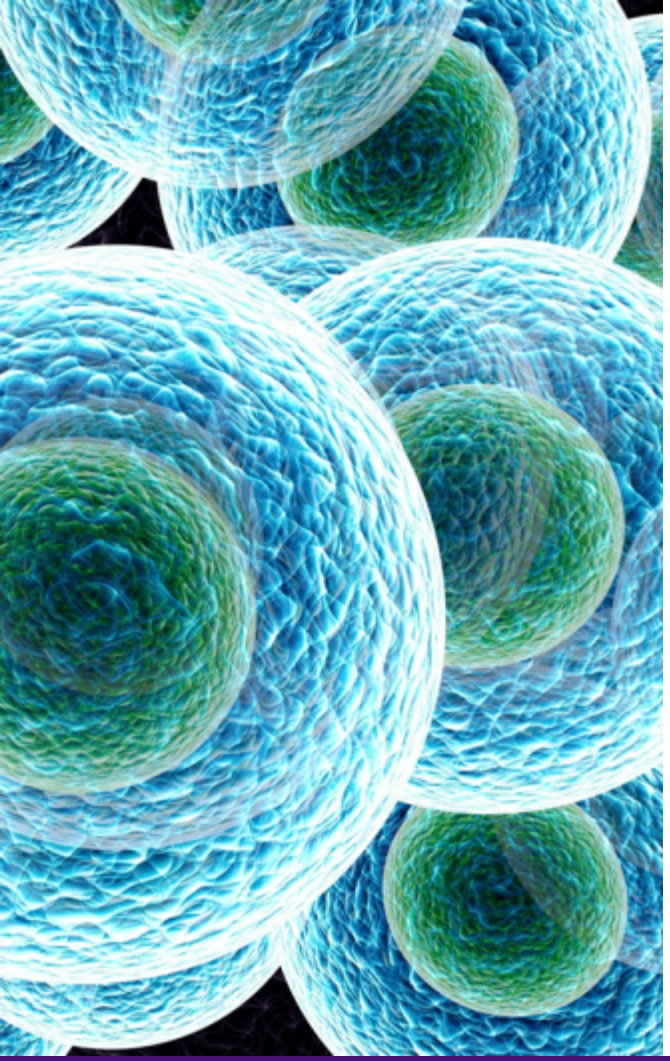


Is ADA by LBA/LC-MS/MS Realistic for Routine Analysis?

A Practical Route for a Validated LCMS Assay

Omnia Ismaiel, Ph.D.
PPD® Laboratories

HELPING DELIVER LIFE CHANGING THERAPIES

PPD®

Anti-Drug Antibody (ADA)

- Biotherapeutics have potential to cause unwanted immune responses and generate anti-drug antibodies (ADAs)
- The immune responses can range from insignificant to serious clinical consequences
- Immunogenicity is a major safety concern and must be evaluated during drug development
- A risk-based approach, fit-for-purpose strategy and case-by-case evaluation are usually applied for different studies

Current standard ADA techniques

- Enzyme-linked immunosorbent assays (ELISA) and electrochemiluminescence (ECL) immunoassays are widely used for ADA detection
- Supersensitive ADA assays have been developed recently, however, the detected ADA levels may be non-clinically relevant

Main challenges in ADA assay:

- Drug tolerance
- Soluble target interference and matrix effects
- Reagent availability
- Several assays are required for:

  Screening, confirmatory, isotyping, magnitude (titer)

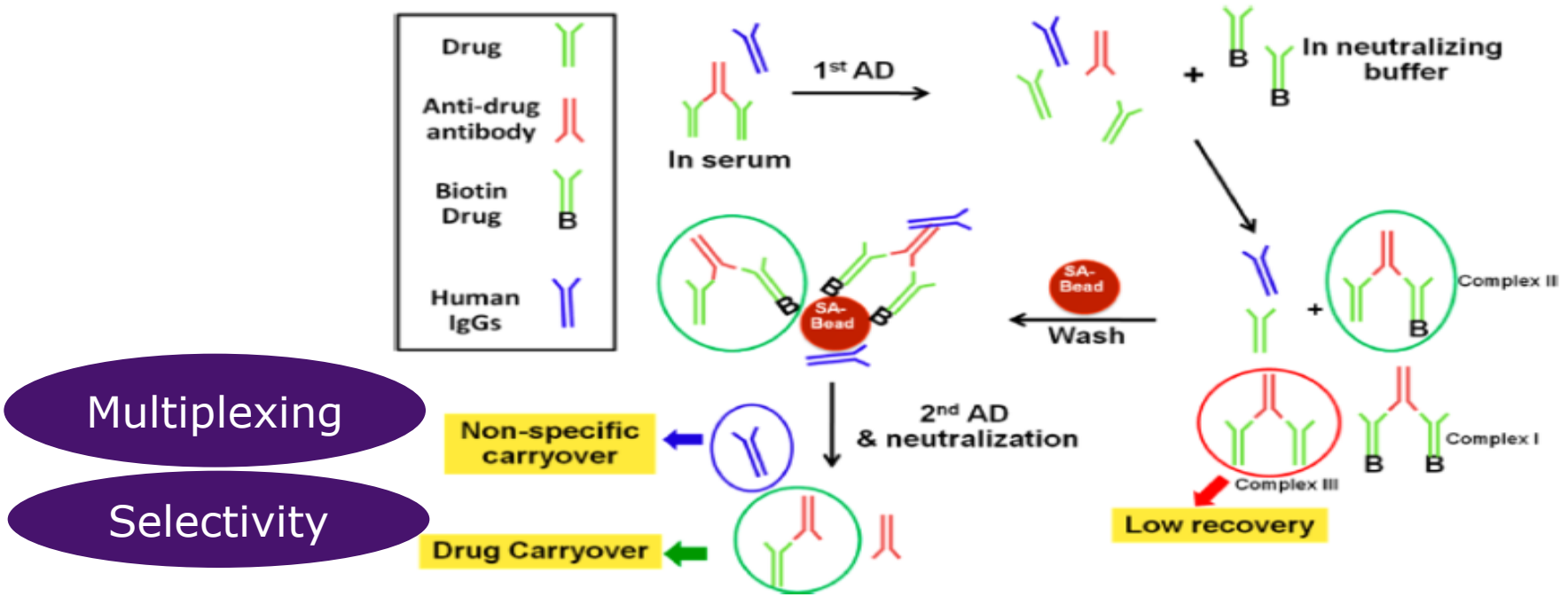
S. Song et al. J. Immunology. Research. 2016

