A drug and target-tolerant hybrid LBA-LC-MS/MS method for (semi-) quantification of ADA isotypes, using a single-tier approach

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Presentation outline

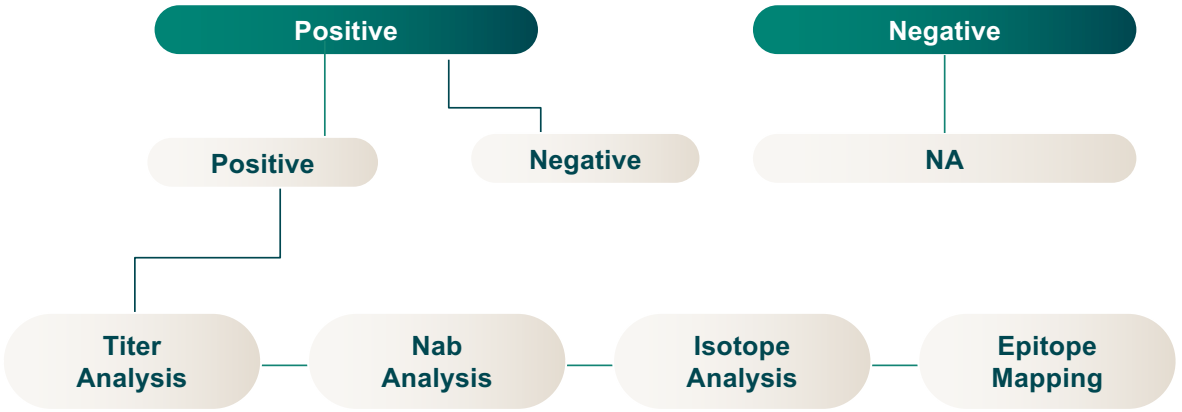
- Introduction
- Case Study
- Hybrid-LBA-LC-MS/MS Method
- Method Validation
- Conclusions
- Acknowledgements

