CHALLENGES DURING ADA ASSAY DEVELOPMENT

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ADA Assay Development

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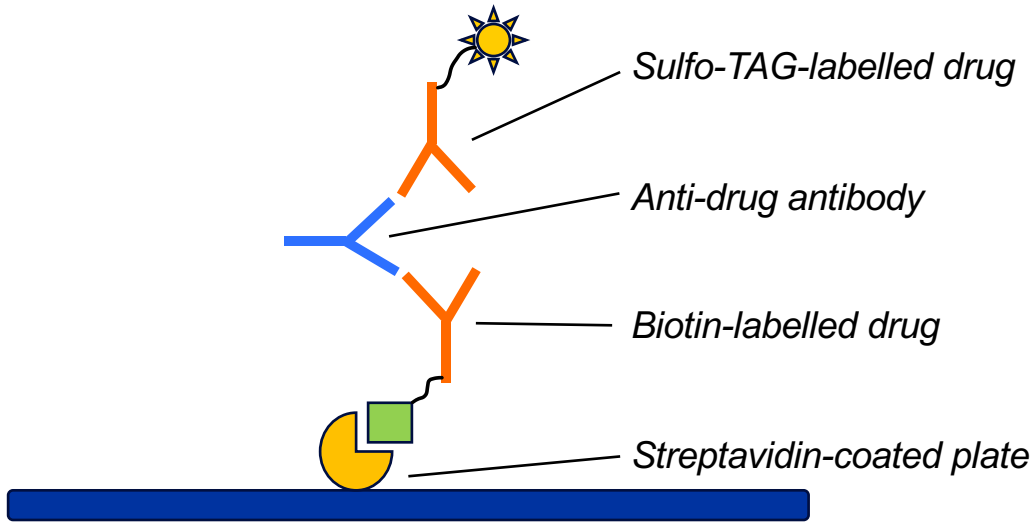
  - Three tier approach (clinical assay)

- Typical challenges
  - Sensitivity
  - Selectivity
  - Drug tolerance



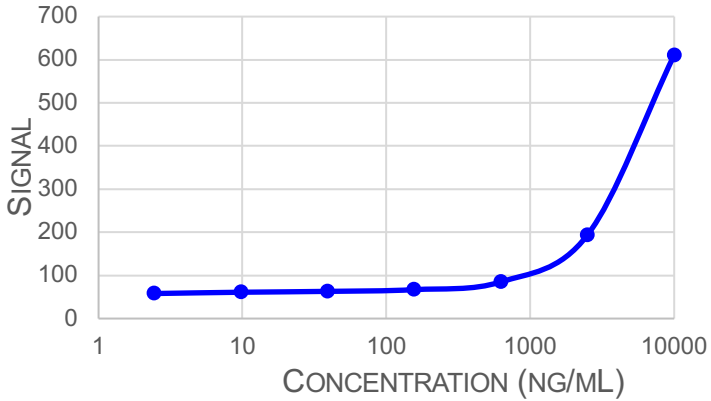
Case study 1

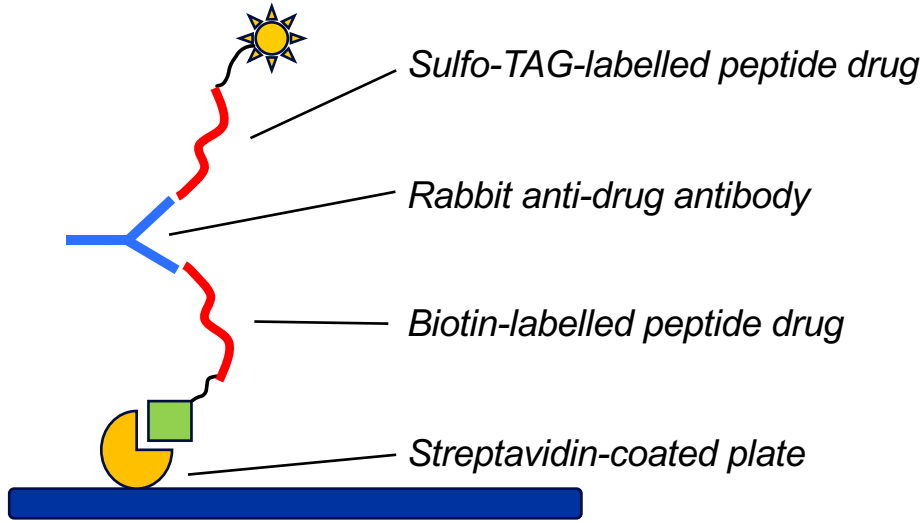
Assay development pre-clinical ADA assay for peptide drug