Why ADA by LBA-LCMS?

- LCMS has been successfully applied for quantitation of a wide range of biotherapeutics
- The main advantages of LC/MS are selectivity and multiplexing capability
- Generic LCMS approaches such as (universal surrogate peptides for animal studies, universal capture procedure using protein A/G and typical protein digestion procedure) are commonly used for biotherapeutics
- Can we get a ***generic or universal*** LC/MS approach(s) for ADA?
- Can ***ONE*** LC/MS assay be used for (screening, confirmatory, isotyping, quantitation of ADA and/or as a complementary technique)

Current LCMS strategies for ADA assay

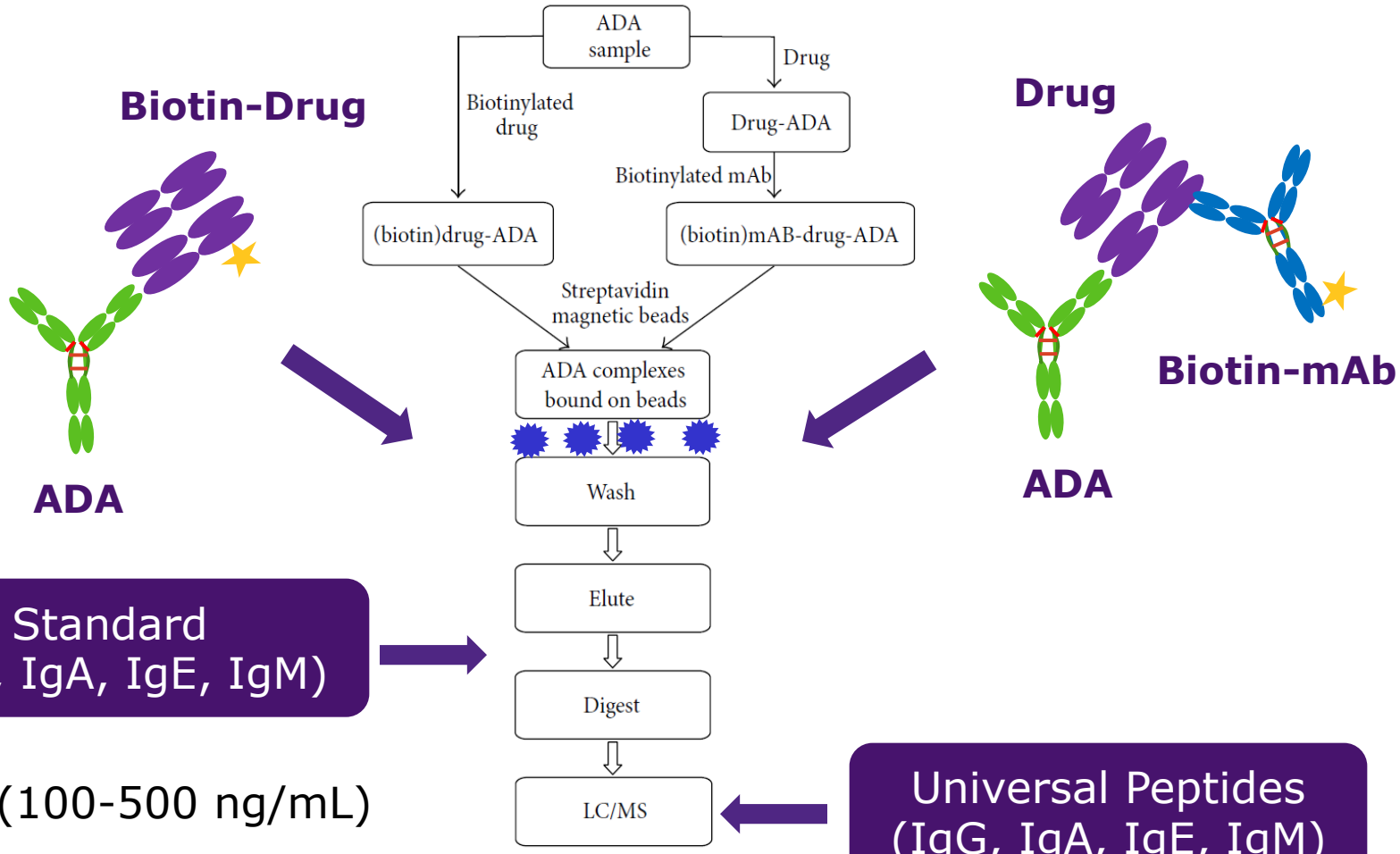
Quantitation of Neutralizing Ab, Residual Drug and Residual Human IgG



