Challenges posed by abundant target in development of free and total PK assays for a therapeutic mAb drug

Birgit Jaitner – Investigator II PK/PD Bioanalytics I

Translational Sciences / Biologics Safety & Disposition

EBF Conference, 02-Dec-2010

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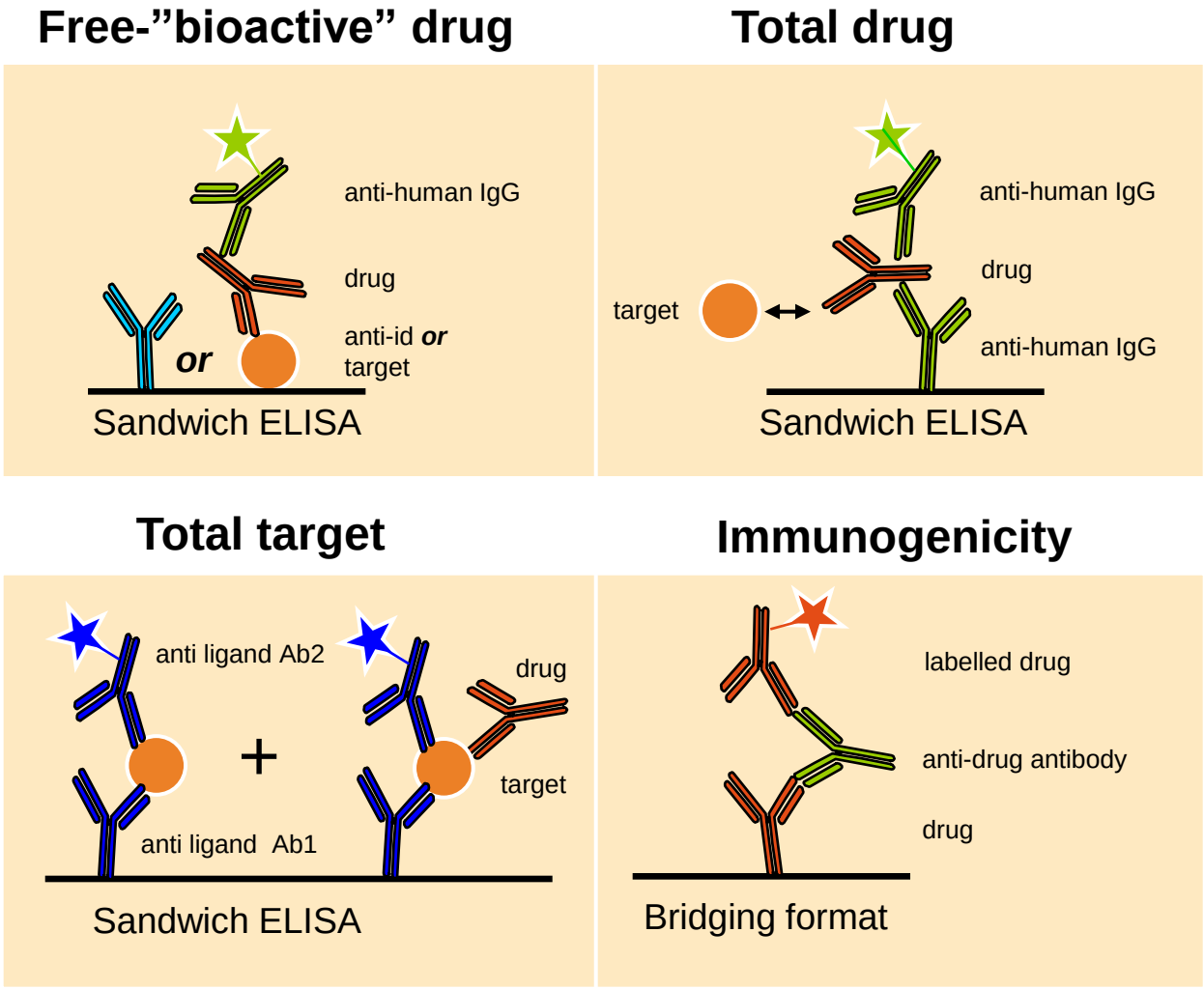
Outline of the presentation

