Risk Assessment for the measurement of Free and Total Drug and Target

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Introduction

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Bioanalytical scientists have become increasingly aware of the potential need to measure Free and Total analyte as well as some of the associated bioanalytical challenges

Despite this awareness there are varied degrees of engagement on this issue by project teams and BA scientists

- Given the rapid progress of projects and need to conserve resources how much effort should be exerted to build and define free/total assays and at what stage?
- What are the risks, how can they be assessed and what are the benefits of doing this?
  - Target Measurement
  - Target/Drug interference
  - Equilibrium Disruption

This talk will discuss analytical risk assessment and mitigation using both theoretical and experimental approaches for monoclonal antibody drugs and their targets.

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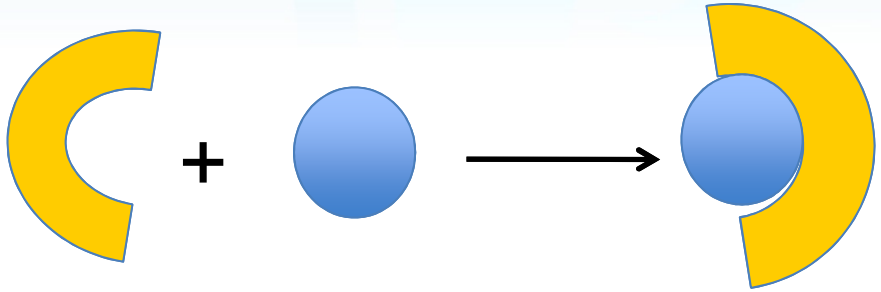
Introduction

Free and Total: Clinical Utility

Free D:

Free L:



Bioanalytical Challenges Re Form of Analyte

- Reagent Specificity: Data
- Free vs. Complex vs. Total: Data
- Matrix Interference: Data

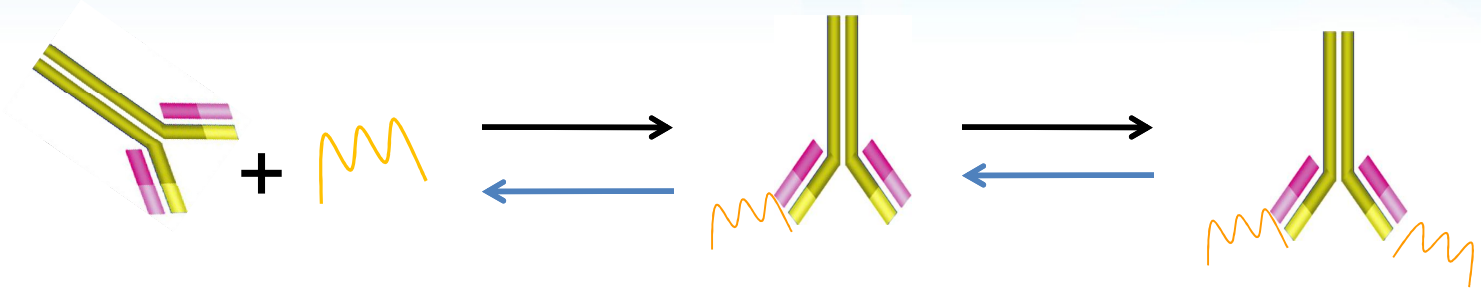
Utility Challenges: Why are we doing this?

- Normal vs. Disease: Data
- Free less than Y = Normal
- Total Greater than X = Clinical Implications

Total : Free + Complex

Introduction

Free and Total: PK : Two site, non-covalent binding



Free D:

Partially Free D:

Free L:

Total : Free + Complex

Bioanalytical Challenges

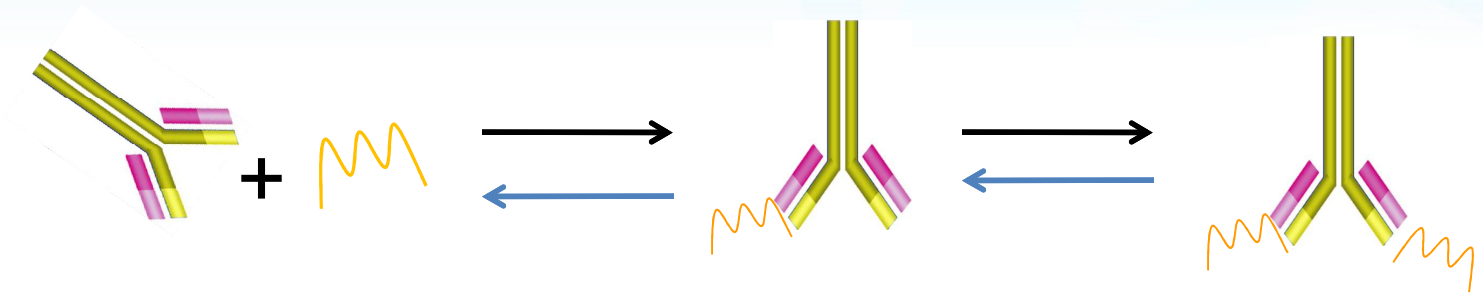
- Reagent Specificity: Data
- Native vs. Cleaved T: ??
- Matrix Interference: Data
- Free vs. Complex vs. Total: ??
- Equilibrium Disruption ??

Utility Challenges: Why are we doing this?

How much effort should we expend?