- ✓ **Drug:** a reengineered mutated IgG4
- ✓ **NAb-PC:** mouse mAb against the drug
- ✓ NAb-PC extraction recovery was **42%**
- ✓ Calibrators (50-10000 ng/mL)
- ✓ Residual IgGs was **1.4 µg/mL**
- ✓ **Signature peptides** were used for quantitation

H. Jiang et al. Anal. Chem. 2014

Isotyping and Semi-Quantitation of ADA



Standard (IgG, IgA, IgE, IgM)

Universal Peptides (IgG, IgA, IgE, IgM)

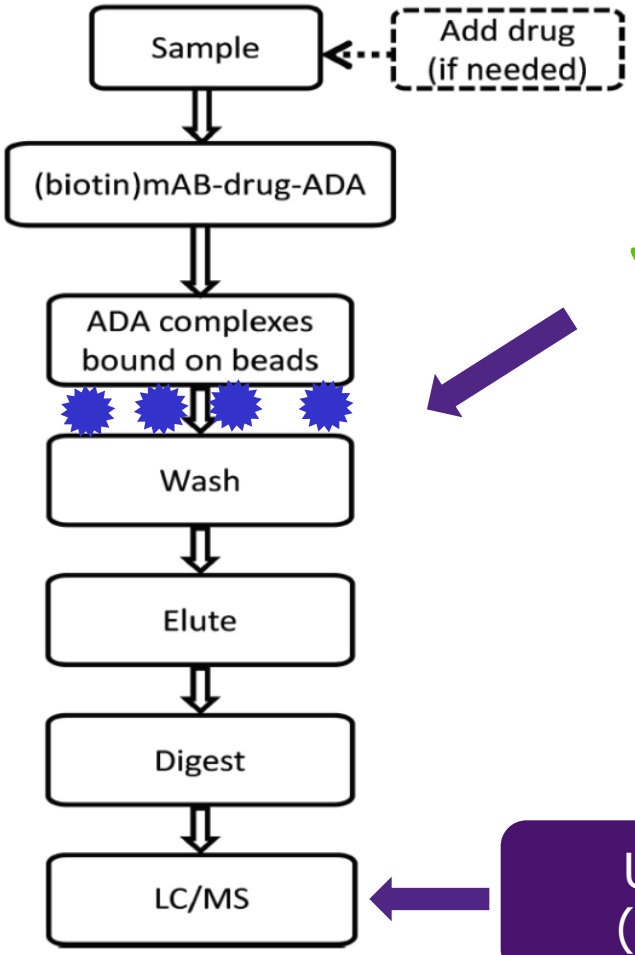
Flexibility

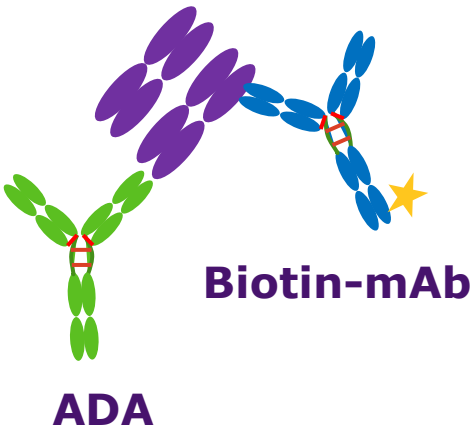
Applicability

- ✓ LLOQ: (100-500 ng/mL)
- ✓ No positive control was available
- ✓ No drug interference was observed

L. Chen et al. J. of Immunology Research, 2016

Detection of Monkey ADA





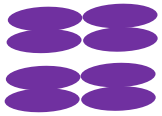
Universal Peptide
(Monkey IgG 1-4)

- ✓ No positive control was available
- ✓ No drug interference was observed
- ✓ LOD: (1.0 µg/mL)

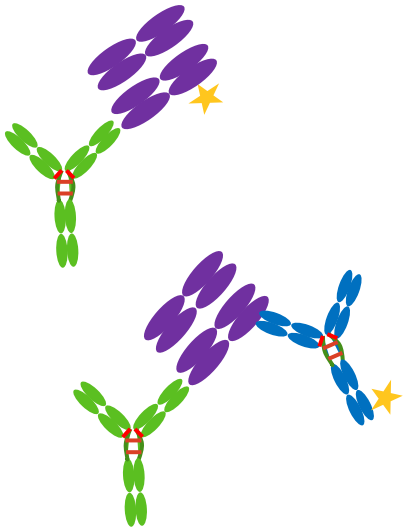
D. Roose et al. J. App. Bioanal, 2016

Is ADA by LBA/LC-MS/MS Realistic for Routine Analysis?