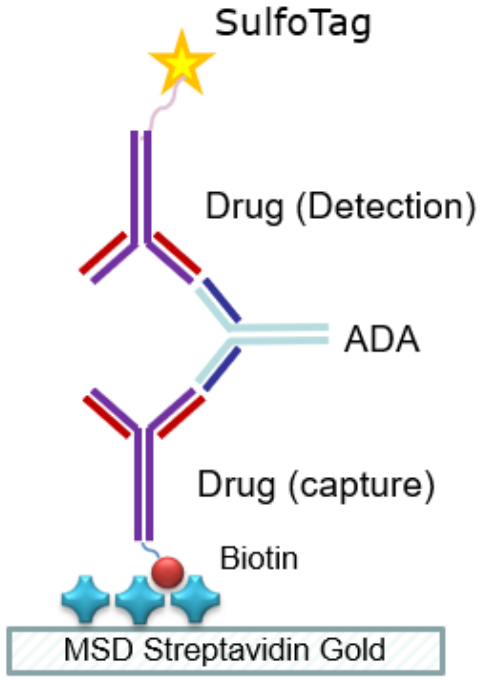
Immunogenicity assessment

Screening assay

Confirmatory assay

Characterization assay





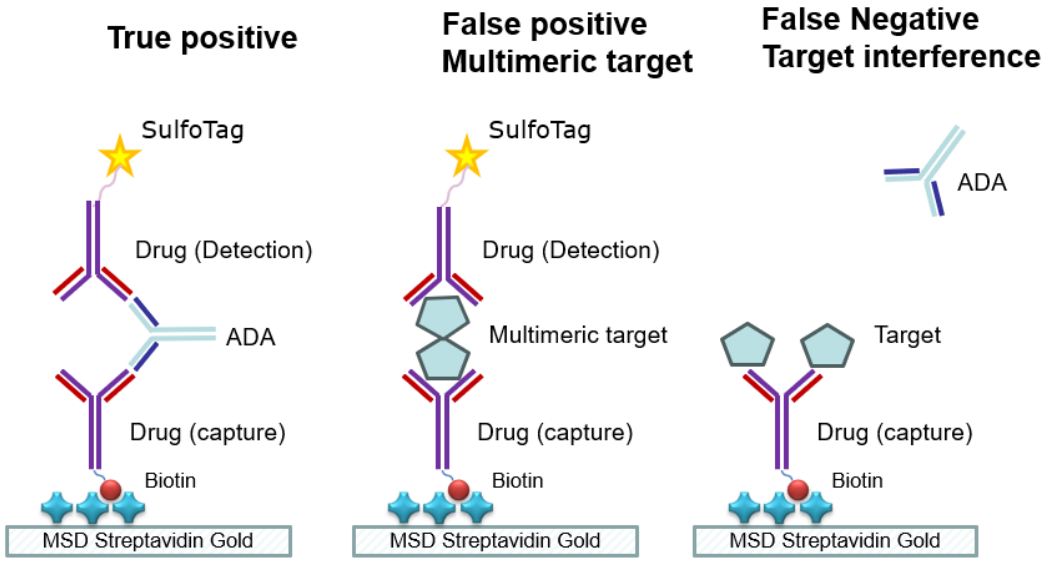
Classical bridging format

Case study

The sponsor asked us to set up an ADA assay

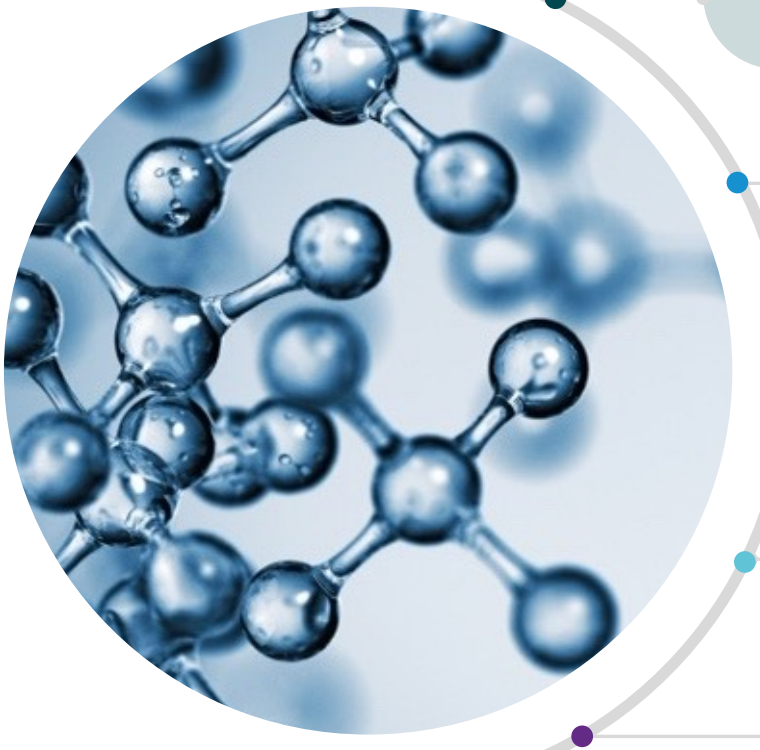
Complications:

- Samples contain relative high concentrations of the target (>10 µg/mL)
- The target can form multimers
- Protein A/G detection not possible
- SPEAD and PANDA are less suitable for high target concentrations



Solution: a hybrid LBA LC-MS/MS method

Hybrid-LBA-LC-MS/MS



Immuno-affinity extraction (LBA) of ADA with LC-MS/MS detection

LBA → Selectivity and specificity in extraction
LC-MS/MS → Separation, Detection and Quantification

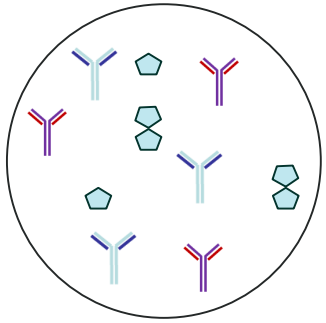
Tryptic digestion to release subclass specific peptides
(from the Fc region of the ADA isotypes)

Calibration standards, analyzed as separate samples

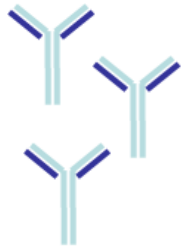
Quantification of extracted ADAs → Semi-quantitative