CASE STUDY 1

ADA assay for peptide drug

- Drug: peptide
- Positive control: rabbit anti-drug antibody
- Bridging assay format with ECL detection



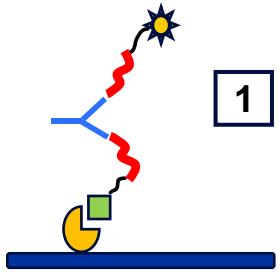


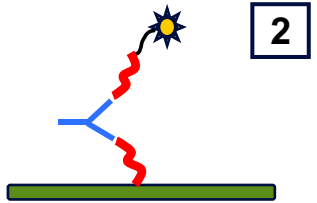
→ Signals low

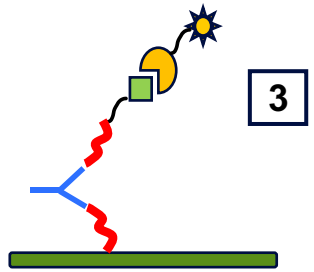
→ Sensitivity potential issue

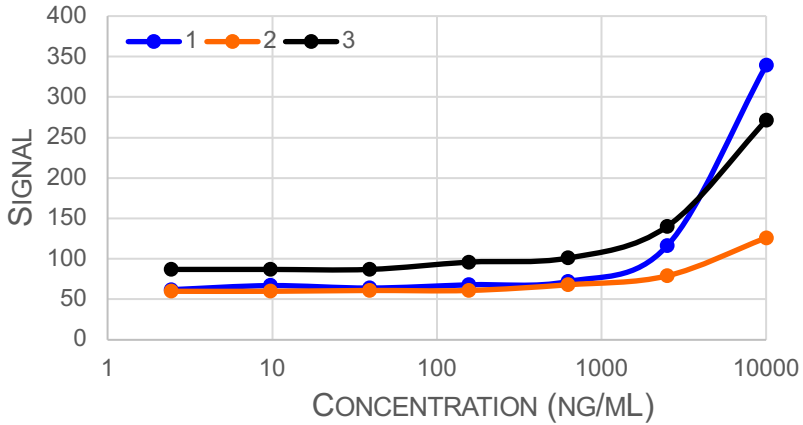
CASE STUDY 1

ADA assay for peptide drug









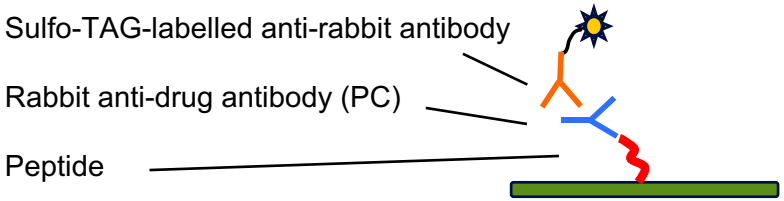
→ Signals low

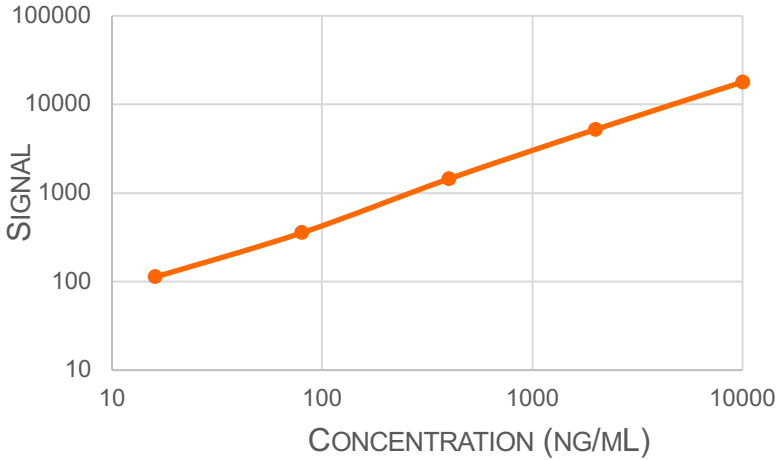
→ Poor sensitivity

→ Steric hindrance of labelled peptide

CASE STUDY 1

ADA assay for peptide drug



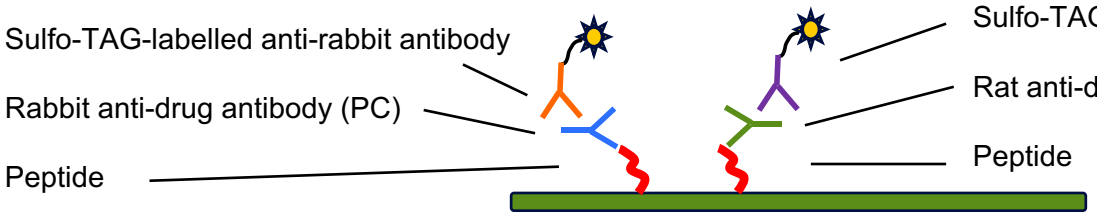


- MRD tested → 50 (2% matrix)
- Sensitivity assessment → <250 ng/mL