- Characteristics of drug and target
- Bioanalytical strategy
- Challenges of the PK assay strategy
- Summary

Characteristics of drug and target

- Fully human IgG1 mAb
- Drug target is a highly abundant (> 50 μg/mL) and large (> 140 kDa) serum protein

Bioanalytical strategy – pharmacokinetics (PK), pharmacodynamics (PD) and Immunogenicity



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Challenges of the PK assay strategy

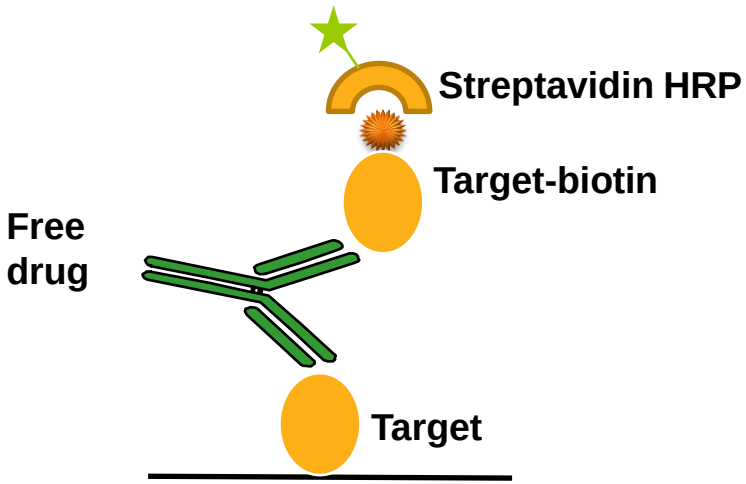
- Bearing in mind very abundant target, are immunoassays feasible to assess PK?
- Free drug assay
  - Which matrix should be used for preparation of standard curve and QC?
  - Is there dilution linearity when diluting samples into the assay working range? Feasible to maintain equilibrium of $drug_{free}$ + $target_{free}$ $\rightleftharpoons$ drug:target complex in diluted sample?
- Total drug assay
  - Feasible to develop drug-specific reagents capable of binding with high affinity to both, $drug_{free}$ and drug:target complex?

Selection of matrix for free drug assay

- Human matrix: Standard curve with very poor apparent sensitivity (> 100 µg/mL) since drug binds to endogenous target.
- Surrogate matrix (goat or rabbit serum): Standard curve with good dynamic range and sensitivity, QCs in same matrix recover well.

Free drug assay is not feasible due to abundant target.

