



The best-laid schemes o' mice an' men: when clinical assumptions go awry

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To a Mouse, by Robert Burns (November 1785)

- **After a farmer plows up a mouse's nest, he apologizes to the tiny creature while assuring it that he means no harm**
 - The best-laid schemes o' mice an' men, gang aft a-gley
 - *The best-laid schemes of mice and men, go often awry*

Sometimes a similar experience in assay development!

Case study

- **Development of anti-drug antibody (ADA) assays for an evolving clinical trial program**
- **Monoclonal antibody against a soluble target**
- **Patient samples difficult to source**

Best-laid scheme

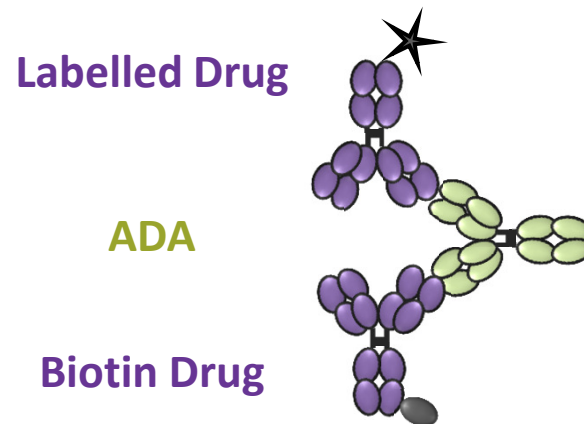
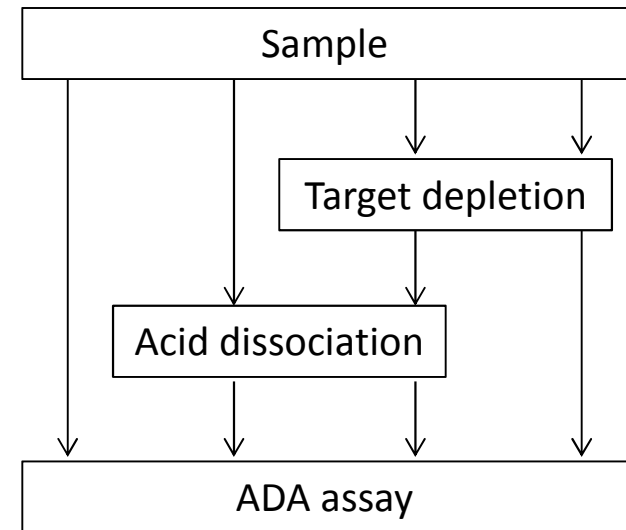
- **Anticipated drug levels based on dosing regimen**
 - 10-20 $\mu\text{g}/\text{mL}$ trough levels after multiple infusions
- **Anticipated target levels in clinical samples**
 - Literature review
 - Different assays used - different (relative) concentration measurement
 - Access to healthy volunteer samples
 - Not reflective of the disease-state
 - Limited access to patient clinical samples
 - Some information, but a very small cohort
 - Accumulation in the presence of drug

Best-laid scheme

- **Anticipated drug levels based on dosing regimen**
 - 10-20 $\mu\text{g}/\text{mL}$ trough levels after multiple infusions
- **Anticipated target levels in clinical samples**
 - 50 ng/mL target with accumulation