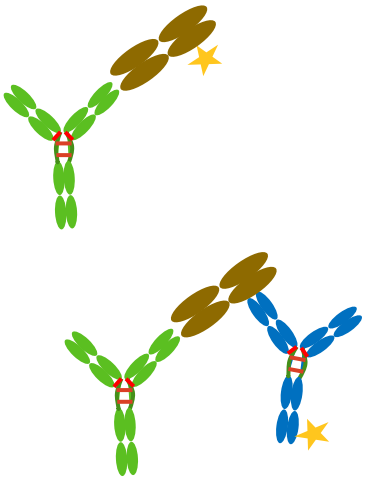
Complexity of the Biotherapeutic

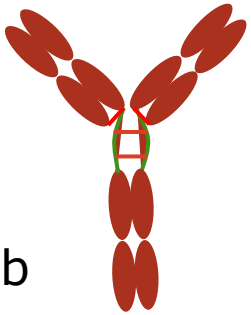


- Protein
- No human Fc
- No universal ADA peptides

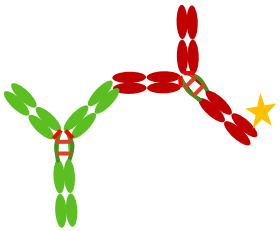


- Fragment (e.g. Fab)
- No human Fc
- Contains universal ADA peptides or not





- mAb
- Contains human Fc
- Contains universal ADA peptides



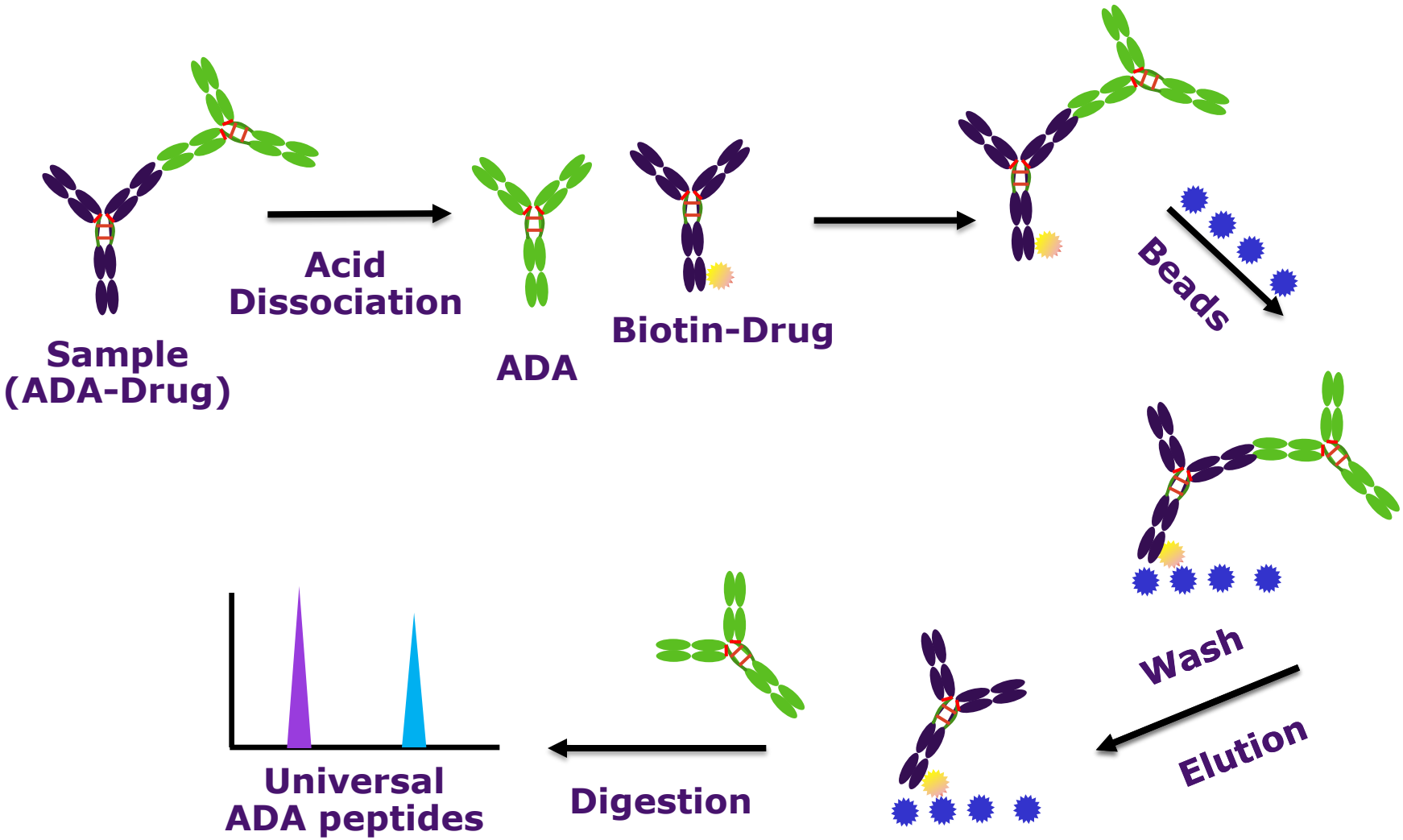
Experimental Design

- **ADA immunocapture:** biotinylated mAb or Fab
- **Positive control (standard ADA material):** anti-drug antibody (human IgG1)