- Response single individuals above response NC
  - Other blocking buffer
  - Higher coating concentration
- Acid dissociation step to improve drug tolerance

CASE STUDY 1

ADA assay for peptide drug

DETECTION POSITIVE CONTROL



DETECTION RAT ANTI-DRUG ANTIBODIES

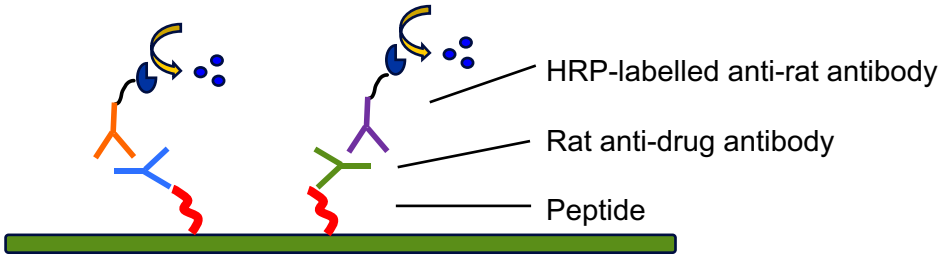
	Anti-rabbit antibody	Anti-rabbit antibody + Anti-rat antibody
NC	200	75,000
LPC	400	80,000
HPC	7,000	90,000

- Very high background levels caused by the Sulfo-TAG-labelled anti-rat antibodies
- Other blocking buffer reduced background only 5- to 10-fold

CASE STUDY 1

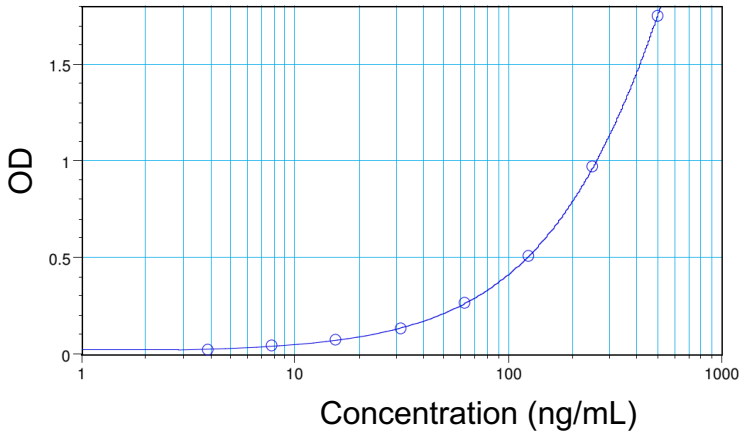
ADA assay for peptide drug

- Switch from ECL to ELISA



- Tweaking capture and detection concentrations:
  - Good sensitivity
  - Good drug tolerance