Hybrid-LBA-LC-MS/MS schematic overview

Sample



Extract ADAs

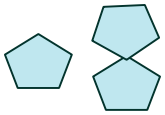


Add internal standards for each isotype

Tryptic digestion

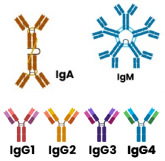
LC-MS/MS Analysis

Reduce/Remove



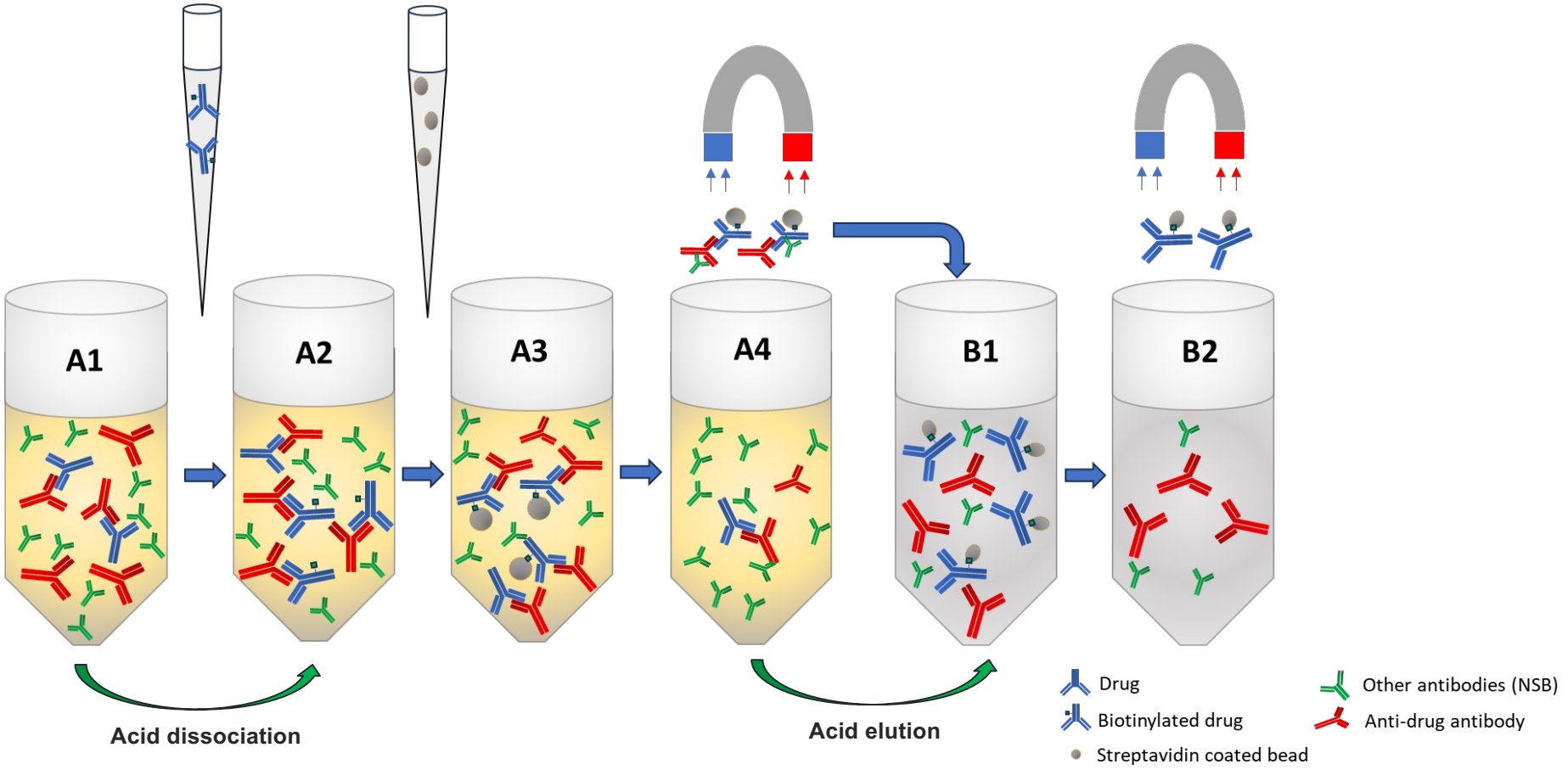
Mono- and Multimeric drug-target (not detected)