Bridging ELISA

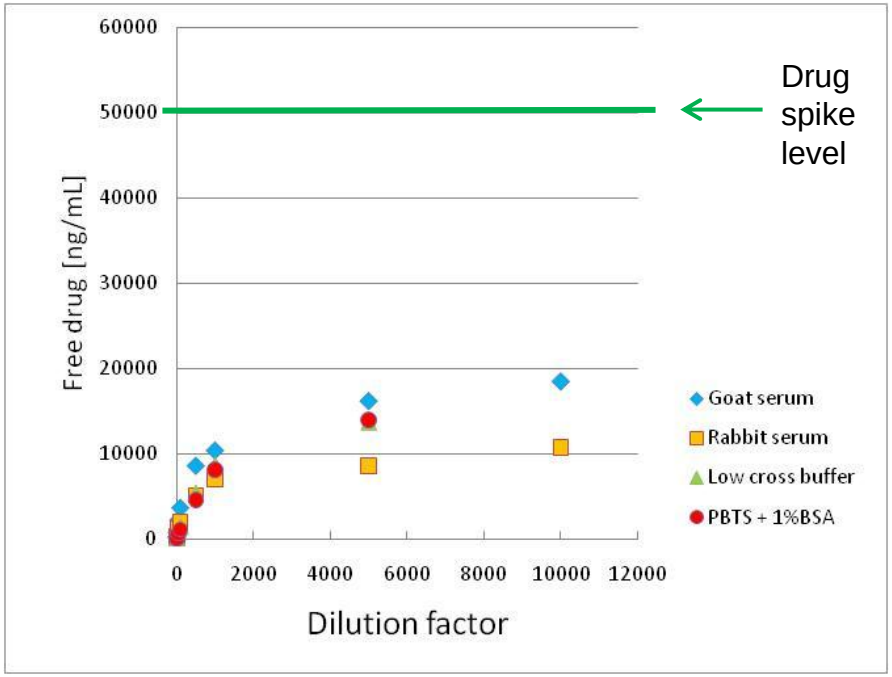


➢ QCs prepared in human serum fail due to missed dilution linearity.

Conclusion: Free drug assay is not viable.

Dilution of Cs and QCs in depicted surrogate matrix.

Spike 50 µg/mL drug into neat human serum. Dilute spiked sample in surrogate matrix or assay buffer and quantify free drug levels.



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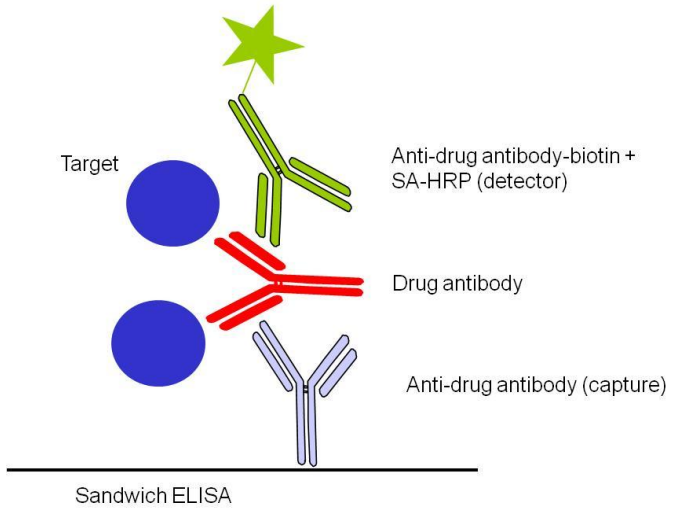
Total drug assay

Challenges:

- To develop an immunoassay capable of quantifying both free drug and drug:target complex with equal efficiency.
- High target abundance (> 50 µg/mL serum) and large molecular weight (> 140 kDa)
- Strategy
  - Select tool reagent tools
  - Identify sample pre-treatment step to overcome target interference



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Select anti-drug specific Fab fragments by use of Biacore technology

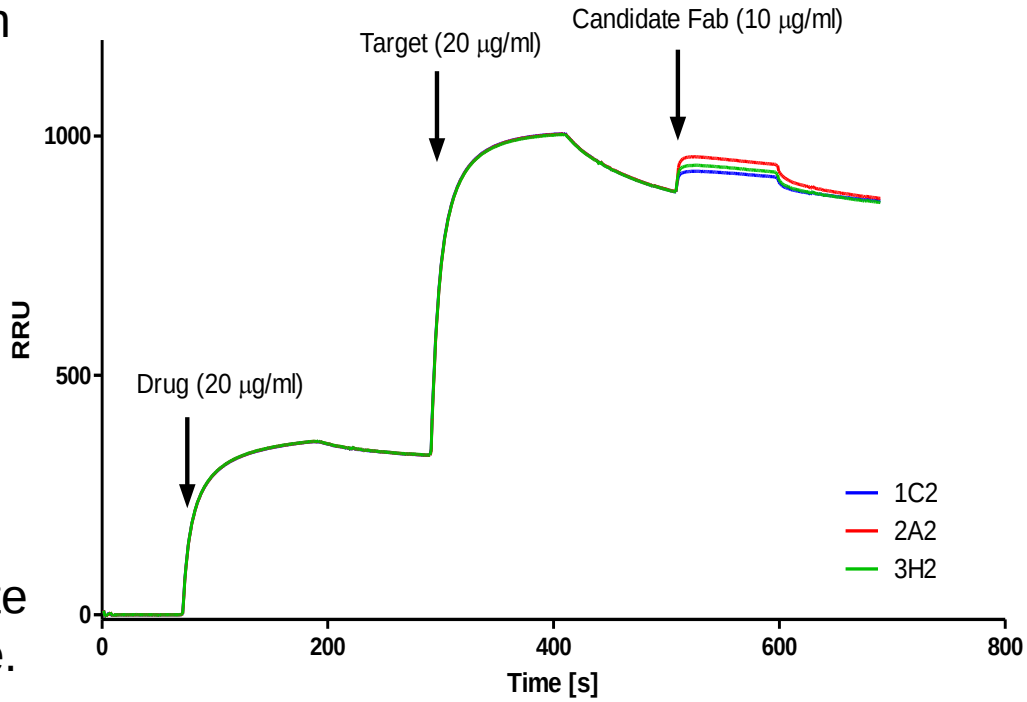
Immobilize drug (20 µg/mL) on protein G chip

