Approach to simultaneous detection, semi-quantification and isotyping of ADA in serum samples by LC-MS/MS

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Inspired by patients.
Driven by science.

Introduction

ADA (anti-drug antibodies)

Are formed as the result of an immune response against a biologic agent.

- May be highly specific for the drug.
- May lead to adverse events (hypersensitivity, cross reactivity with native proteins).
- May impact PK parameters.
- May reduce efficacy if neutralizing.
- May interfere with PK assays.

ADA analysis is a critical part of large molecule drug development

Immunoglobulin Isotypes

Immunoglobulin G (IgG)

- About 80% of total Ig.
- Major component of humoral immune response.
- 4 subclasses (igG1,IgG2,IgG3,IgG4).

Immunoglobulin M (IgM)

- Expressed on B-cells (monomer) and secreted (pentamer).
- Important in primary immune response.

Immunoglobulin E (IgE)

- Allergen binding, histamine release, allergy.

Immunoglobulin A (IgA)

- Mostly mucosal areas.

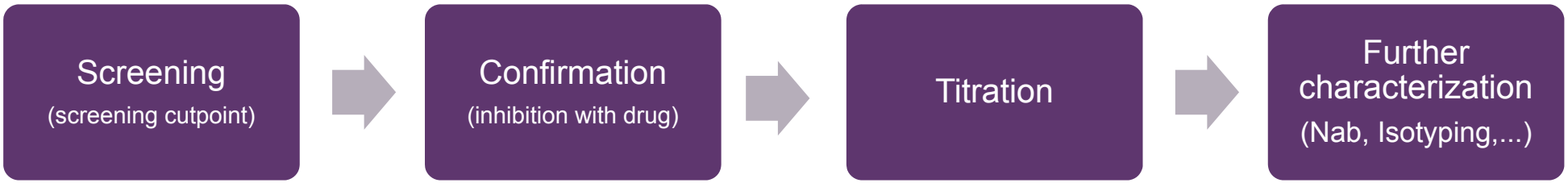
Immunoglobulin D (IgD)

- Mostly membrane bound on B-cells.

Standard Approach

Historically, ADA have been analyzed by ligand binding assay techniques, which remain the “gold standard” for immunogenicity testing.

Usually, a tiered approach in sample analysis is used.

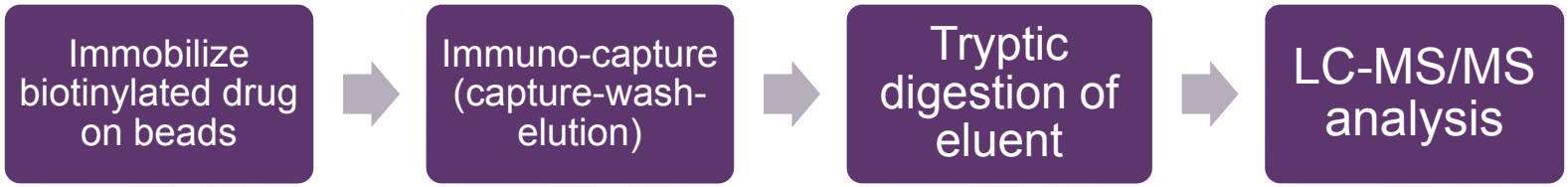


- Quasi-quantitative assays.
- Isotyping requires development of multiple assays.

Could LC-MS/MS add value/provide faster answers in ADA analysis?

Our strategy

Analysis is based on immuno-capture using streptavidin coated magnetic beads



Selective peptides were chosen for monkey IgG, IgM and IgE and analyzed by LC-MS/MS

- Must be universal.
- Must be unique within the matrix (cynomolgus monkey).
- Must display adequate sensitivity and chromatographic properties.

In this case data was compared to those obtained by ligand binding assay (reference method)

Analytical method

Sample preparation

- 1µg of biotinylated drug in 100µL buffer (TBS, Tween 20, BSA) coated onto 100µg of beads.
- 10µL plasma diluted in 90µL of buffer .
- Elution in 0.2M Glycine buffer pH2.
- Wash between each step with buffer.

Peptides

- IgG: DTLMISR, common to all subclasses.
- IgE: GTVNLTWSR.
- IgM: GQPLSPEK.

LC-MS/MS

- C_{18} chromatography (ACE chromatography, 250x2.1mm, 2.5µm), API5500 QTrap in MRM mode.
- Quantification ranges from 500 – 10000ng/mL.

Standardization

A difficult question with multiple answers

- In this case the easiest strategy was used: pure IgG, IgM and IgE standards in elution buffer without any extraction procedure. This means there is no compensation for losses during immuno-capture.

Precision and Accuracy (3 levels, n=5, 3 runs)

Isotypes	Inaccuracy (%)	Precision (RSD %)
IgG	≤5.7	≤4.8
IgM	≤2.9	≤5.4
IgE	≤10.5	≤7.0

Extraction yield

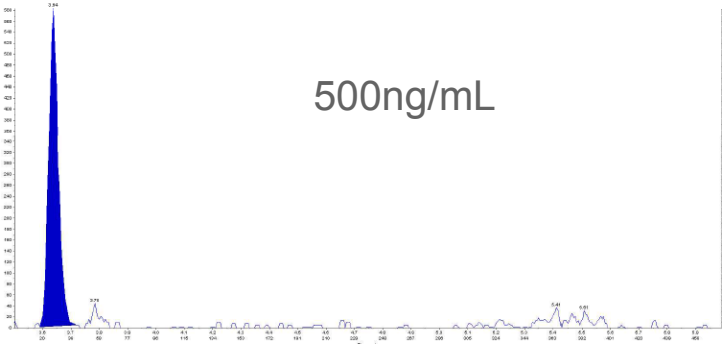
Since losses during immuno-capture are not compensated for by the calibration standards extraction yield should be determined

- Performed extraction yield experiment using a monoclonal rabbit IgG (used as positive control in the LBA assay) and compared signals between eluents from spiked serum subjected to immuno-capture to solutions prepared in elution solvent.