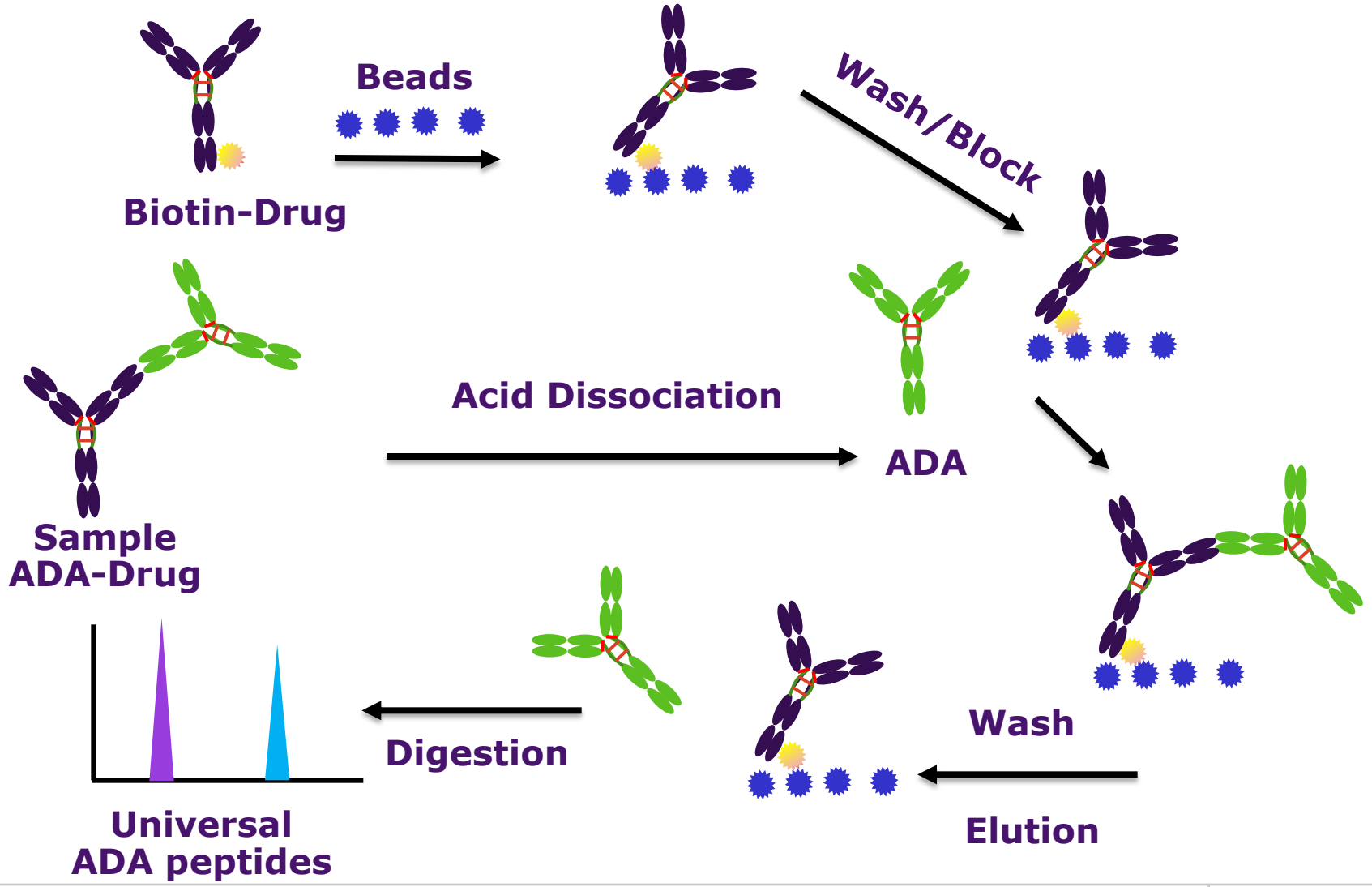
(many ready-made anti-biotherapeutic antibodies are commercially available)

- **Calibrators:** Standard IgG(1-4) materials
- **Detection:** Universal peptides for human IgG(1-4)
- **Internal standards:** SIL-IgG1, SIL-IgG4, SIL-universal peptides (IgG2, IgG3)
- Immunocapture was optimized using standard ADA material
- Digestion was optimized using ADA and IgG(1-4) standard materials

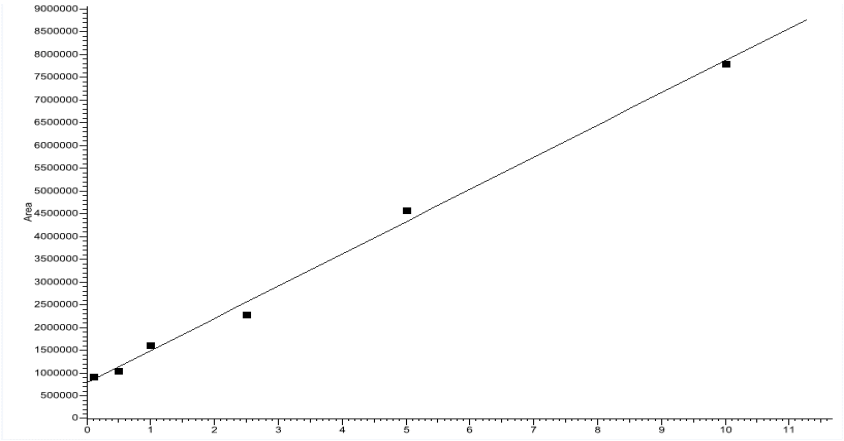
Biotinylated Drug-ADA immunocapture
(Direct Capture)