Drug

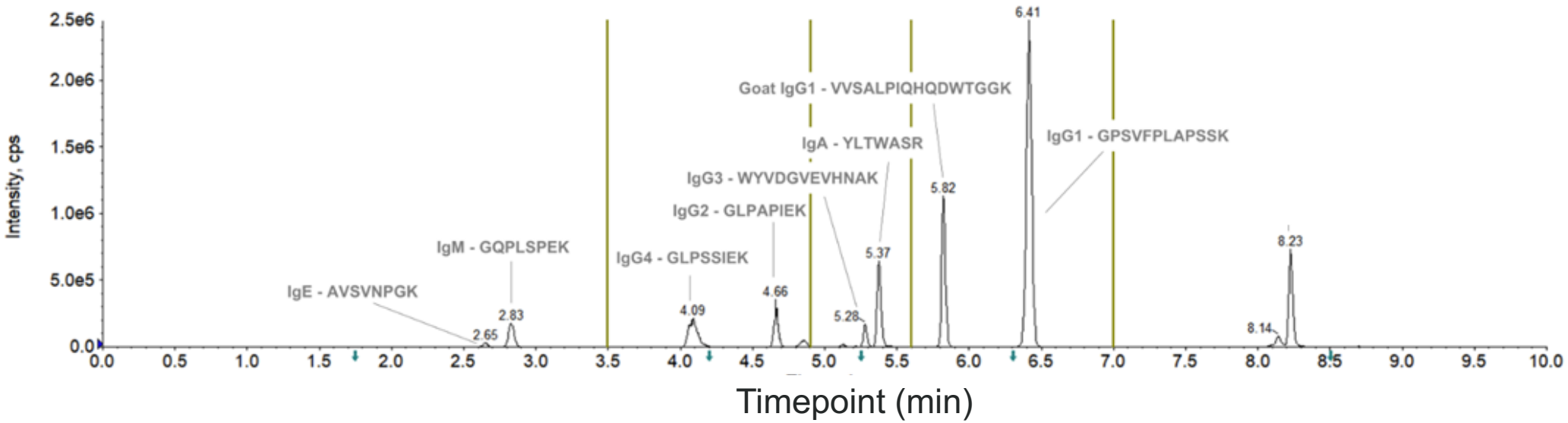


Non-ADA immunoglobulins

Bead-based Extraction Method - Details



Chromatogram, one method for all isotypes



LC-MS/MS chromatogram of the calibration standard at the highest concentration after digestion, showing the signature peptides of each immunoglobulin isotype.

Data submitted for publication Frank Schalk et al 2026

Method Validation Experiments

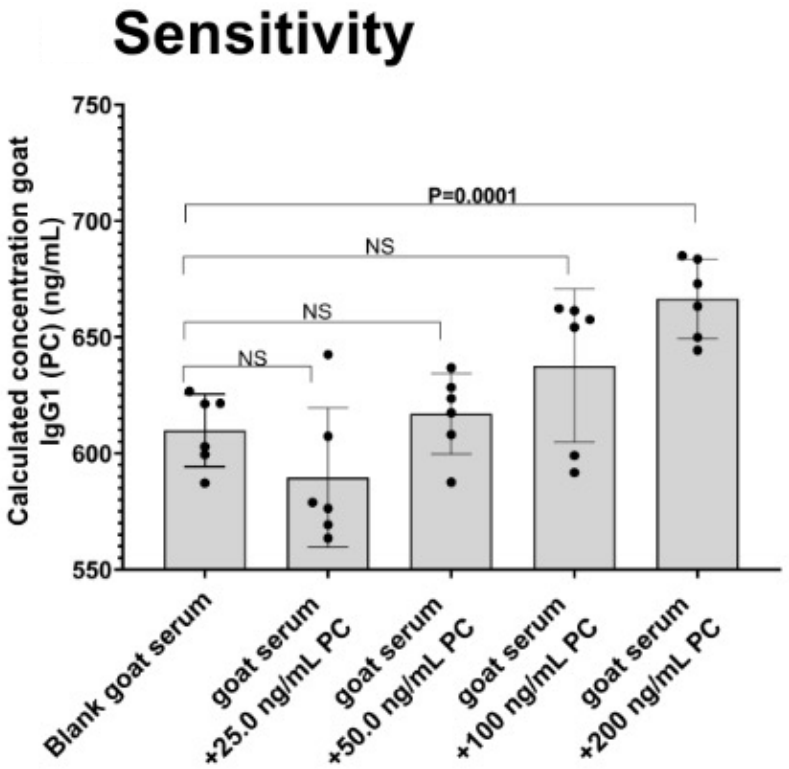
Cut point	Human citrate plasma (50 individual lots), 3 runs, 1 replicate per sample per run
Sensitivity	Goat serum, 1 run, 6 replicates per run
Drug Tolerance	Human citrate plasma (spiked at relevant drug levels), 1 run, 6 replicates per run
Target Tolerance	Human citrate plasma, 1 run, 6 replicates per run
Selectivity	Human citrate plasma, 1 run, 3 replicates per run
Precision	Goat serum, 3 runs, 6 replicates per run
Stability	Goat serum, 1 run, 3 replicates per run (BT, F/T and frozen storage)
Specificity	Amount of non-specific bound immunoglobulins
MRD	Not relevant for Hybrid-LC-MS/MS
Prozone-effect	Not relevant for Hybrid-LC-MS/MS