Introduction

Free and Total: Target :



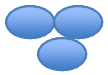
Free D:
Partially Free D:

Bioanalytical Challenges Re Form Target

Free L:

1) Potential for Disulfide bridge formation

2) Trimeric Target



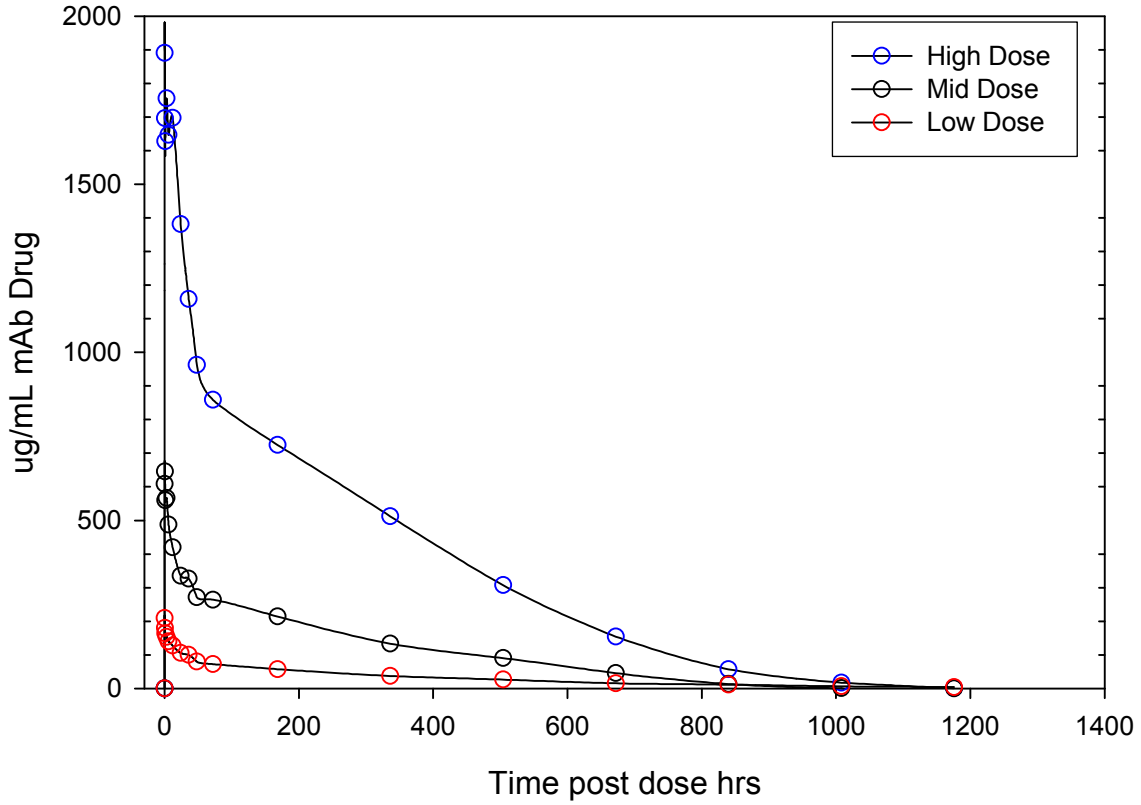
Total : Free + Complex

3) Weak bound heterogeneous aggregates

When Should We Be Concerned ?

Ignorance is Bliss ?

Time vs Concentration Profile



Total or Free Assay?

Clinical Phase Question

What is [target] ?

Can it interfere in PK assay?

PK Assay

Impact of lack of confidence in data

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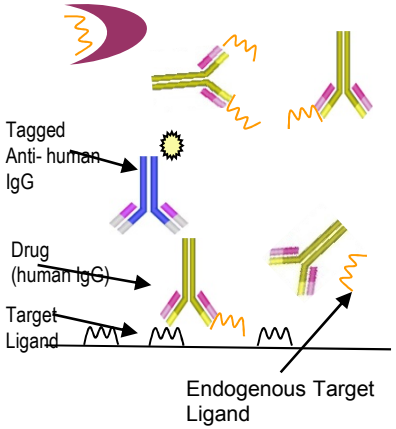
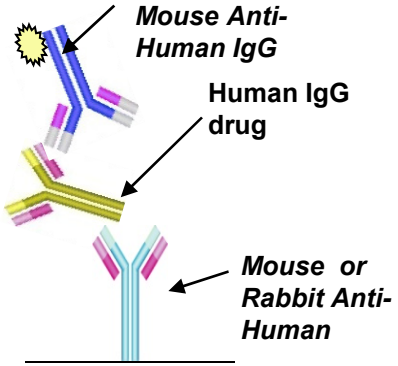
Enlightened PK/ PD Driven Bioanalytical support for IVT study

