) → 8.4 - 41.9 ng/mL	III	rash, dyspnea, and hypotension.
Very high (17.50–49.99) →41.9 – 120 ng/mL	IV	anaphylaxis Life threatening consequences
Very high (50.00–100.00)	V	
Extremely high (> 100.00)	VI	

Williams P et al., Clin Exp Immunol. 2008 Jul;153(1): 10-8
Chung CH et al., N Engl J Med. 2008 Mar 13;358(11): 1109-17
Stubenrauch K et al., Clin Therap. 2010 Aug ;32(9): 1597-1609
Bloem, K et al., Therap Drug Monit. 2017 Aug; 39(4); 327-332

Summary and perspective

LCMS offers flexible assay format (direct and indirect), tolerance to interferences and possibility to measure MK-A ADA IgE

ADA LC MS assay qualification for sample testing

Potential for LCMS semi-quantitation of ADA levels to help characterize ADA kinetics and aid PK/PD modelling

Potential for expanding to total ADA and NAb assay applications

ADA LCMS assay - ADA incidence and magnitude comparison

Proposed “Cut-point”: assay LLOQ as the threshold for ADA+ incidence

Magnitude reported as conc. equivalent to surrogate reference standard

Correlation between LBA S/N/titer and LC MS conc.

- Read-out from in vitro PCs
- Read-out from in vivo study samples

Consider the totality of evidence - assay usefulness depending on clinical relevance in the context of PK, efficacy and safety

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Thank you