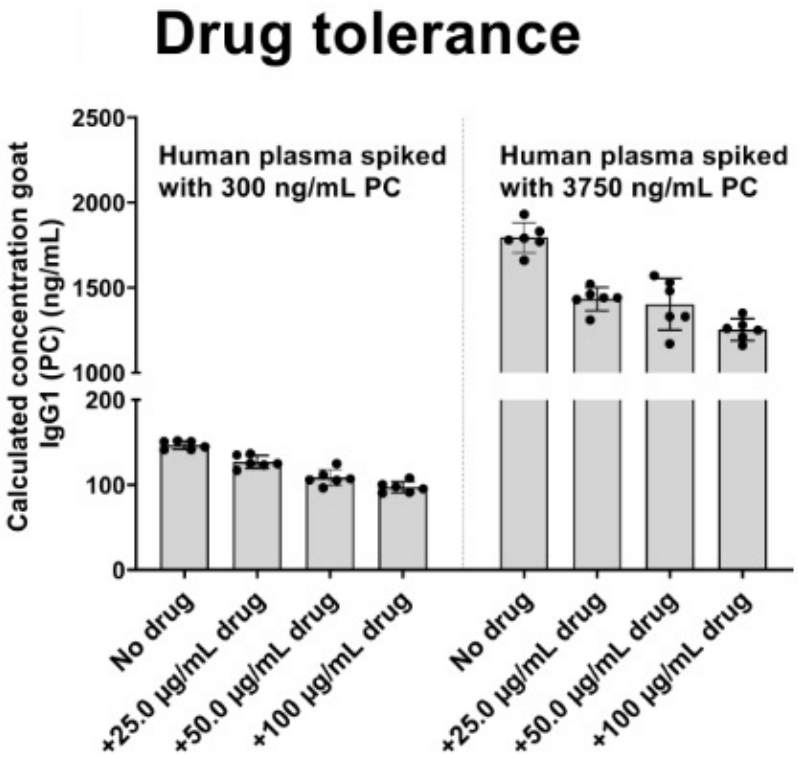
Cutpoint setting for each Isotype

Subclass	LLOQ (Calibrators) Buffer ng/mL	Average background Human plasma ng/mL	Cut-point 1% FPR ng/mL
IgG1	100	162	288
IgG2	50.0	78.3	136
IgG3	25.0	56.0	81.0
IgG4	N/A	57.8	247
IgA	25.0	34.4	64.0
IgE	5.00	N/A	N/A
IgM	25.0	51.7	112