Inject 20 µg/mL of target

Subsequently, inject anti-id Fab (10 µg/mL)

<u>Selection criteria:</u> In the presence of target, highest on-rate & lowest off-rate while similar decay of signal over time.

<u>Result:</u> Three out of 12 Fabs tested selected for further evaluation



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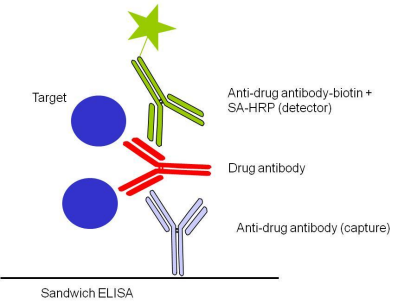
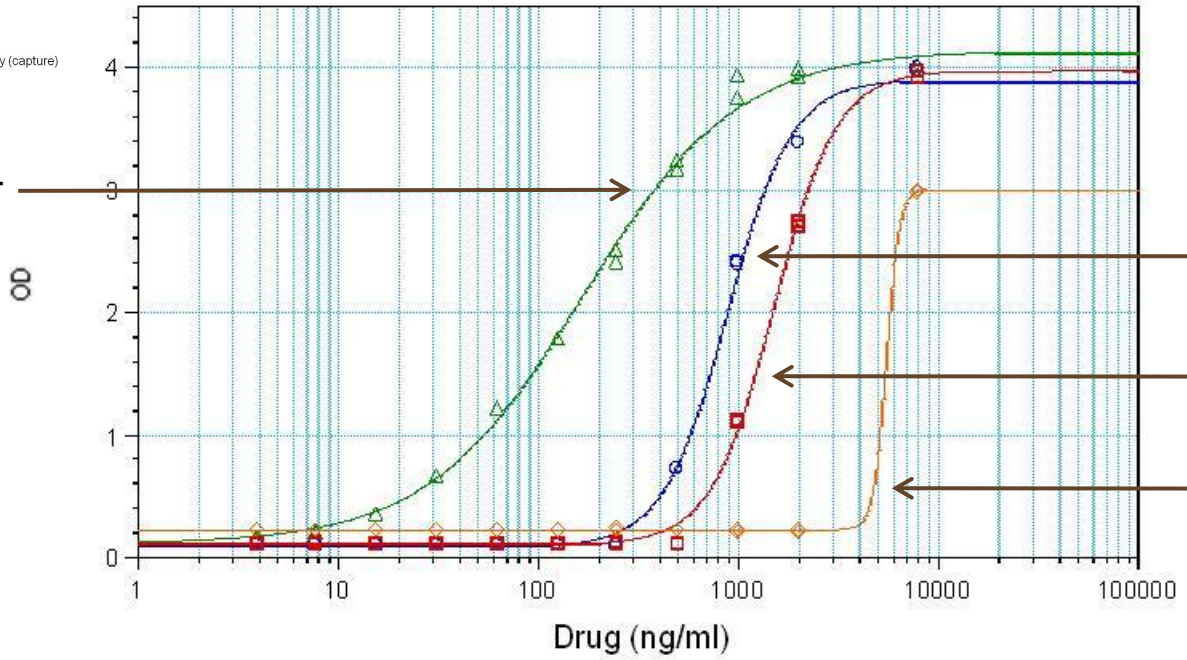
Strong matrix effect due to target abundance in human serum.