Free and Partially Bound

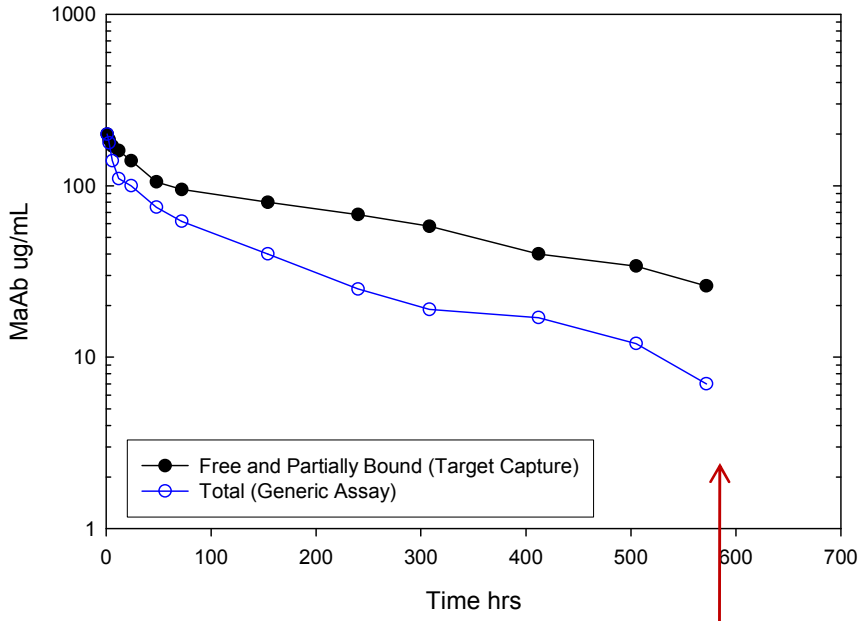


Generic Total Assay



Adapted from King 2010 and Clarke et al 2010

Assay format defines form of analyte?



Which data set is correct?

Target interference ?

Complex Dissociation?

Other?

Potential Target Interference:
Key Questions

- Is the target is soluble or shed
- What is the target concentration in normal and disease

PK

- Can the target can interfere in PK assay
- When can the target interfere in PK assay

Target PD

- Can the drug interfere in PD assay
- When can the drug interfere in PD assay

PK and PD Assays

- Is equilibrium disruption a real concern

Binding Equilibrium

Law of mass action

Foundation of the binding relationship of the drug (mAb) to the target ligand (L):

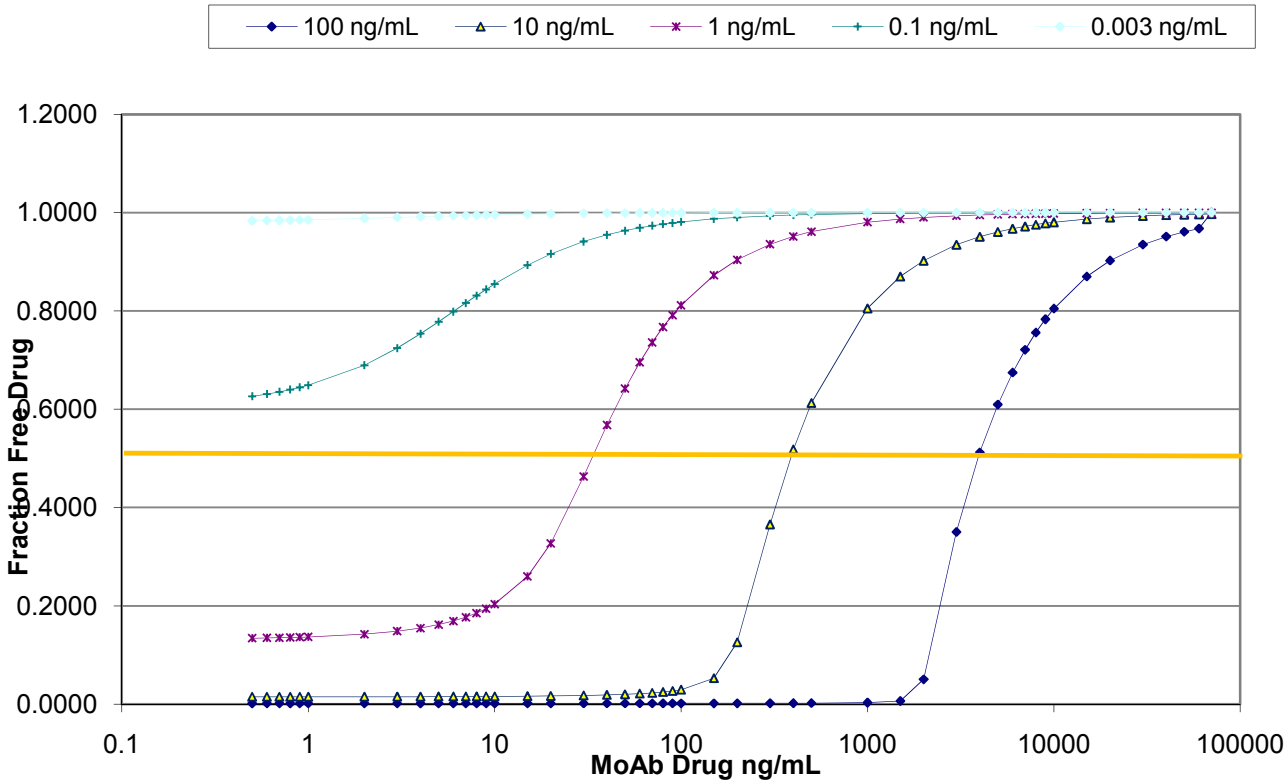
$$mAb + L \rightleftharpoons mAb^*L$$

$$K_d = \frac{1}{K_a} = \frac{[mAb]_{free} \times [L]_{free}}{[mAb^*L]_{bound}} = k_{off} / k_{on}$$