- Rat IgG and IgM as controls for performance anti-rat antibodies



CASE STUDY 1 - DISCUSSION

ADA assay for peptide drug

- ADA assay for peptide drug more challenging due to small peptide and bulky labels
- Check early in method development if positive control can bind to labelled peptide

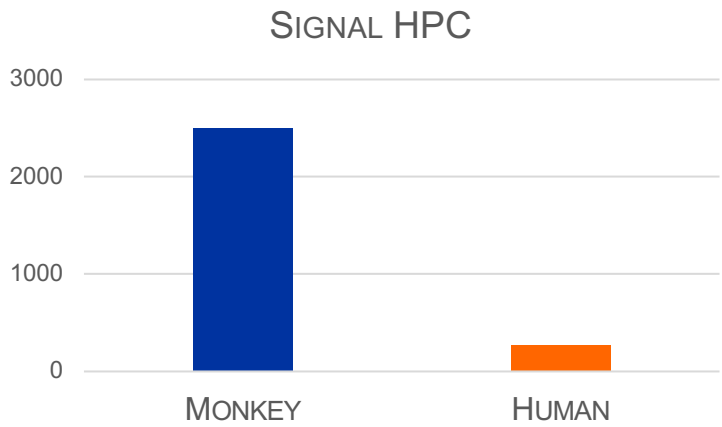
Case study 2

FROM PRE-CLINICAL TO CLINICAL ASSAY: LOSS OF SIGNAL

Case study 2

From pre-clinical to clinical assay: loss of signal

- ADA assay monkey serum
- Bridging assay
- Positive control: purified rabbit polyclonal
- Drug: humanized mAb
- ECL

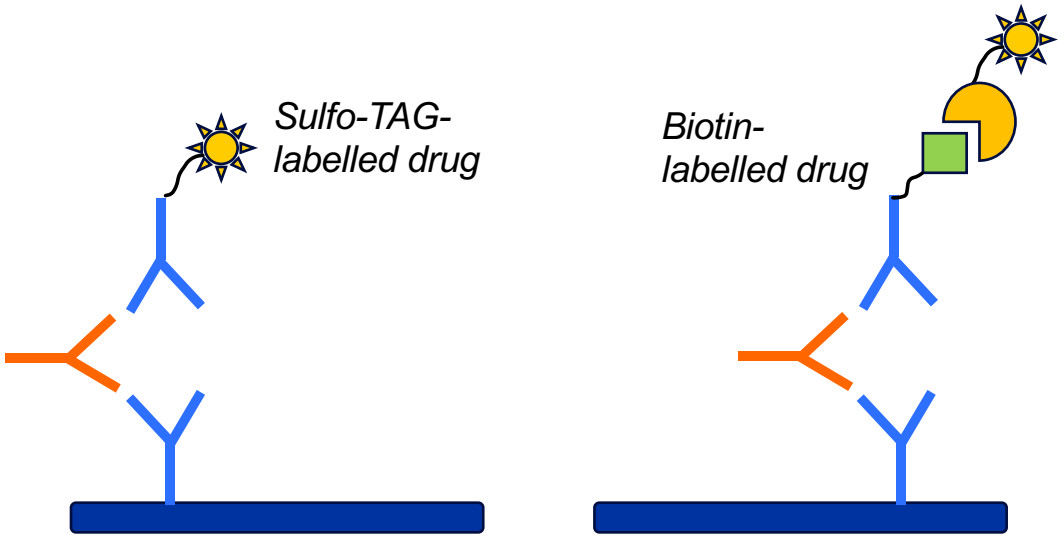


- **Transfer of assay from monkey serum to human serum** : loss of signal

CASE STUDY 2

FROM PRE-CLINICAL TO CLINICAL ASSAY: LOSS OF SIGNAL

- Performance issues capture or detection reagents?