Recovery

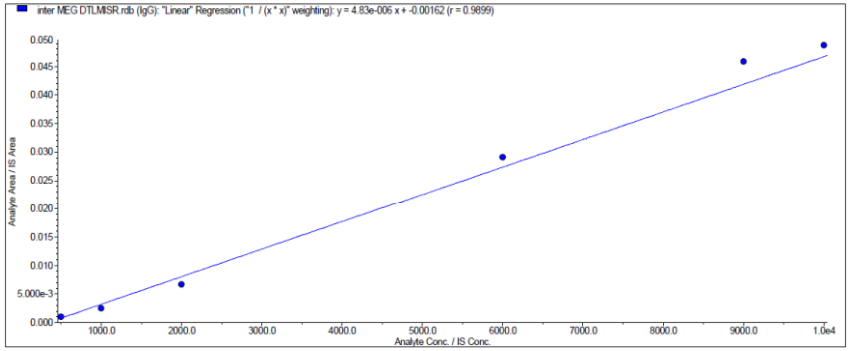
Isotype	Extraction yield (%)	Precision (SD %)
IgG	47.8	12.5

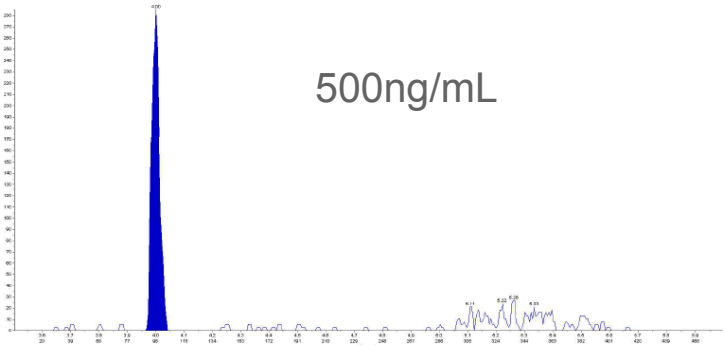
LC-MS/MS assay



500ng/mL

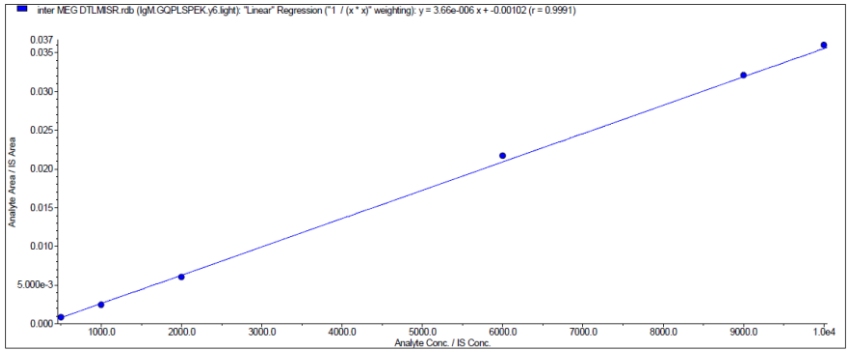
IgG

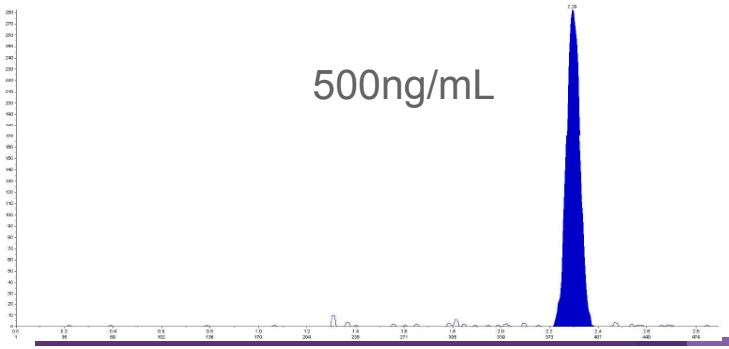




500ng/mL

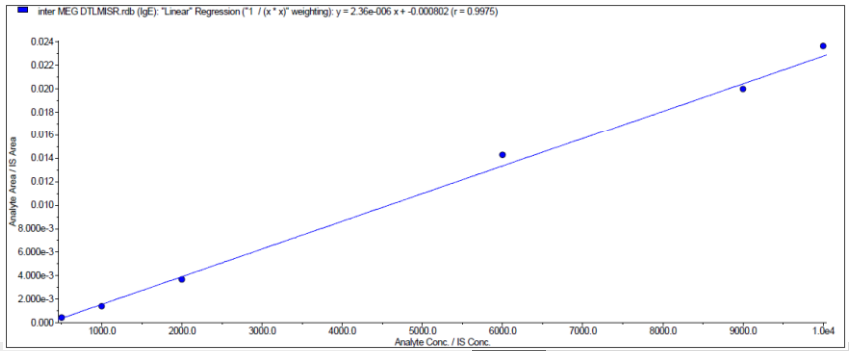
IgM





500ng/mL

IgE



Reference method

- Homogeneous Bridging assay with an ECL endpoint on the MSD platform.
- Fit-for purpose characterization.
- 30 individual monkey sera used to establish cut-point (4 qualification batches, 2 operators).
- Original ECL signals normally distributed. Batches means and variances equal.