The dynamics of target ligand-mAb binding may shift with dose and time

Assessment of Target Interference

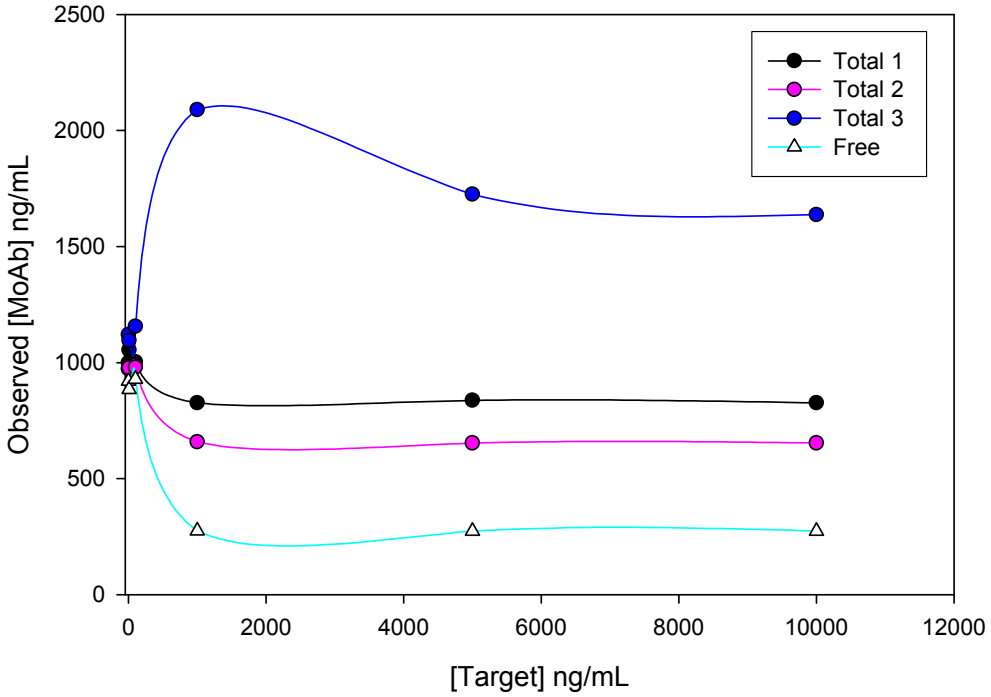
Free and Partially Bound



Simulation based on Biacore derived Ab – Target KD

Assessment of Target Interference

Effect of Target on Three Candidate Generic Total and a Free/Partially Bound PK assay



What we did right:

- Equilibrium
- Defined Edges
- QC neat matrix +control

What we might do different:

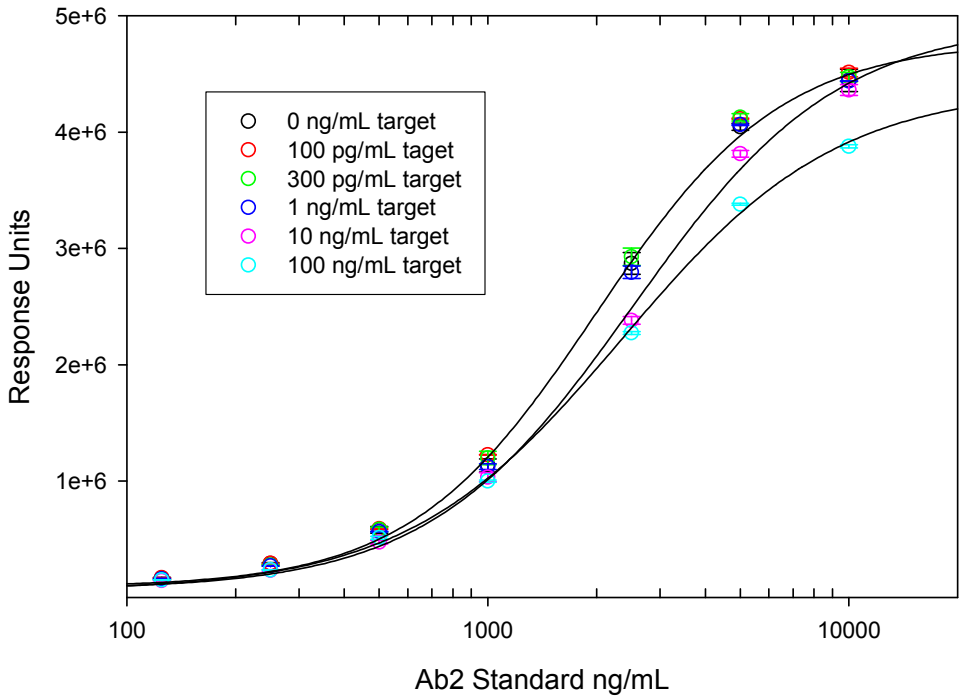
- Conduct interference testing before Sample Analysis: Assay utility?

Guo J, King L, Wang HF (2009)Development of generic total assays for quantitation of therapeutic human IgG2 monoclonal antibodies in nonclinical species using ligand binding assay. AAPS Journal 11(S1), 315.