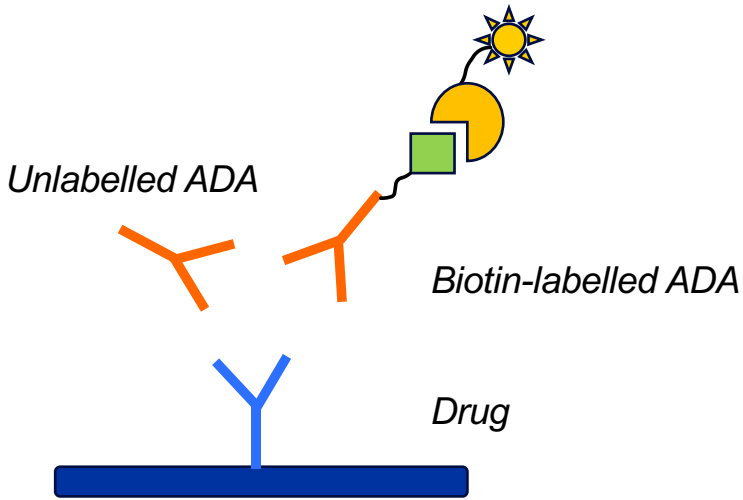


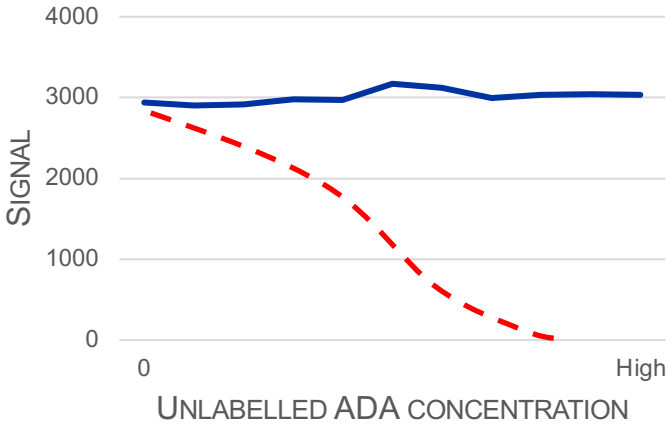
→ Low signals

CASE STUDY 2

FROM PRE-CLINICAL TO CLINICAL ASSAY: LOSS OF SIGNAL

- Alternative assay set-up: competitive ADA assay



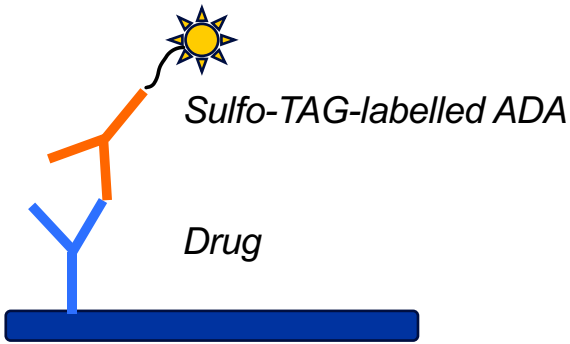


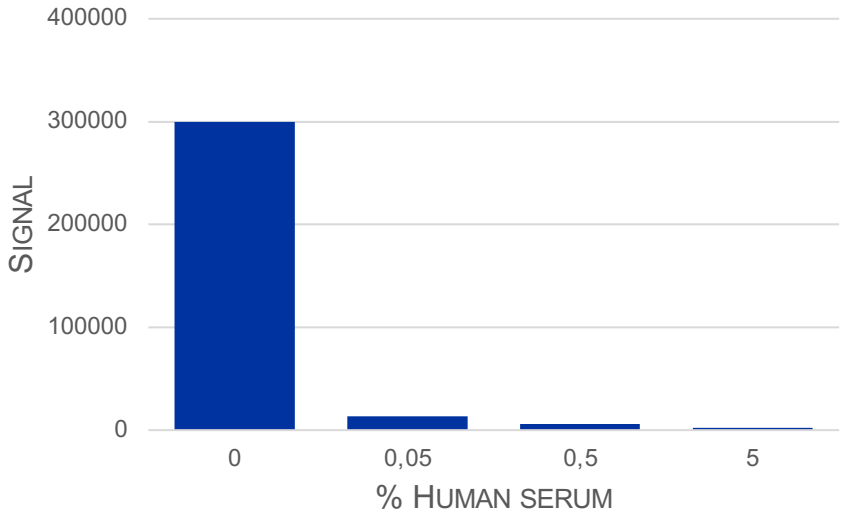
→ Signal in assay buffer 100-fold higher.
→ No competition. A-specific signal.
→ Matrix effect?

CASE STUDY 2

FROM PRE-CLINICAL TO CLINICAL ASSAY: LOSS OF SIGNAL

- Evaluation impact human serum



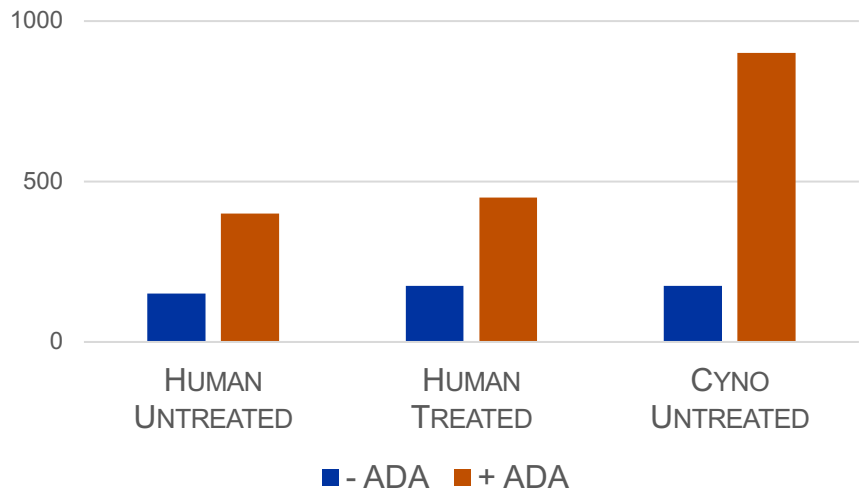


→ Interaction human serum components and ADA

CASE STUDY 2

FROM PRE-CLINICAL TO CLINICAL ASSAY: LOSS OF SIGNAL

- Loss of signal caused by serum protein interference in human serum?
- Removal of serum proteins from human serum through thiophilic resin antibody purification



→Antibodies interfering with ADA

Case study 2 - Discussion

From pre-clinical to clinical assay: loss of signal

- Positive control fractions
  - hIgG column eluate → used as positive control in monkey assay
  - hIgG column flowthrough

- Positive control in eluate fraction
  - Binds to all human IgG antibodies
  - Will only bind to antibody drug in monkey serum
  - Will bind to drug and other IgG antibodies in human serum

- Positive control in flowthrough fraction
  - Includes the idiotype-binding antibodies
  - Used for development of ADA assay for human serum