Assay buffer



1 % human serum

2 % human serum

10 % human serum

4-P Fit: y = (A - D)/(1 + (x/C)^B) + D	A	B	C	D	R^2
Curve1 (Curve in LCB: Concentration vs OD)	0.106	1.12	167	4.13	0.996
Curve2 (Curve in 1% human Ser: Concentration ...	0.0746	2.71	882	3.89	0.998
Curve3 (Curve in 2% human Ser: Concentration ...	0.0931	2.76	1.51e+03	3.97	0.998
Curve4 (Curve in 10% human Ser: Concentration...	0.219	13.7	5.5e+03	3	1

Curve Fit Option - Fixed Weight Value

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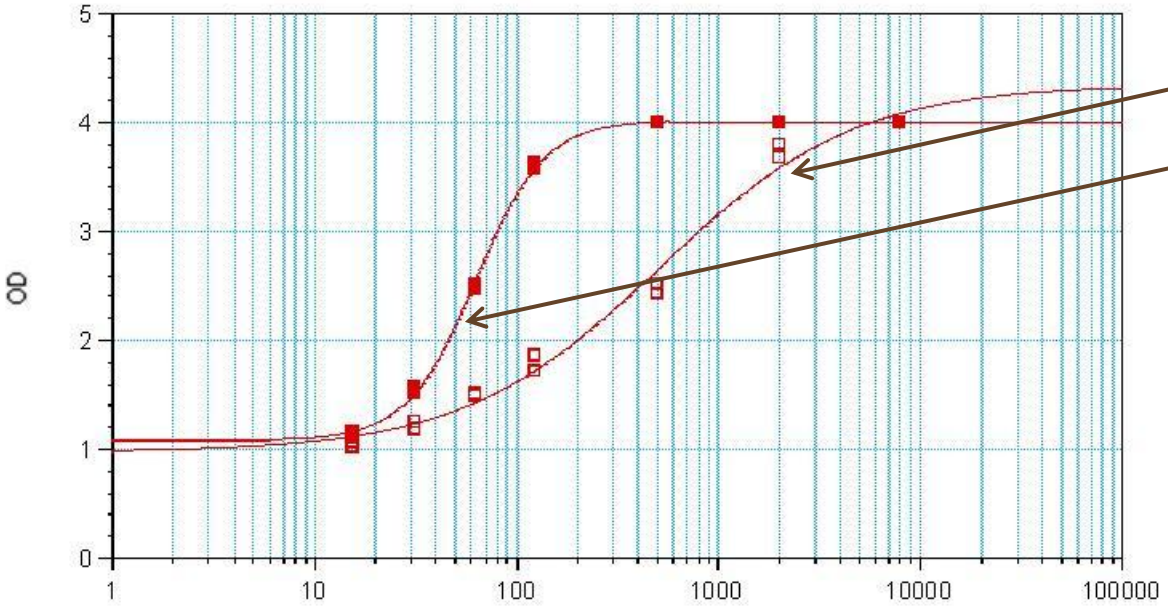
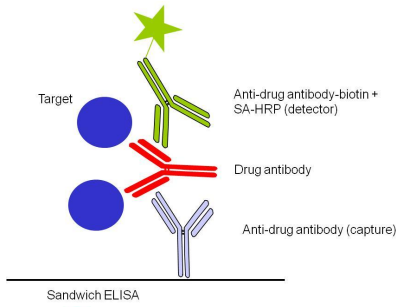
Sample pre-treatment with acid overcomes target interference in 10 % human serum with a modified set of tool reagent.

- Procedure: Add 100 µL 0.08 N HCl to 100 µL Cs, QCs and unknown sample, incubate 10 min at RT. Neutralize with 100 µL 0.08 N NaOH, load samples onto 3H2 coated 96-well plate and perform ELISA with 1C2-biotin and SA-HRP as detectors.