Anti-Id "Total" Assay

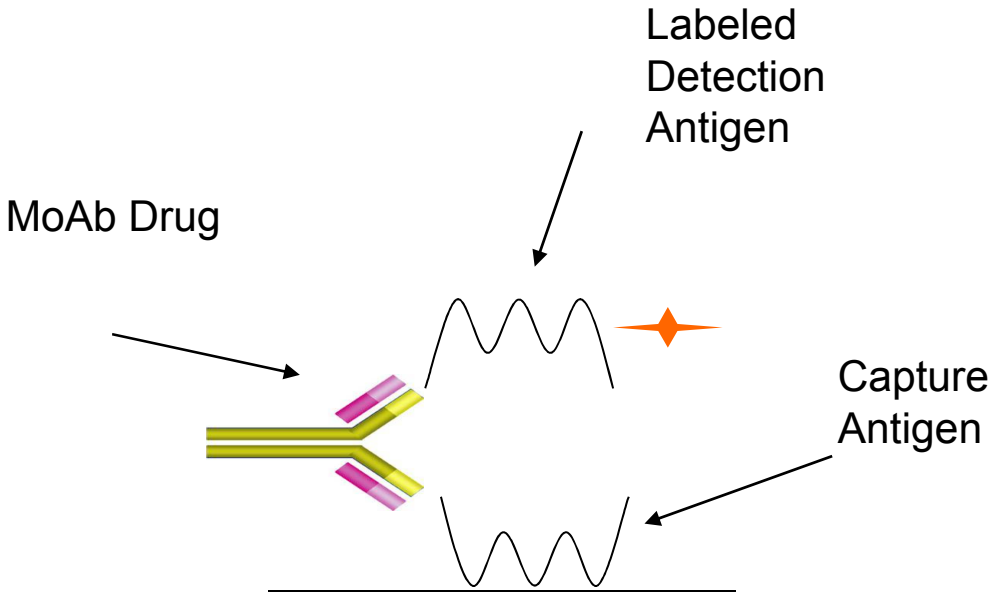
Unexpected Target Interference

Non Competing Anti-id based on Octet



Free PK Bridging ELISA

In principal assay measures only free drug



In practice risk of overestimating Free?

Assessment of Target Interference

MoAb Nominal µg/mL	0.5	0.5	0.5	0.5	1.2	1.2	1.2	1.2	1.75	1.75	1.75	1.75
rhTarget Spiked ng/mL	0	1	10	50	0	1	10	50	0	1	10	50
MoAb Observed µg/mL	0.669	0.601	0.529	0.308	1.35	1.26	1.116	1.11	1.85	1.83	1.84	1.52
Percent Recovery From Nominal	133.8	120.2	105.8	61.6	112.5	105.0	93.0	92.5	105.7	104.6	105.1	86.9
Free MoAb Predicted µg/mL	0.5	0.499	0.487	0.433	1.2	1.199	1.186	1.129	1.75	1.749	1.736	1.678
Binding Site Molar Ratio MoAb to target	NA	340	34	6.8	NA	816	81.6	16.3	NA	1190	119	23.8

Result

Assay appears to measure total drug at less than 10 ng/mL Target Antigen

"Apparent Free"

Soderstrom et al

What data are most important to the team?

- PK data of Ab
- Antigen Baseline concentration measurement
- Turnover rate constant (T1/2 of Antigen)
- Free Antigen level after mAb dosing
- Complex level after mAb dosing
- Total Antigen concentration (=free+bound)

Can be modeled

- Wide dose range

Target Assays

- Expected PD profile essentially validates assay is fit for purpose
- Free and Total target informs PK/PD relationship
- Drug interference testing needed

Total target data can be used

- to model the potential for target interference in the PK assay
- Trigger PK data review based on target interference data from PK assay validation

Free target can be used

- to define target suppression and subsequent return to baseline after dosing

Equilibrium shifts during sample analysis can result in an overestimation of the amount of free observed

Total Target as Biomarker
Fit for Purpose

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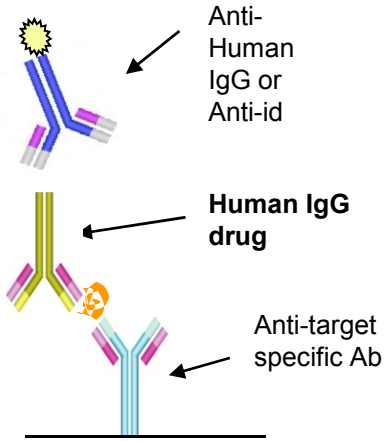
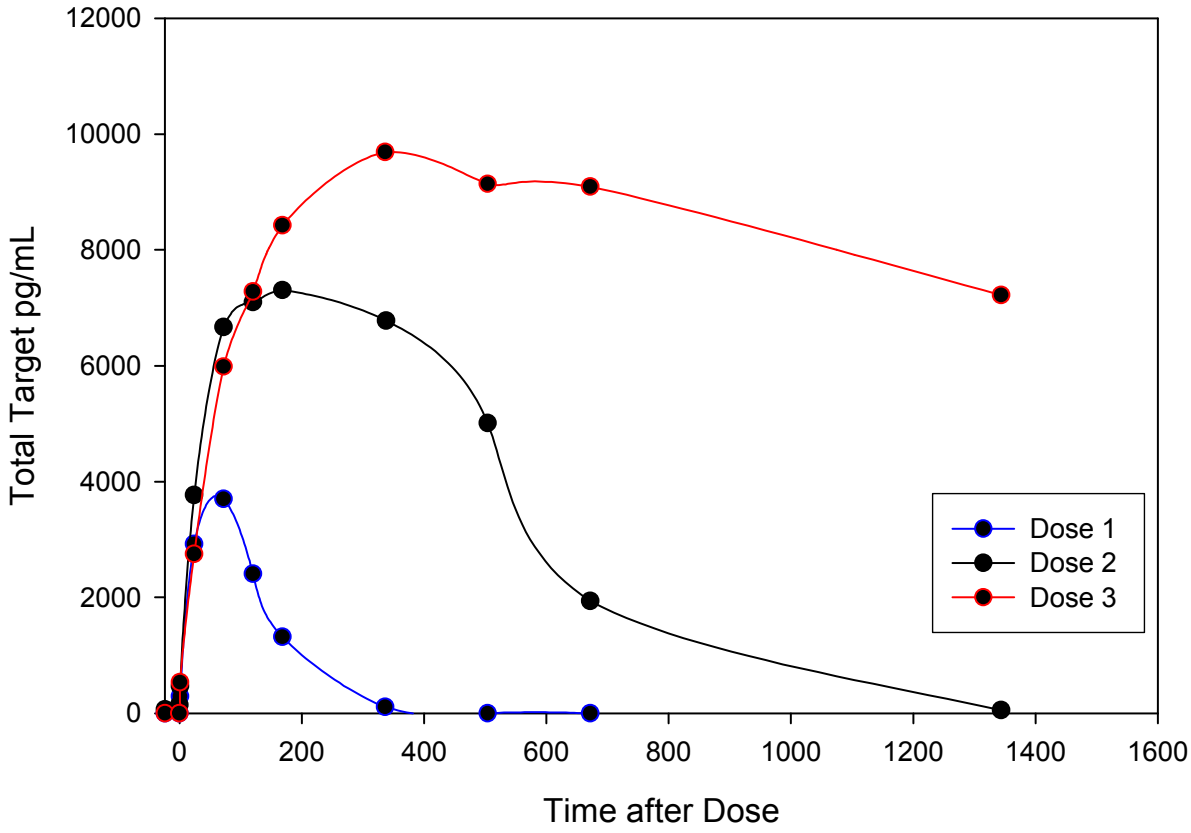
Total Target Assay