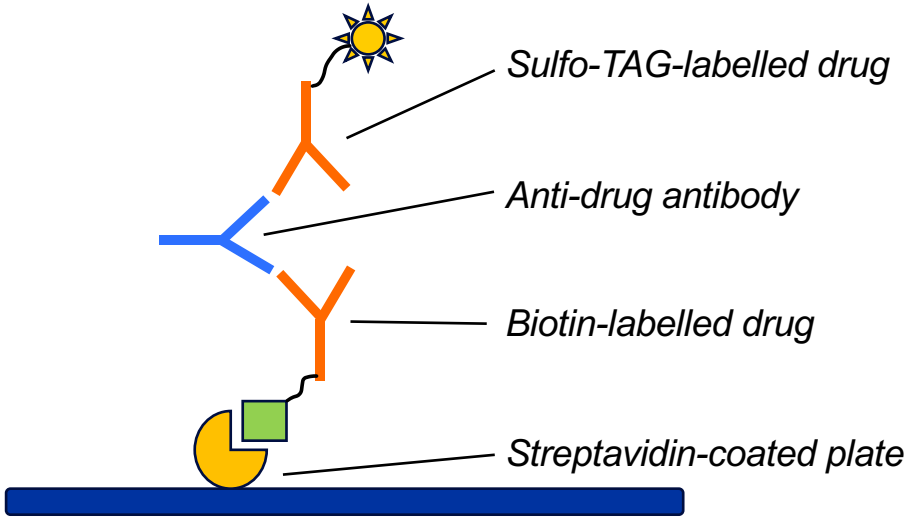
Case study 3

Impaired assay performance over time

CASE STUDY 3

IMPAIRED ASSAY PERFORMANCE OVER TIME

- Validated ADA assay for human serum on ECL
- Over time assay performance diminished
  - → Poor range (NC ↑, HPC ↓)
  - → Lower sensitivity
  - → Plate-specific cut point too high