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D	A	B	C	D	R^2
Curve1 (Curve in 1%BSA PBST- No HCl: Concent...	0.967	0.892	510	4.34	0.99
Curve3 (Curve in 1%BSA PBST- 0.027N HCl: Con...	1.07	2.55	63	4.01	0.999

Curve Fit Option - Fixed Weight Value



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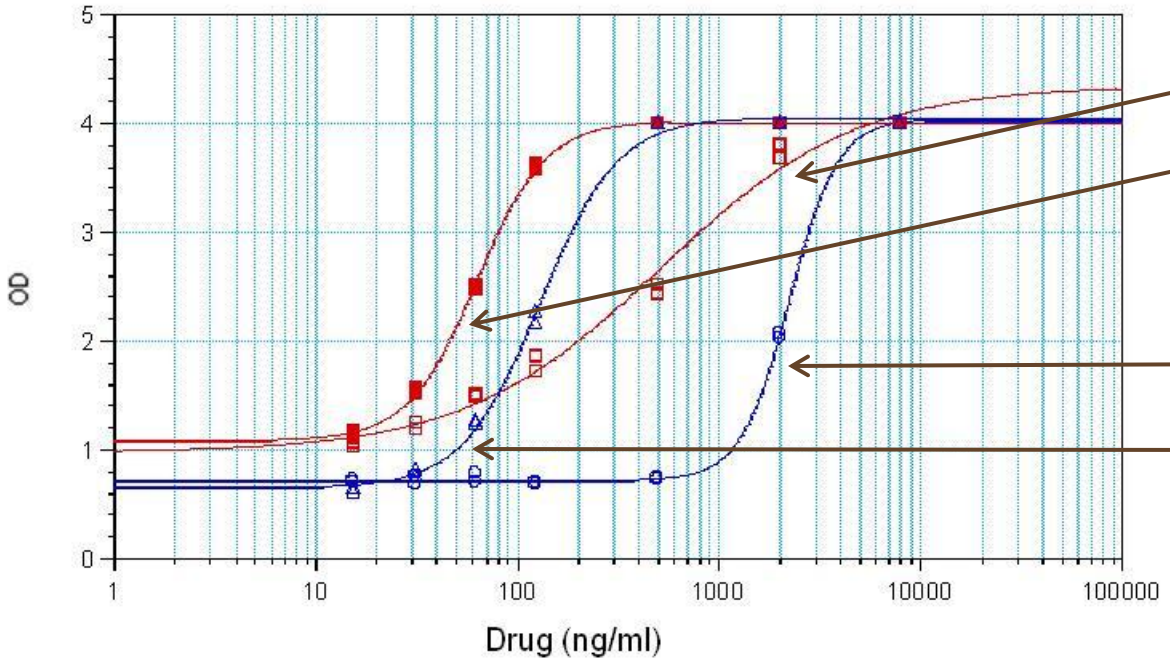


Buffer without acid

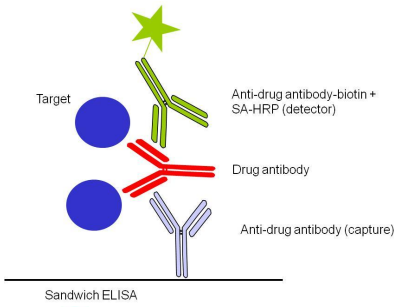
Buffer with acid

10% human serum without acid

10% human serum with acid

4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
Curve1 (Curve in 1%BSA PBST- No HCl: Concent...	0.967	0.892	510	4.34	0.99
Curve2 (Curve in 10% hum ser 0 HCl: Concentrat...	0.708	3.6	2.24e+03	4.03	0.999
Curve3 (Curve in 1%BSA PBST- 0.027N HCl: Con...	1.07	2.55	63	4.01	0.999
Curve4 (Curve in 10% hum ser 0.027N HCl: Conc...	0.64	2.26	130	4.05	0.998