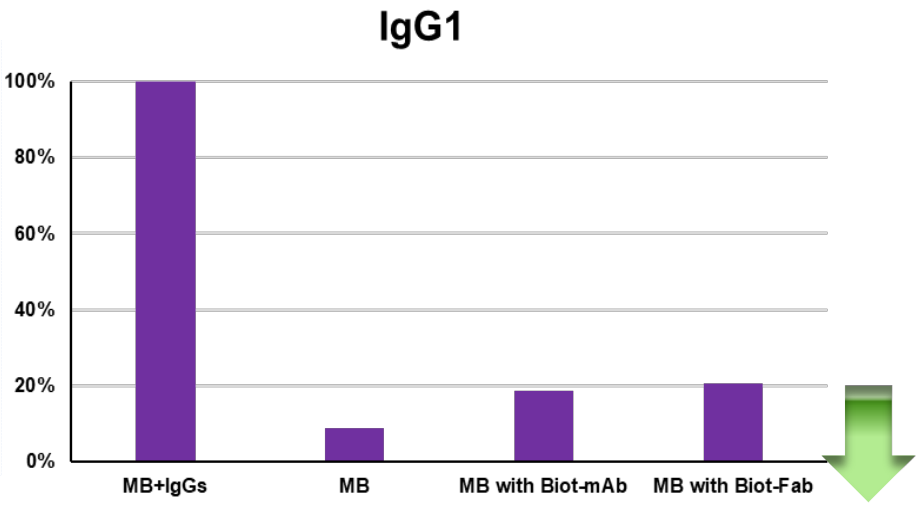


Biotinylated Drug-ADA immunocapture
(Indirect Capture)



Preliminary Results



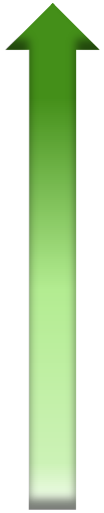


- Residual endogenous IgG1: (~ 1-2 µg/mL)
- Endogenous IgG1 Conc.(mean serum level): 6.98 mg/mL
- % IgG1 depletion: 99.98-99.97%

Sources of Background

Background → Non specific binding of endogenous IgGs

Human serum IgG mean Conc.*	
IgG1	**6.98 mg/mL**
IgG2	3.8 mg/mL
IgG3	0.51 mg/mL
IgG4	0.56 mg/mL



Background

The main challenge

*G. Vidarsson et. al. Front. in immunology. 2014

Key Factors to Minimize Non-Specific Binding (Residual IgGs)

- **Binding and wash buffers**
  - Additives: NaCl, TBS, Tween 20, CHAPS.
  - Blocking agents:

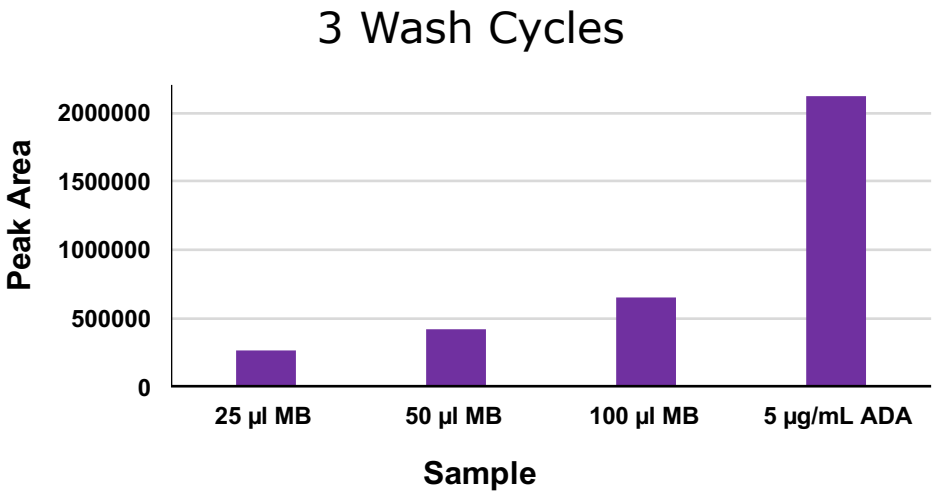
    BSA (30-50% *lower ADA recovery*)

    Casein (*30% lower background*) 
  - Dilution: with animal matrix
  - Washing steps: longer time and mixing at high speed
- **Sample volume and dilution**
- **Amount of beads**
- **Elution solution**

Effect of Wash Cycles Before Elution

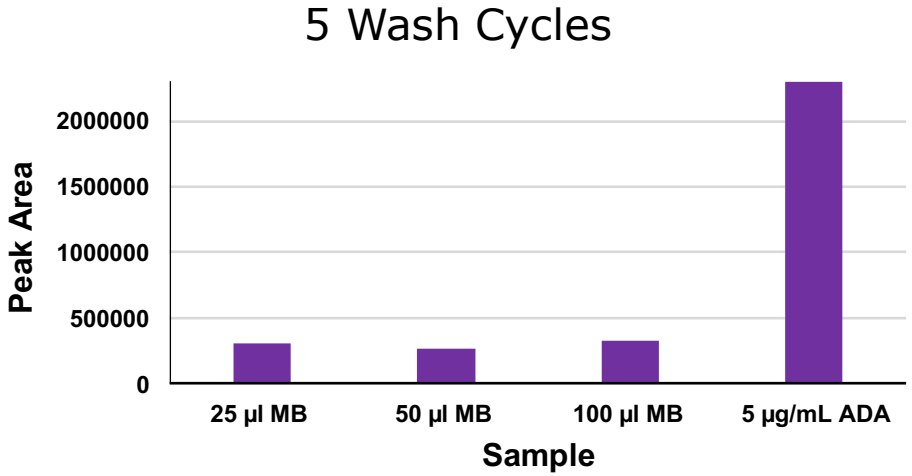
3 Wash Cycles
Medium speed for 20 sec.