- A fixed screening cut point (SCP) of 85 ECL units was established (parametric approach AVG + 1.645 x SD) and used to screen “potentially positive samples” (allowing for 5% false positive rate).
- A fixed confirmation cut point (CCP) of 15.4% (parametric approach AVG + 2.33 x SD) signal reduction was used in the confirmatory set-up (allowing for 1% false positive rate).
- No titration performed, ECL units reported as a quasi-quantitative measure

Sample analysis

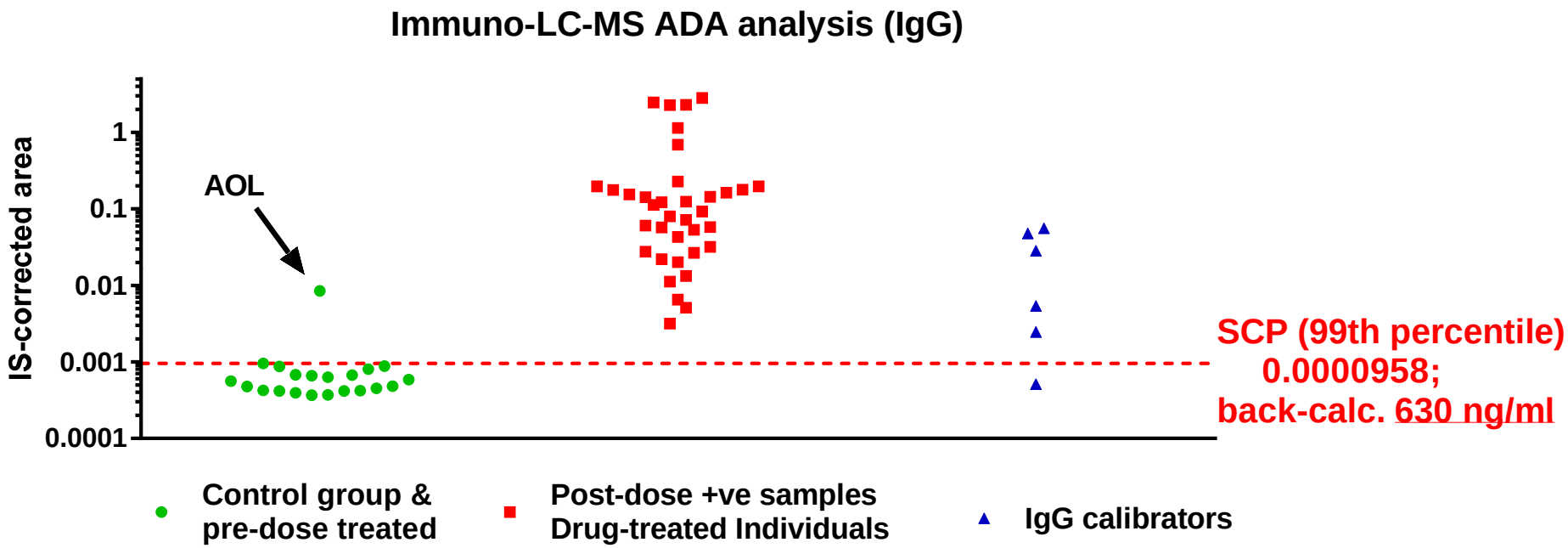
Samples from a 4-week tox study were analyzed for ADA both by LBA (MSD) and hybrid immuno- capture LC-MS/MS.

- Three dose groups, Control, low and high dose.
- Dosing every week.
- Samples taken at pre-dose, day 15, day 22 and day 28.