Data submitted for publication Frank Schalk et al., 2026

Sensitivity and drug tolerance





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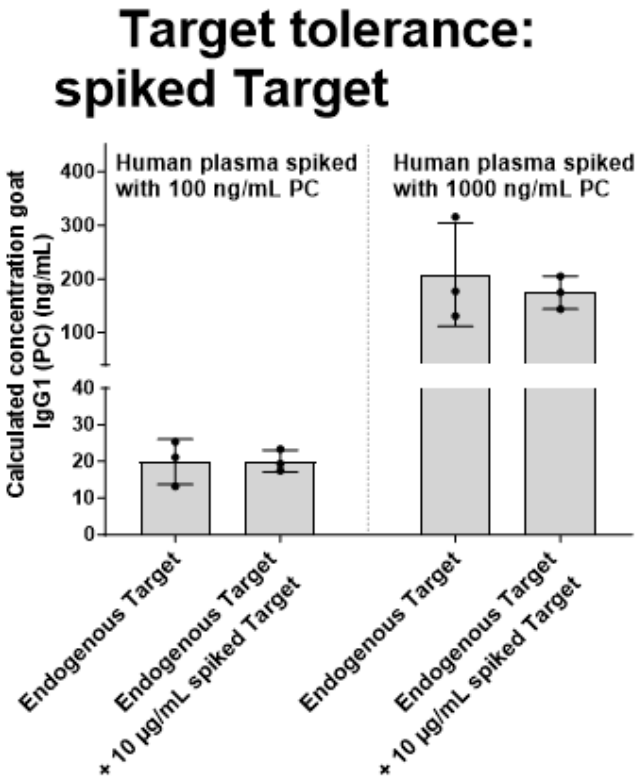
Target tolerance



Data submitted for publication Frank Schalk et al., 2026

Benefits of this Method over the Conventional Bridging Format

Single tier	No separate screening, confirmatory and characterization assays
Throughput	Comparable to classical ADA assessment, at a 20% confirmed positive
Specific	Can also detect IgG4 with only one available Fab-arm
High target tolerance	Limited interference of both mono- and multimeric target
Isotype characterization	Deeper insight into immune response and relation to clinical outcomes
High drug tolerance	Limited drug interference
Singlicate analysis	Higher throughput, lower sample volume required
Blueprint	Flexible framework for future immunogenicity assay development

Isotyping & subclass characterization

Five different isotypes, differ in biological functions and physiological location

IgG
- Most abundant antibody (~78% of plasma antibodies)
- Divided in 4 subclasses IgG1 (53%), IgG2 (18%) IgG3 (6%) and IgG4 (3%)
- Produced by memory B-cells and plasma cells and secreted in the blood
- Good indicator of secondary immune response

IgM
- Pentameric or hexameric antibody (~10% of plasma antibodies)
- First response on initial exposure to antigen
- Produced by B-cell either on cell surface or secreted in blood

IgE
- Low abundant (<0.01% of plasma antibodies)
- Responsible for allergic reactions, capable of triggering anaphylaxis

IgD
- Mainly location on B-cell surface
- Low abundant in plasma (~0.2% of plasma antibodies), not well understood

IgA
- Dimeric (~12% of plasma antibodies)
- Most abundant in secretions such as mucus, saliva, and tears
- Produced by mucosal lymphoid tissue

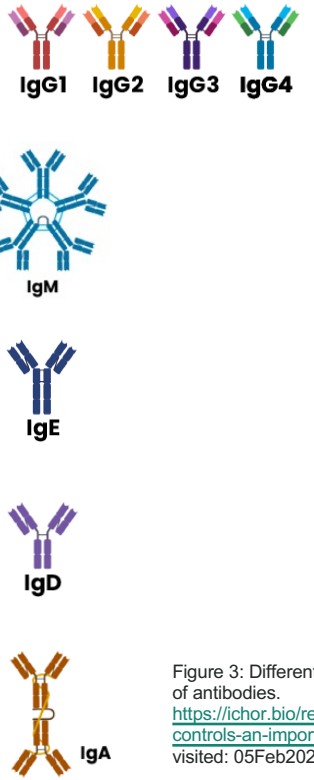


Figure 3: Different isotypes of antibodies. https://ichor.bio/resources/isotype-controls-an-important-experimental-tool/ visited: 05Feb2024

FDA (2019) and EMA (2017) Guidelines and ADA isotyping

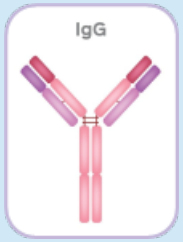
Non-mucosal routes of administration,
IgE isotype ADAs are not relevant

Allergic response → Class Switch

- Mild
  - Fever or skin rashes
- Serious
  - Anaphylactic-like reactions
  - Cytokine storm.