Curve Fit Option - Fixed Weight Value



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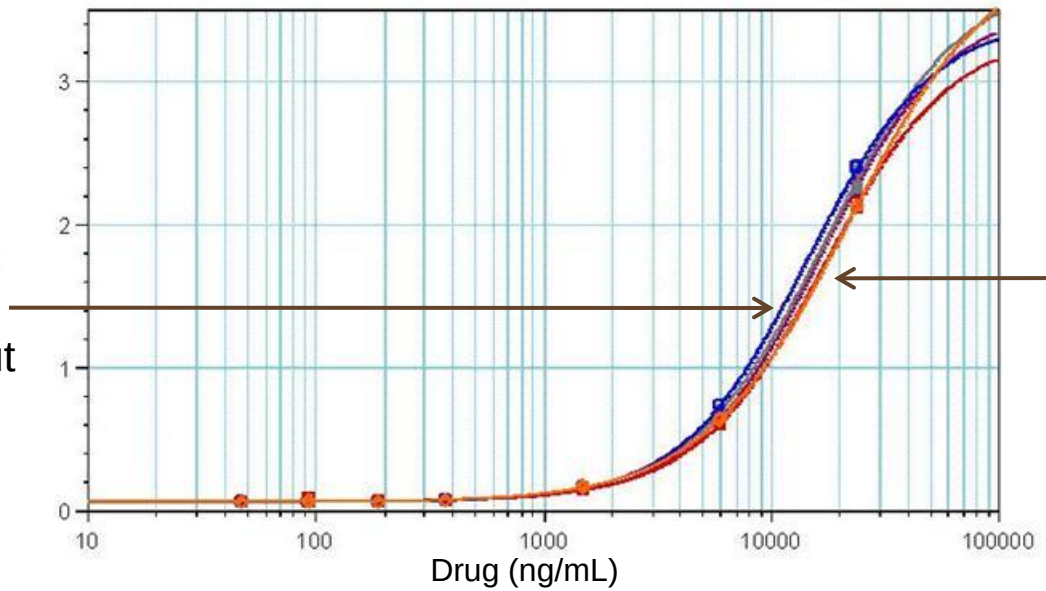
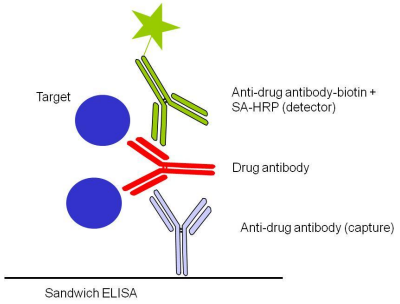
Overcome target interference

Add increasing amounts of target (0, 100 up to 400 µg/mL) into drug spiked 10% human serum and resolve target interference of the 3H2/1C2 assay by acid treatment.

Calibrators in 10% human serum without spiked target.



Calibrators in 10% human serum, spiked with 100, 200, 300 and 400 µg/mL target.