Drug interference testing conducted

Effect of Dose on Total Target Levels



Guo et al., 2009

Sample manipulation and Handling

Equilibrium Disruption

- Sample dilution can disrupt sample equilibrium
  - MRD in assay buffer 1:10.
  - Dilution in matrix pool; PK samples diluted significantly before the MRD: ULOQ 2 ug/mL
- Incubation time can disrupt sample equilibrium
  - Incubation time after MRD or matrix dilution
  - After sample dilution incubation time on plate
- Antigen coat concentration may contribute to equilibrium shift during plate incubations.
  - Locally high target concentration
  - Avidity contributes (binding of first site facilitates binding of second)

- Reality potentially a combination of above. **How Significant an issue is it?**

Sample Equilibrium Disruption: Time

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Rate of Binding Reaction

$d[sL]/dt = k_1[s]*[L] - k_2[sL]$

[s] = Concentration of binding sites

[L] = Concentration of ligand

k_1 = association rate

k_2 = dissociation rate

If all free drug and ligand is removed expect exponential decay curve

$[sL] = [sL]_0 e^{-k2*t}$

Can use this "worst case scenario" to simulate dilution and time effects on sample that started off at equilibrium (t=0)

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Simulating the effect of Time

PK Risk Assessment

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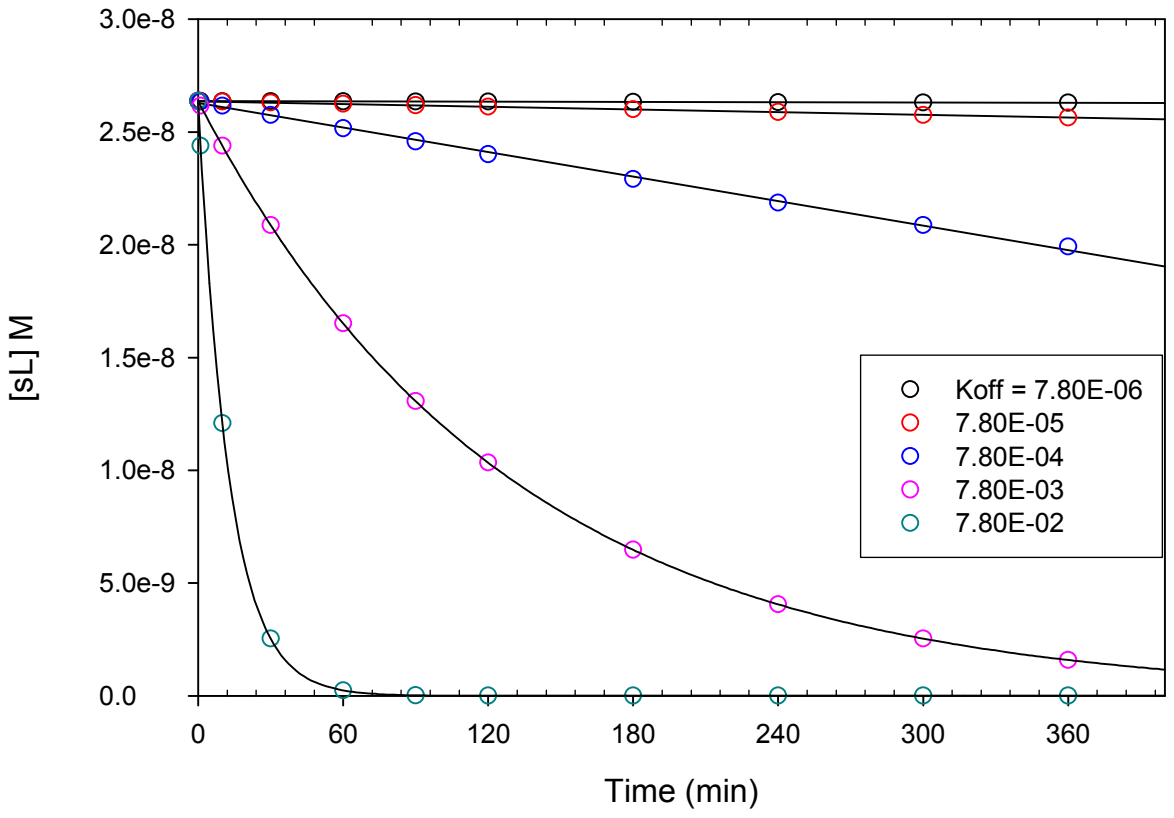
Dissociation of Complex over time
Off Rate Simulations