Sample analysis

IgG

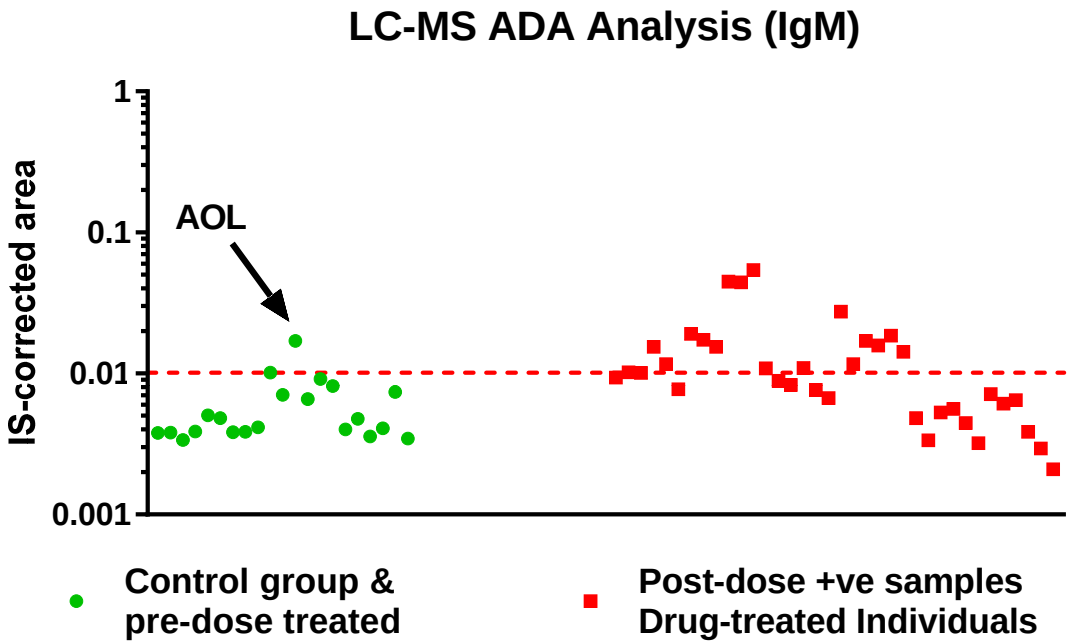
Cut-point determination



Sample analysis

IgM

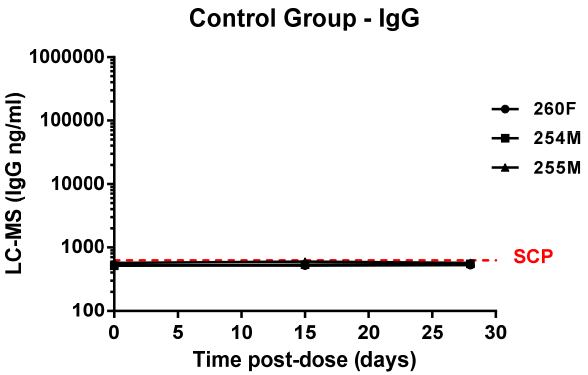
Cut-point determination

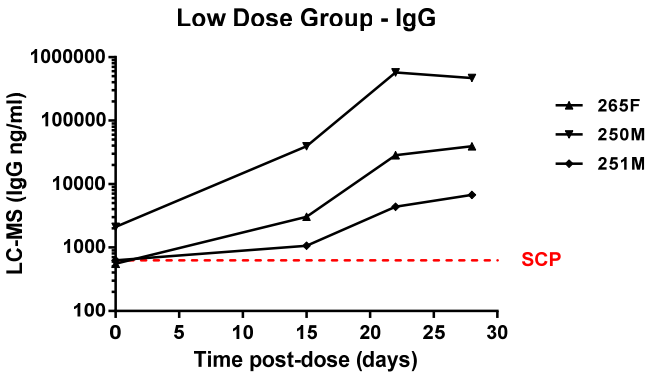


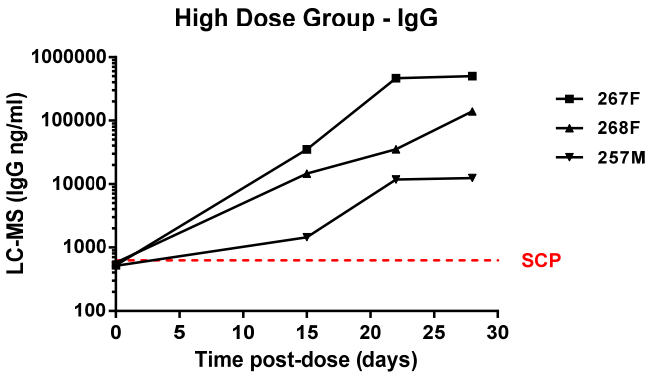
SCP (99th percentile)
0.01015;
back-calc.: 2870 ng/ml

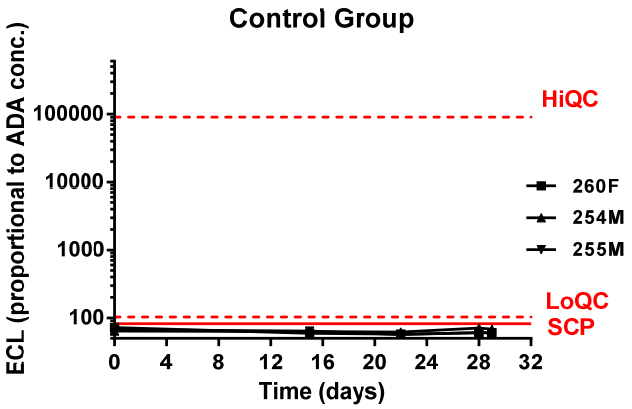
Sample analysis

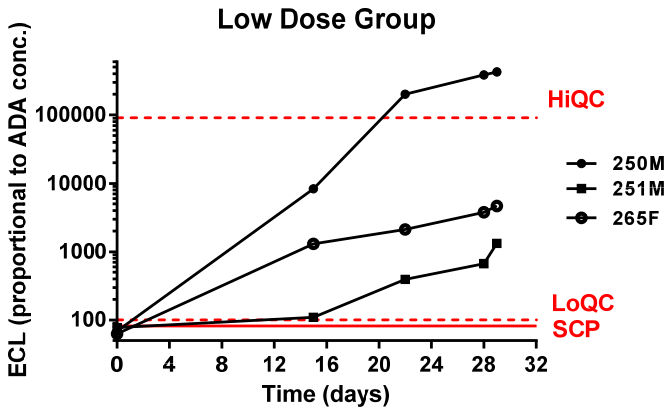
IgG Concentration measurements (top panel) compared to LBA (MSD) data (bottom panel)