Residual IgG1
~ 1.6 µg/mL
(in 100 µl sample)

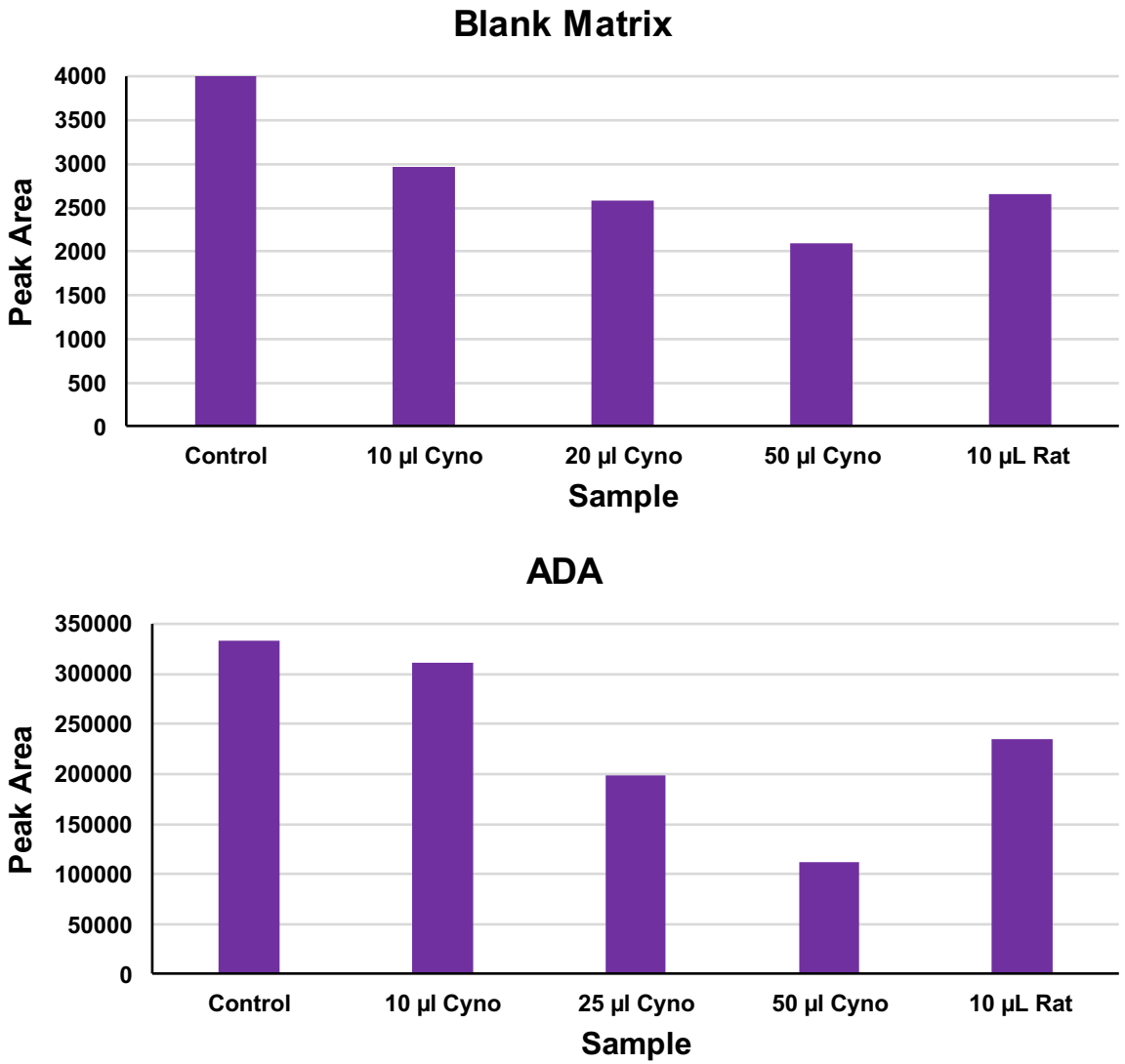


5 wash cycles
High speed for 1.0 min.

Residual IgG1
~ 0.7 µg/mL
(in 100 µl sample)



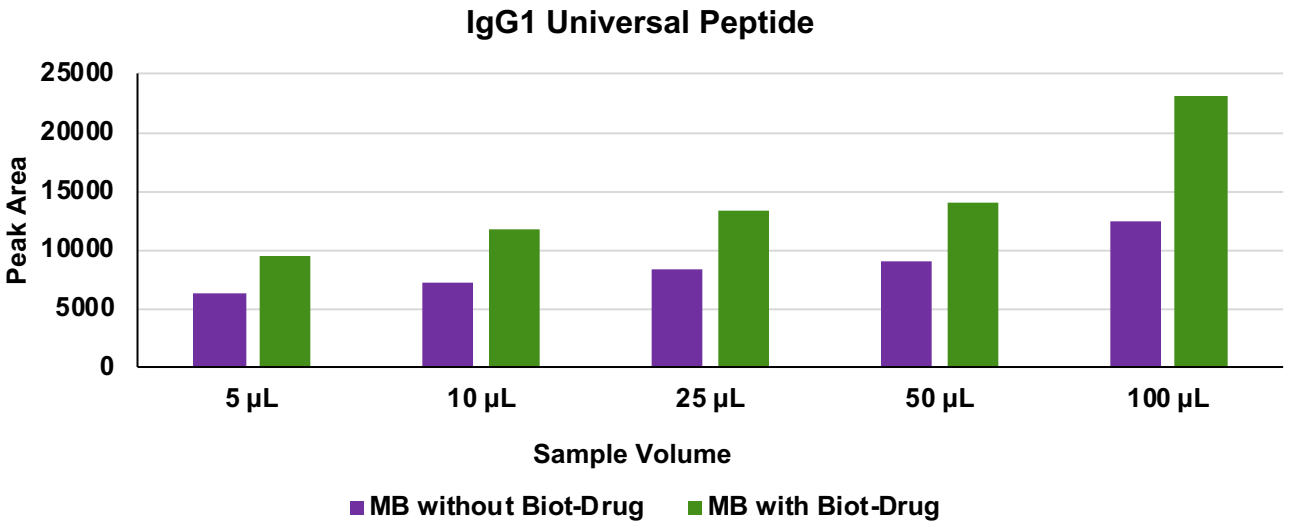
Dilution with Animal Matrix (Other Species IgGs)