Molar Ratio = 2.56

Drug Binding Sites to Target

10 µg/mL drug

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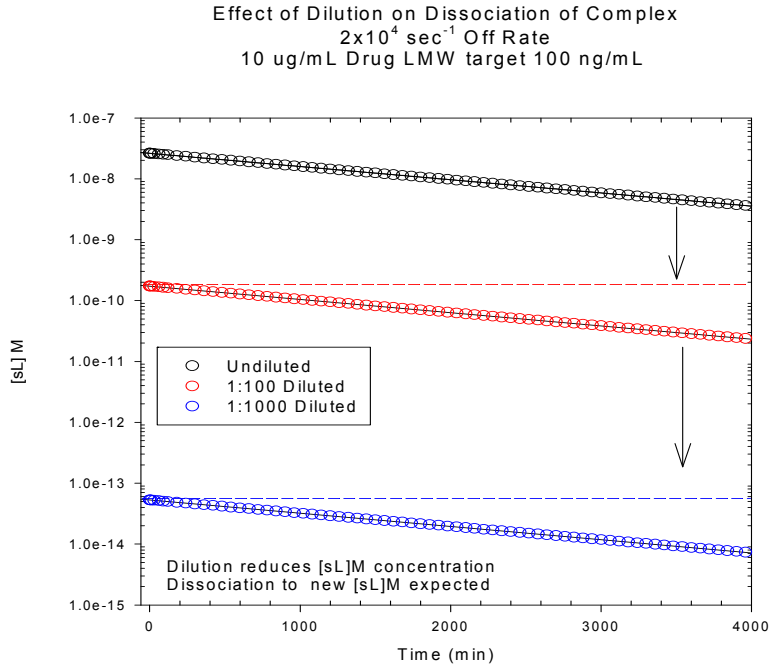
Dilution Induced Complex Dissociation

Is it really an issue?



PK Eg.

Experimental: Dilute Drug/Target Interference QC

1:5, 1:20, and 1:50

Test within 60 min

Results: No Change in observed result

Conclusion: Complex dissociation not a factor

Experimental data confirm simulation

What you need to know to better assess risk

Capture and Detection Ab/Reagents/ Drug-Target Kinetics

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Assay Format Implications		
[Target Ligand]:	10-50 pg/mL	1-50 ng/mL
Target Kinetics:	Unknown	Rapid clearance and synthesis
Structure:	Monomer	Dimer/Trimer/Aggregates
Drug -Target	KD : ≈ 10 pM	KD ≈ 1nM
	On Rate : fast	On Rate : moderate
	Off Rate: slow	Off Rate: moderate
**********	**********	**********
Drug –Capture Ab KD :	?	KD ≈ nM
	On Rate : ?	On Rate : moderate
	Off Rate: ?	Off Rate: ?
Target–Capture Ab	KD : nM	KD ≈ pM
	On Rate : medium	On Rate : moderate
	Off Rate: medium	Off Rate: slow