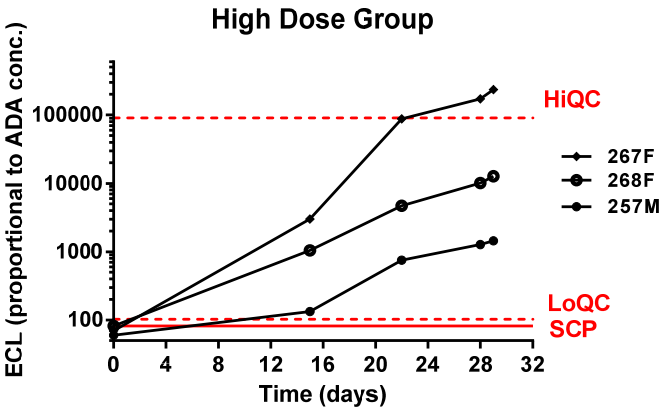






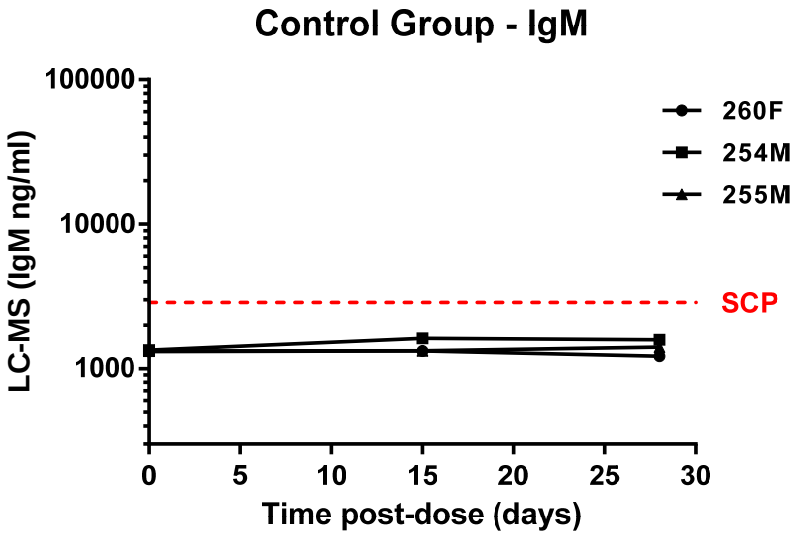


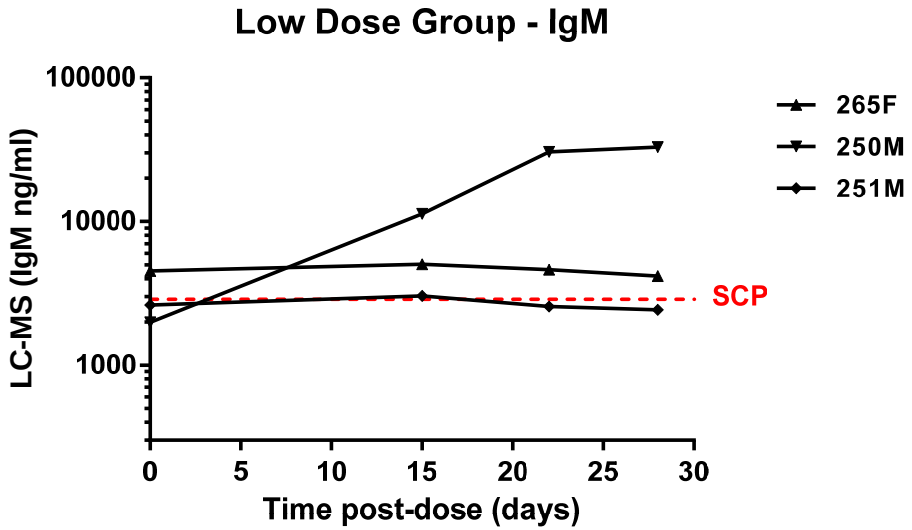




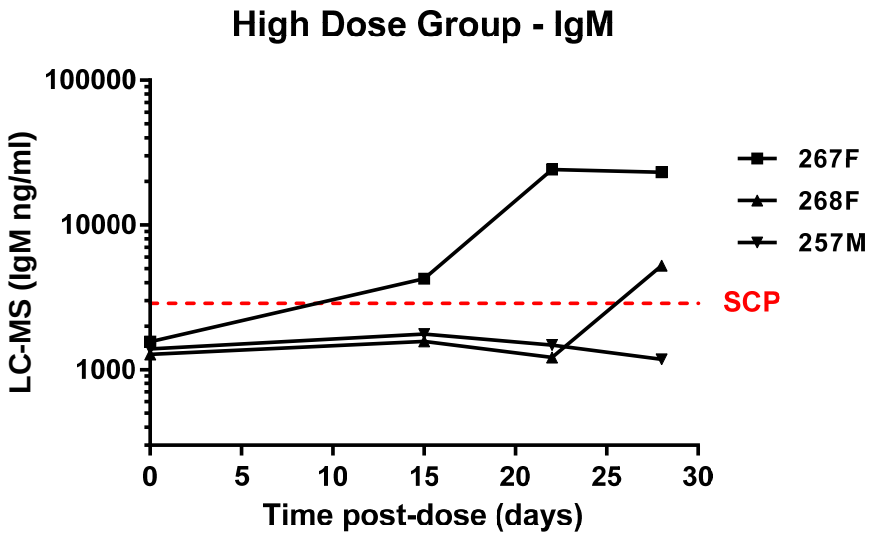
Sample analysis

IgM: Concentration measurements



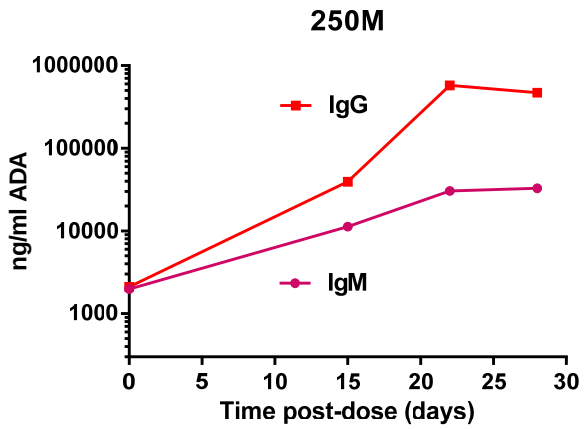


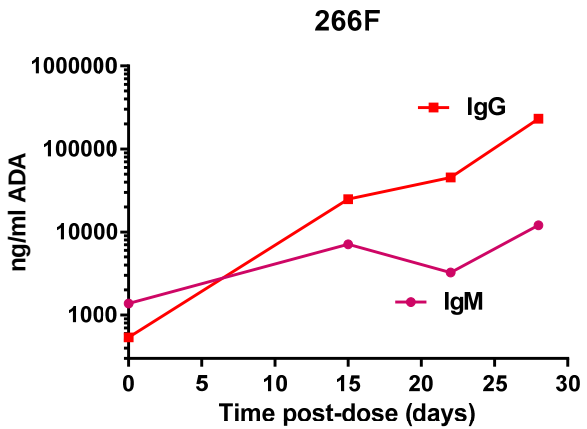
MUCH LOWER than IgG concentrations. Up to 30-fold differences