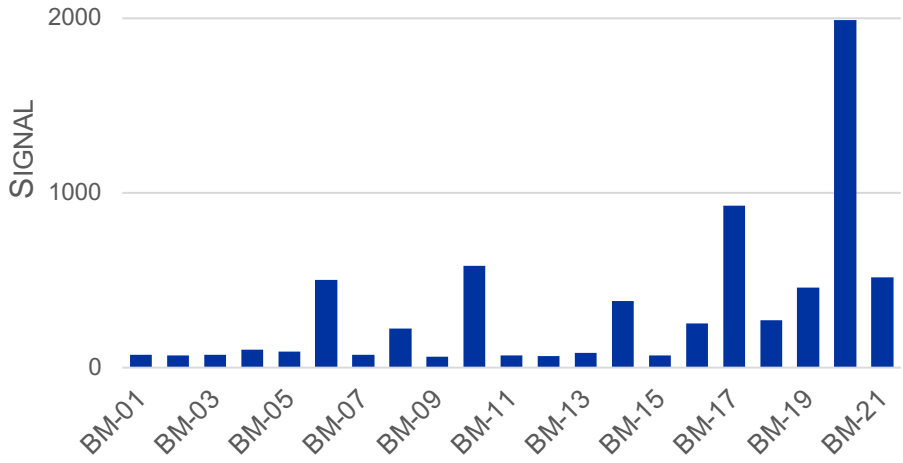


CASE STUDY 3

IMPAIRED ASSAY PERFORMANCE OVER TIME

- Impaired performance of ADA assay

	Validation	Modified
NC	150	360
LPC	290	430
HPC	9000	4000

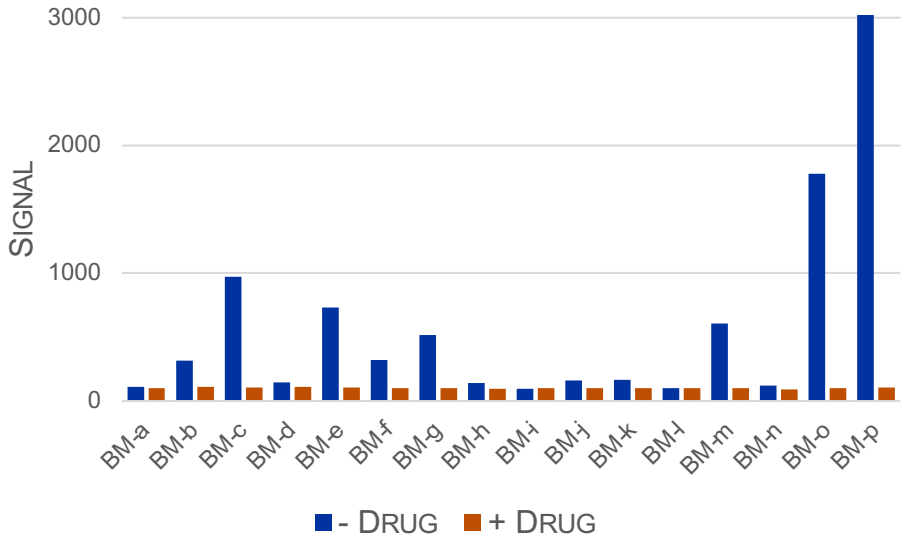


- New biotin- and Sulfo-TAG-labelled drug
- New negative control pool needed
- Testing individual blank human serum samples
  - →High variation between individual samples
  - →Acid dissociation did not improve results: pre-existing antibodies?

CASE STUDY 3

IMPAIRED ASSAY PERFORMANCE OVER TIME

- Confirmation high variation caused by pre-existing antibodies in blank human serum samples

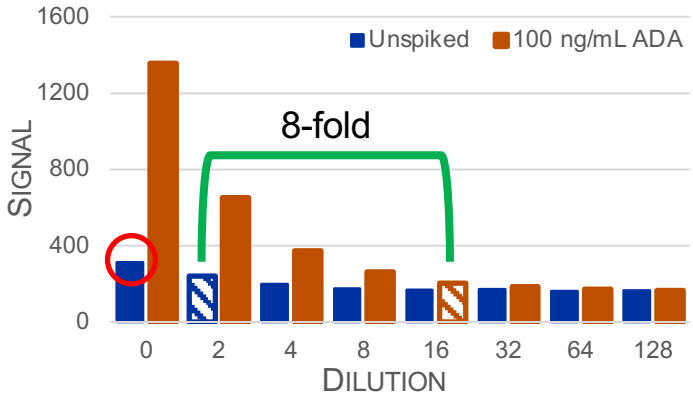


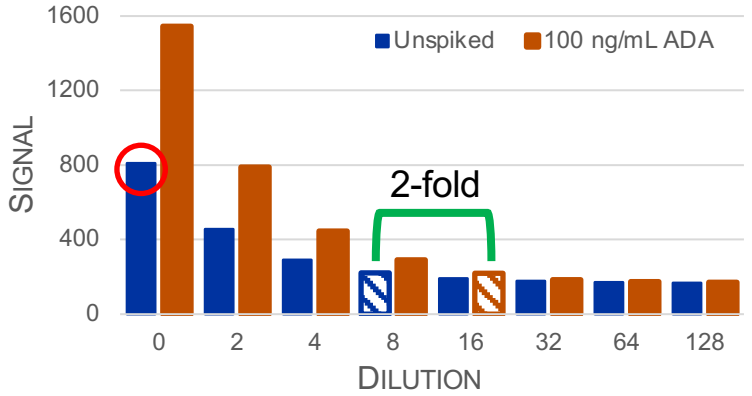
→ 40-50% of samples have pre-existing antibodies

CASE STUDY 3

IMPAIRED ASSAY PERFORMANCE OVER TIME

- Approach cut point determination
  - Pre-screen large number of blank human serum samples
  - Select samples with low response
- Identification of drug-induced ADA response
  - Titer sensitivity will be determined using individual human serum samples with pre-existing antibodies





→ The drug-induced antibody response may not be detectable at very high pre-existing antibody concentrations

Case study 3 - Discussion

Impaired assay performance over time

- Better sensitivity of new labelled drug resulted in increased background of negative control
- Higher negative control response due to more pronounced pre-existing antibody signals
  - → *Pre-screen of blank human serum samples needed before cut point determination*
  - → *Titer sensitivity experiments needed for interpretation of study sample data*
- Impaired HPC performance not related to pre-existing antibodies

Thank You

Acknowledgements
- Lard de Jong
- Sandra Henkelman
- Dian Spakman
- Duco Schriemer
- Monique Putman
- Jelger Lijcklama a Nijeholt