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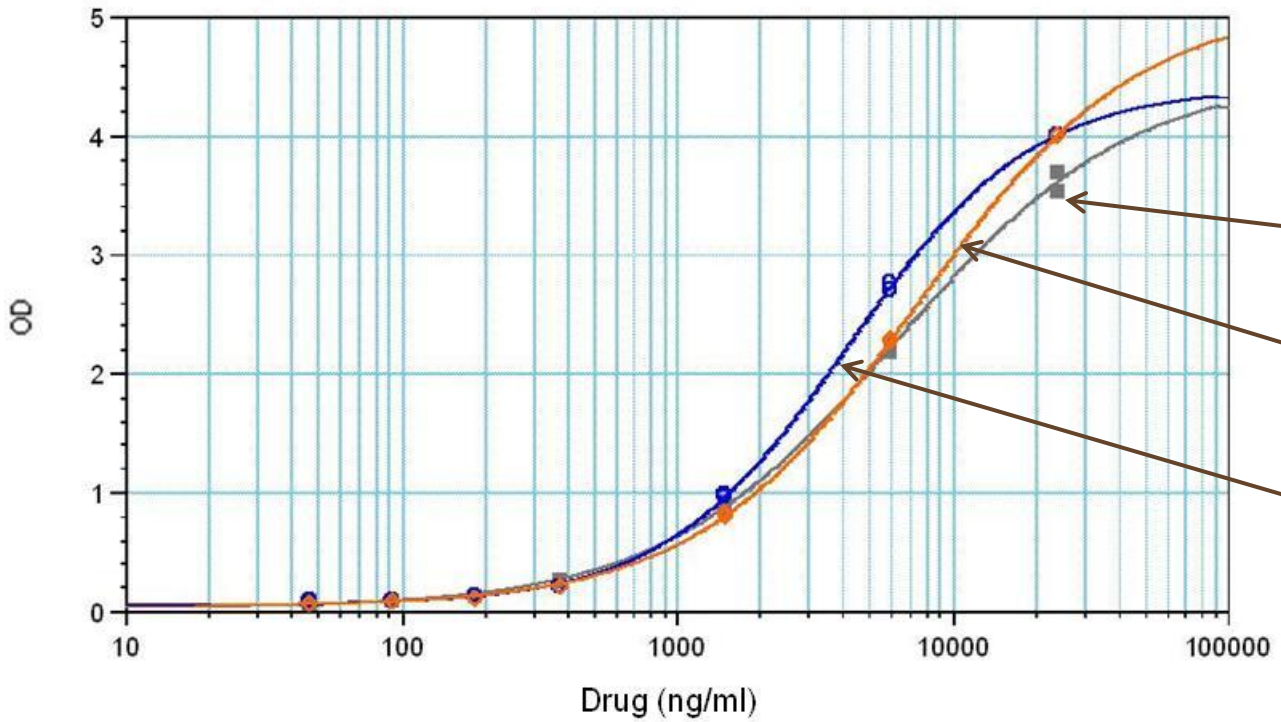
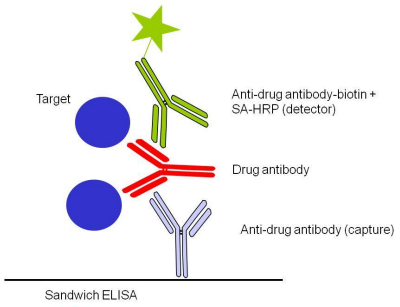
Minimal matrix effect when calibrators are prepared in 5% up to 20% human serum.



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
Curve6 (Neat serum dil 1/5: Concentration vs OD)	0.024	1.02	6.26e+03	4.52	0.999
Curve2 (Neat serum 1/20: Concentration vs OD)	0.0467	1.3	4.18e+03	4.41	1
Curve4 (Neat serum dil 1/10: Concentration vs OD)	0.0334	1.08	7.42e+03	5.11	1

Curve Fit Option - Fixed Weight Value



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Assay validation

- Assay dynamic range: 1.88 µg/mL (LLOQ) to 50 µg/mL (ULOQ) in neat human serum.
- QC high, mid, low: 3.75 µg/mL, 25 µg/mL, 37.5 µg/mL
- Accuracy & precision: Bias +/- 20%, CV (%) $\leq$ 20
- Dilution linearity
- Selectivity for 15 individual normal human serum samples
- No target interference
- No hook effect

Summary & conclusions

- Developing a free PK assay for human serum samples in the presence of abundant target is not feasible due to missing dilution linearity.
- Developing a total PK assay for the same matrix is challenging when the target is abundant and bulky.
- Identification of (i) anti-id antibodies for such an assay and (ii) sample pre-treatment conditions (acidification and neutralization prior to ELISA) overcame these hurdles.
- A sensitive and specific ELISA to quantify total drug in human serum samples (sum of free and target-bound drug) was developed. This assay will support PK analysis of the First in Human Trial of a new drug antibody candidate.

Thank you for your attention

Acknowledgement:

Andrew Warren

Peter Lloyd

Jennifer Sims

Anita Auger, Sandra Haile

Igor Vostiar, Kyo Chung

Stefan Ewert, Nicole Balke

Mark Milton

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