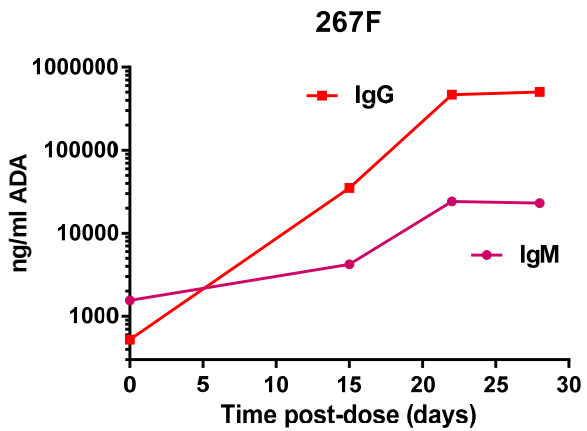


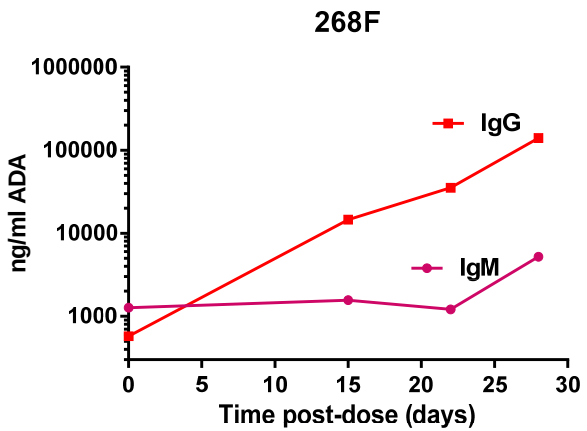
Sample analysis

IgM vs. IgG Concentration measurements in 4 individuals



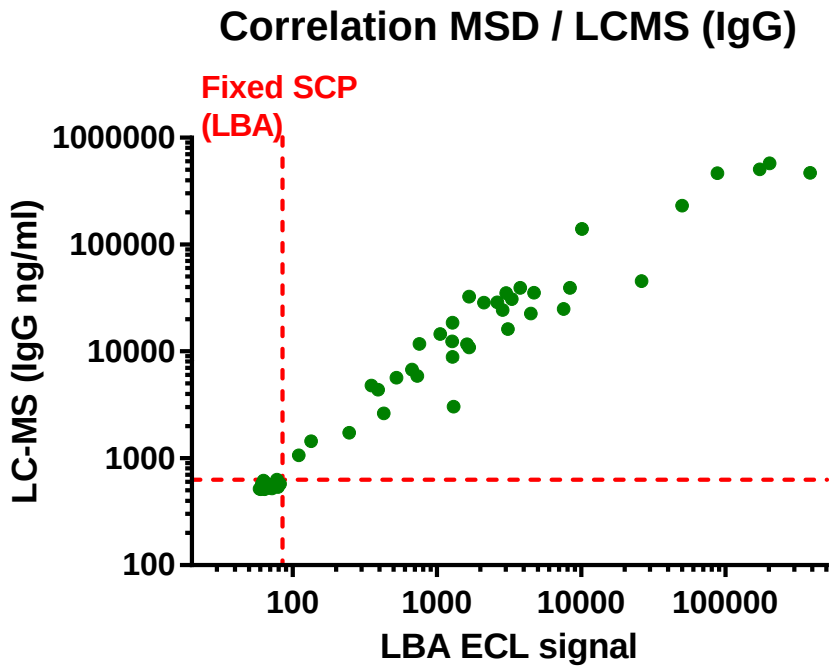






Sample analysis

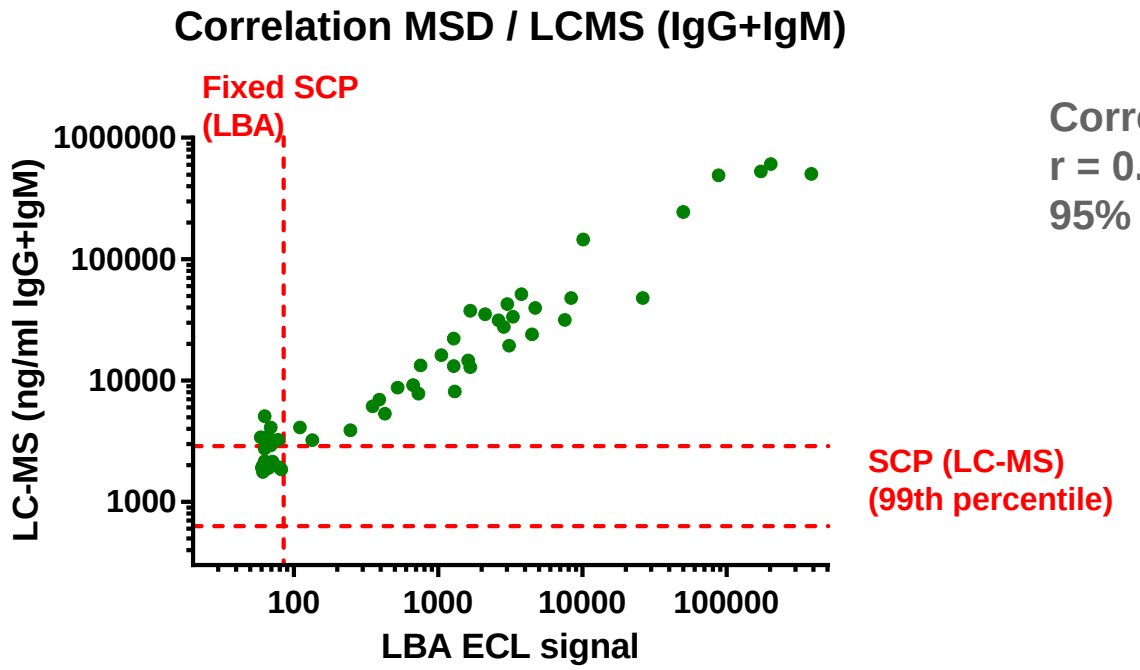
IgG: Correlation LBA vs. Immuno-capture LC-MS/MS



Correlation results
r = 0.8618
95% CI (0.7743 – 0.9169)

Sample analysis

IgG + IgM: Correlation LBA vs. Immuno-capture LC-MS/MS



Correlation results
r = 0.8656
95% CI (0.7813 – 0.9189)

Conclusion

ADA analysis by immuno-capture LC-MS/MS is feasible and generates data which are comparable to LBA data.

The method allows for simultaneous isotyping and semi-quantification.

A combination of LBA (screening/confirmation) and hybrid immuno-capture LC-MS/MS (quantification/isotyping) may be a viable proposal for the future of ADA measurement.

Conclusion

Issues and perspectives

- Standardization, multiple options, needs to be further evaluated.
  - Establish curves in animal plasma for each isotype and use biotinylated anti-Ig isotypes as capture reagent. May compensate for capture losses. However, no assurance the determined recovery is representative for that of true samples.
  - Well characterized polyclonal serum produced in animal species, probably the most representative of true samples. However, since peptides are not necessarily equal, it may only function as validation experiment/continuous monitoring of assay performance alongside another calibration option.
- A-specific binding (more pronounced for IgM than for IgG) leading to signals in control samples and hampering sensitivity. Ideally would like to be at a point where 250ng/mL is easily measurable without significant interference. Requires a 10 (IgG) or 30-fold (IgM) drop in a-specific binding but would eliminate the need for cut-point determination.
- How do you validate? Against which criteria?
- Further work required for characterizing recovery and drug tolerance.
- Further work for distinction between IgG subclasses is required.