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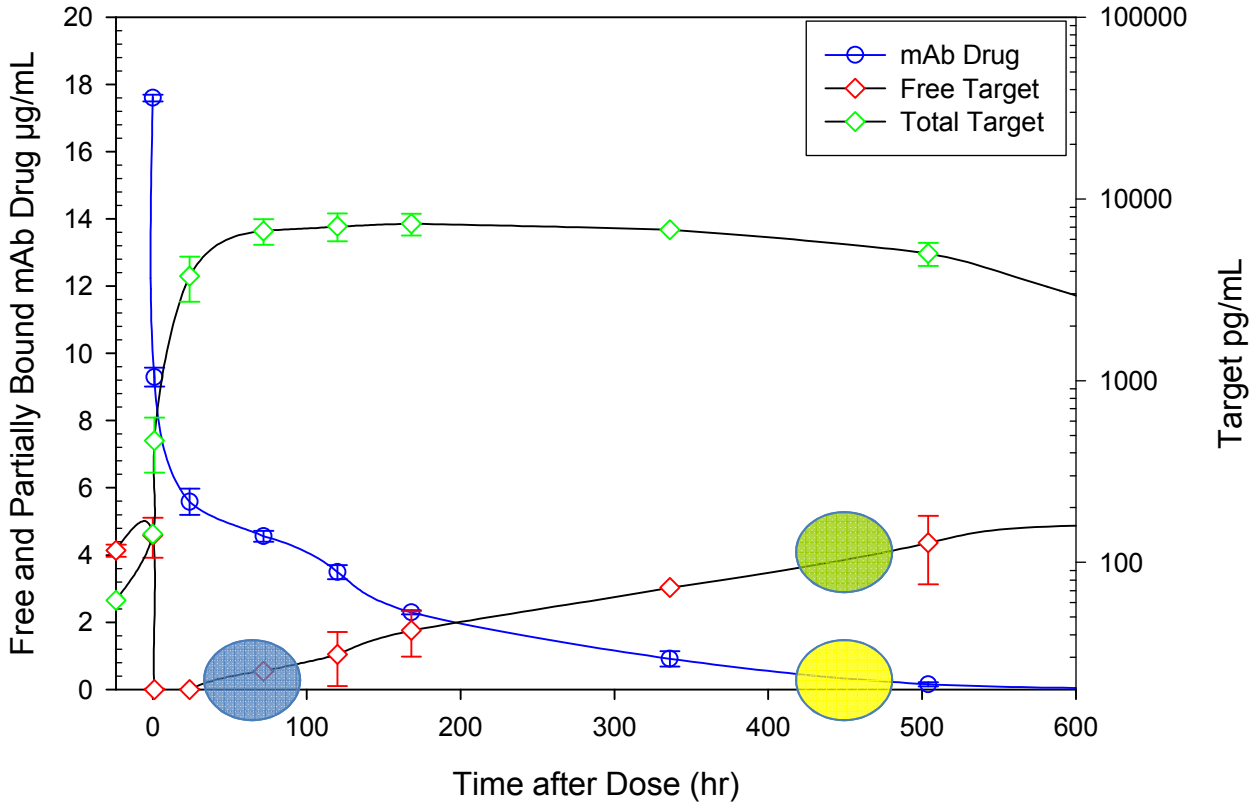
When should we be concerned?

PK/PD Efficacy study

Dose vs Time Plasma Levels

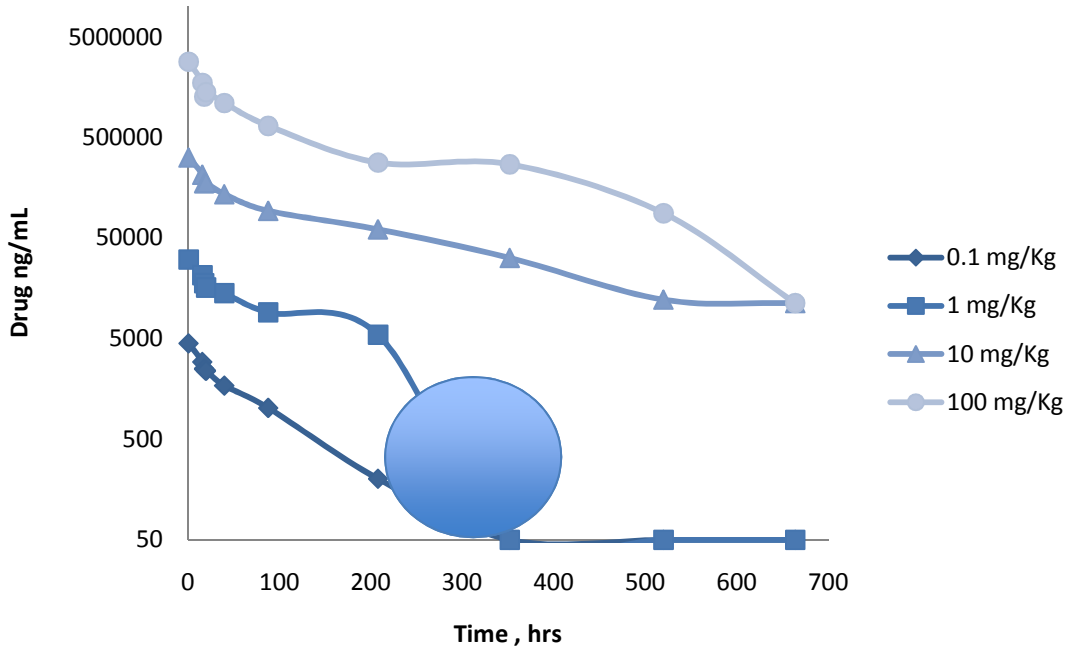


What are the risks ?

1) *Lack of sensitivity to measure free target after dosing*
2) *Under recover free drug. Target interference*
3) *Over recovery of free target*

PK

Target interference; when does it matter?



Can target interference alter PK parameters in a meaningful way?

TMDD, Target Interference or ADA?

Conclusions

Target /drug interference when does it matter?

Having raised awareness regarding Free and Total now need to find the right balance between

- asking for 5 assays and equilibrium disruption phobia
- doing nothing to assess either issue

When there is a potential for target/drug interference it needs to be experimentally defined so it can be managed

If observed total target level reaches a level that has been shown to interfere in the PK assay then the impact can be evaluated.

Conclusions

Bioanalytical scientists can have a significant impact on programs by helping the team understand this issue

- It is better to select assays which can generate the best data and define edges than to attempt to measure multiple forms of analyte with less confidence.
- Risk of not assessing interference is potential for a lack of confidence in data, no matter what the cause, that can lead to significant additional bioanalytical investment to define this risk.
- Sample handling (dilution/ time) and assay format can contribute to equilibrium disruption and result in an under or over estimation of Free analyte **but** important not to overstate this risk
- Knowledge of binding kinetics will help put this issue in perspective and simulation is a useful tool to illustrate this for the team