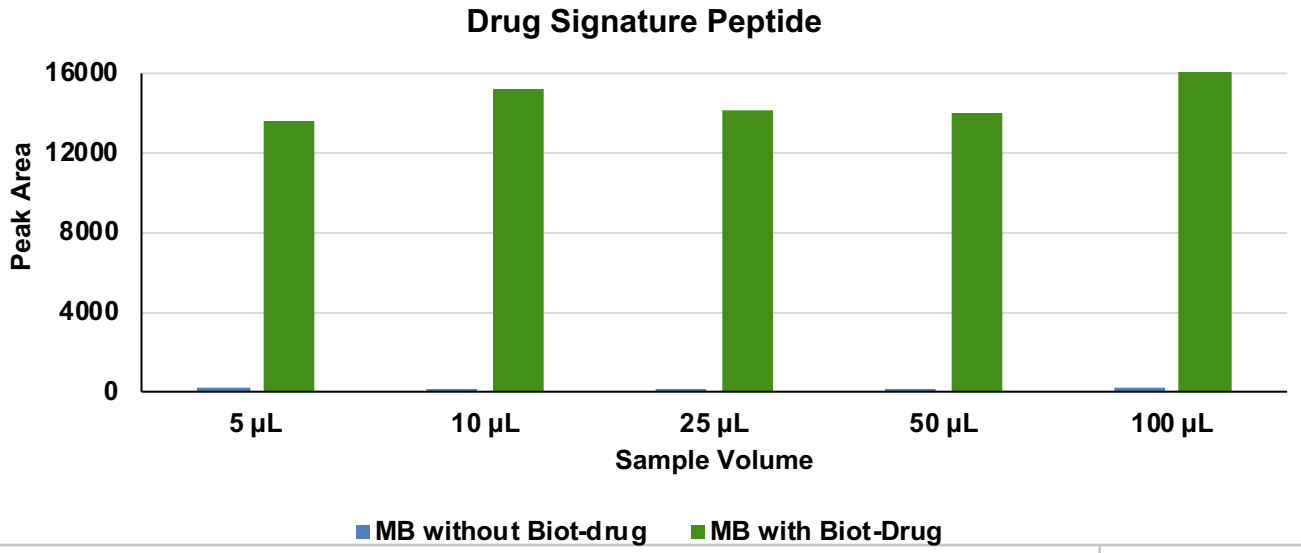


Other Sources of Background: Biotinylated Drug?

Troubleshooting capability

Drug/target Interference

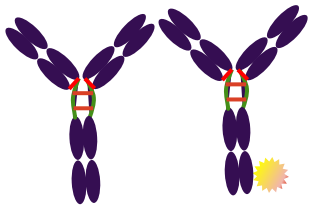




Key Factors to Minimize Background Related to Biotinylated Drug

- **Free biotin**
  - Desalting 2-3 times after biotinylation
- **Elution of the biotinylated drug**
  - Wash with elution solution (*Indirect capture only*) before ADA capture

- **ADA-Drug ratio**
  - 1:2 ratio (~60% ADA recovery)
  - 1:4 ratio (~85% ADA recovery)
- **Drug aggregation**
  - Use the recommended formula to disaggregate the drug*



* S. Giannos et al. Pharm. Res. 2018

Linearity, Precision, Accuracy and Background IgG (1-4)

	IgG1	IgG2	IgG3	IgG4
Linearity	100-10000 ng/mL	100-10000 ng/mL	100-10000 ng/mL	100-10000 ng/mL
Residual IgGs*	50-100 ng/mL	50-70 ng/mL	< 50 ng/mL	< 50 ng/mL
LLOQ	200-300 ng/mL	100-200 ng/mL	100 ng/mL	100 ng/mL
ADA Precision/Accuracy	Within ± 25%	NA	NA	NA
Immunoaffinity capture efficiency	80-90%	NA	NA	NA
Drug Tolerance	100 µg/mL (Direct) < 25 µg/mL (Indirect)	NA	NA	NA
IgG (QC) Precision/Accuracy	Within ± 25%	Within ± 25%	Within ± 25%	Within ±25%

*Residual IgGs in absence of ADA is usually higher than the actual background

Conclusions

- LBA-LC-MS is a very promising technique for ADA confirmatory, isotyping and semi-quantitation assays
- Few successful LBA-LC-MS/MS approaches have been applied for ADA determination
- Selectivity and multiplexing are the main advantages of LC/MS over other techniques
- High background is the main challenge for ADA by LC/MS
- Including LBA/LC–MS/MS as the main methodology for ADA bioanalysis is highly anticipated in the near future as current approaches mature

Acknowledgements

PPD team

- William Mylott
- Moucun Yuan