Acknowledgements

- Antoine Francotte
- Luis Pérez-Tosar
- Claudine Kestelyn

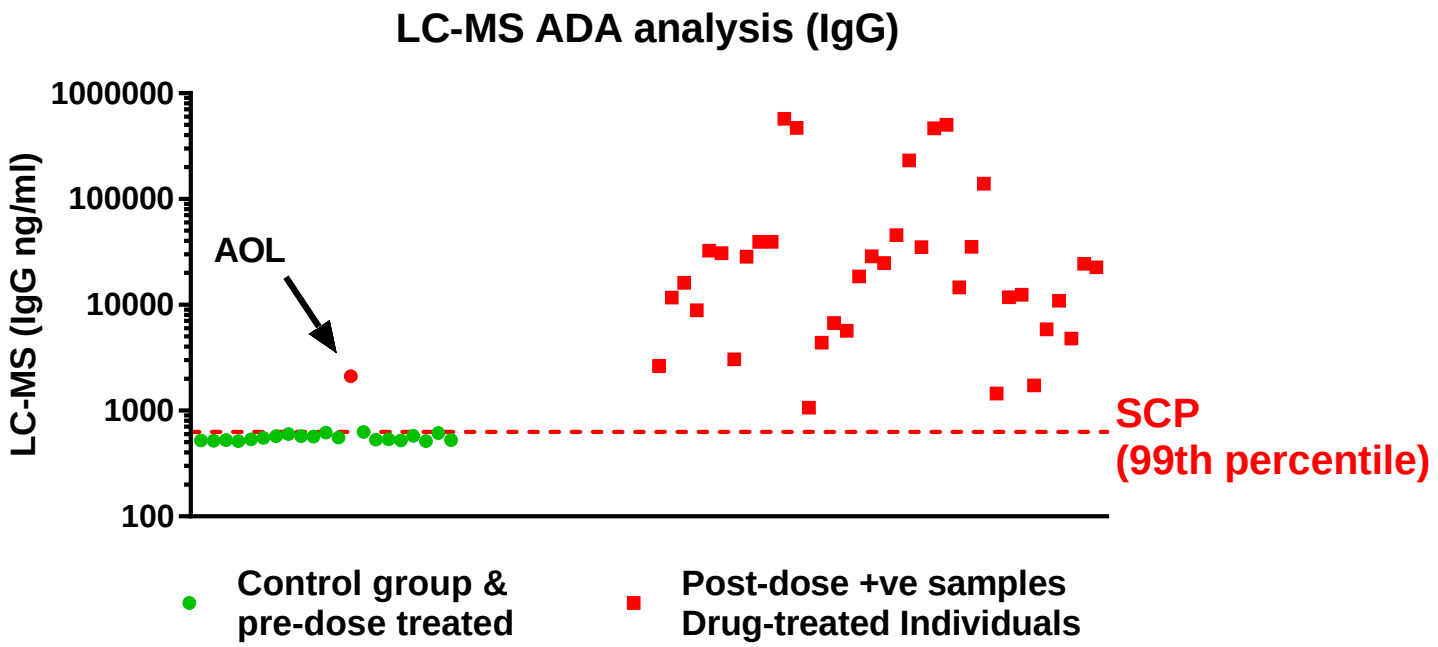
Questions?

Thanks!

Sample analysis

IgG: cut-point setting

Cut-point determination: Back calculated concentrations

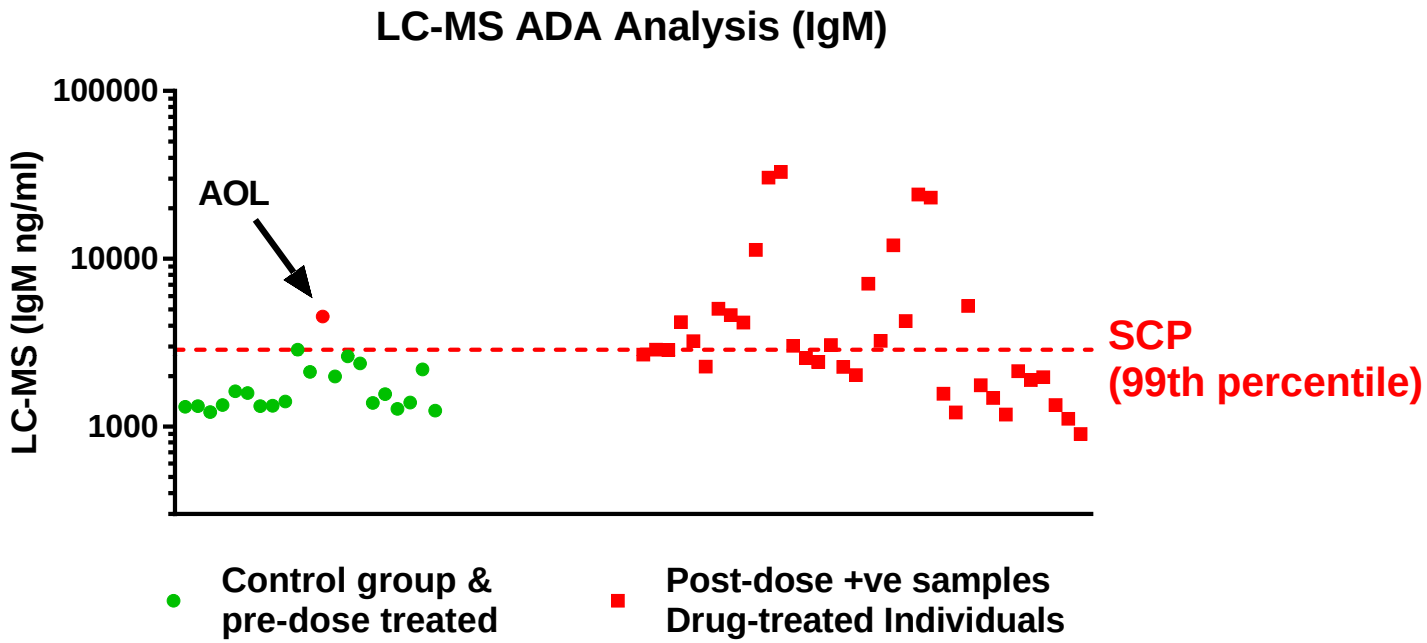


Cut-point: 630ng/mL

Sample analysis

IgM: cut-point setting

Cut-point determination: Back-calculated concentrations



Cut-point: 2870ng/mL

Week 4 TK data