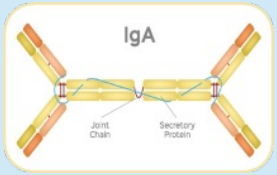
- Drug clearance
- Immune response

Class Switch →



- Rapid drug clearance
- (chronic) inflammation
- Tissue damage

Mucosal Class Switch →



- Local neutralising effect
- No inflammation

Other potential benefits of isotype classification

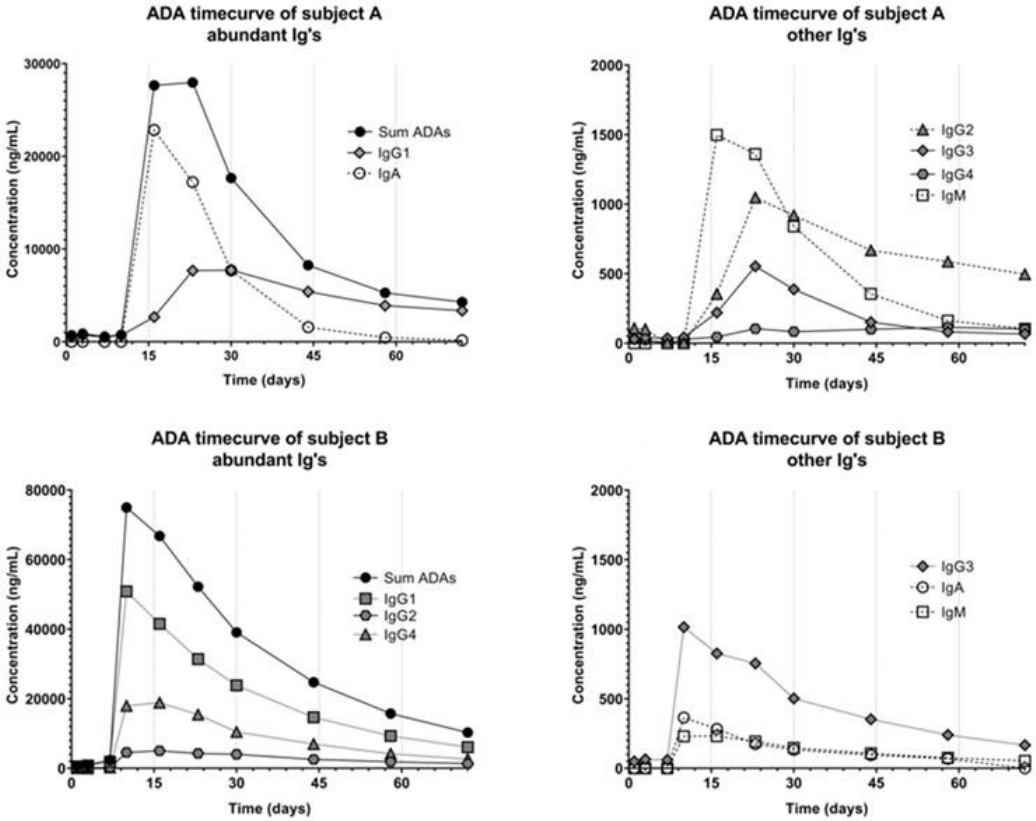
Characterisation of high pre-dose responses

- Cross-reactive antibodies to endogenous homologs
- Antibodies from prior exposure to similar biologicals
- Antibodies to shared epitopes (e.g. FC region)
- High IgM levels (multimeric binding)

IgG subclass matters

- IgG1: Most common clinically relevant ADA (early response, more polyclonal)
- IgG2: Usually lower inflammatory risk (prolonged exposure)
- IgG3: Pro-inflammatory responses, strong complement activation (early response)
- IgG4: Often linked to persistent or neutralizing ADA (prolonged exposure).

Example of variable isotype responses in two subjects



Source: Schalk et al., Bioanalysis, 17(2), 87–98 (2025), published by Taylor & Francis.

Conclusions

- A *novel* hybrid LC-MS/MS method for semi-quantification and isotyping of ADAs is developed
- This is a singlicate, single tier method
- This method is drug & target tolerant
- The throughput is comparable to classical screening, confirmatory, and titer assessments at a 20% confirmed positive.

Discussions & Limitations

- ADA isotyping can provide useful insights, but it may also generate excessive data that isn’t always clinically relevant.
- Relevant human PC would be preferred, the sensitivity of a Goat PC might be different
- The method is less sensitive than 100 ng/mL as described in ADA guidelines.

Acknowledgments

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