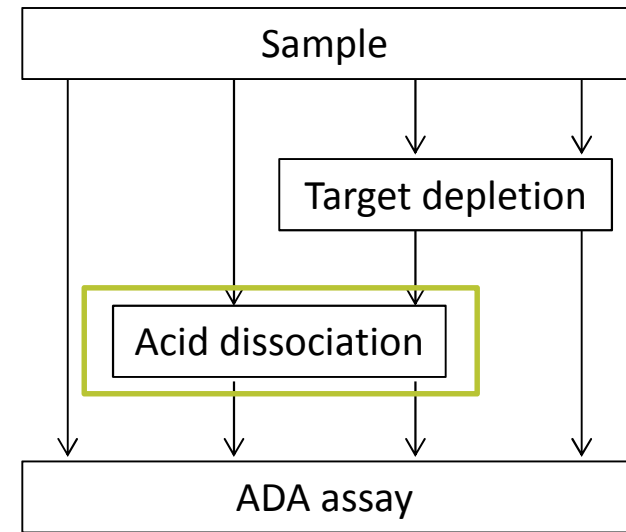
ADA assay development

- Bridging format

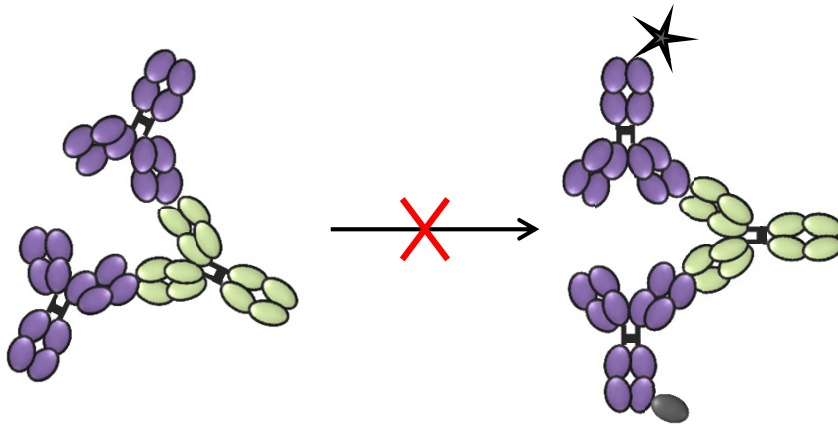


ADA assay development

- **Bridging format**
- **Acid dissociation**
 - 300 mM acetic acid pH 3.0
 - Drug tolerance of 20 $\mu\text{g}/\text{mL}$ (250 ng/mL reference ADA)



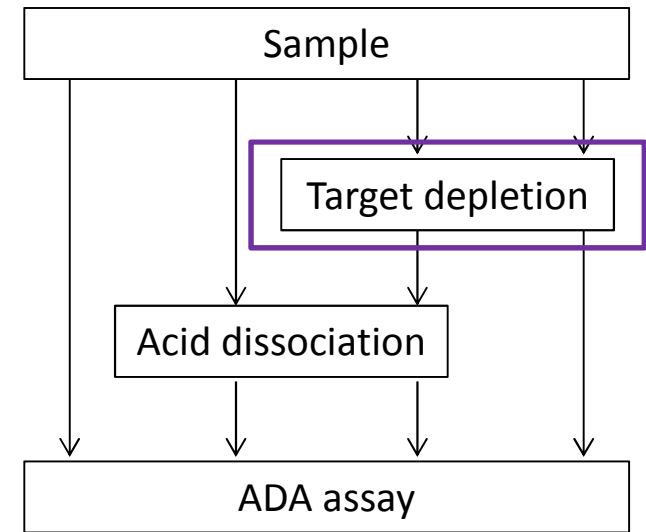
ADA:Drug complexes



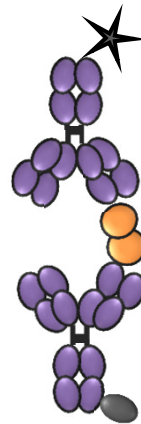
➔ False negative results

ADA assay development

- **Bridging format**
- **Acid dissociation**
 - 300 mM acetic acid pH 3.0
 - Drug tolerance of 20 $\mu\text{g}/\text{mL}$ (250 ng/mL reference ADA)
- **Target depletion**
 - Target tolerance of 50 ng/mL



Labeled Drug:Target complexes



➔ False positive results

For further details see:

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2016, Article ID 5069678, 15 pages
<http://dx.doi.org/10.1155/2016/5069678>



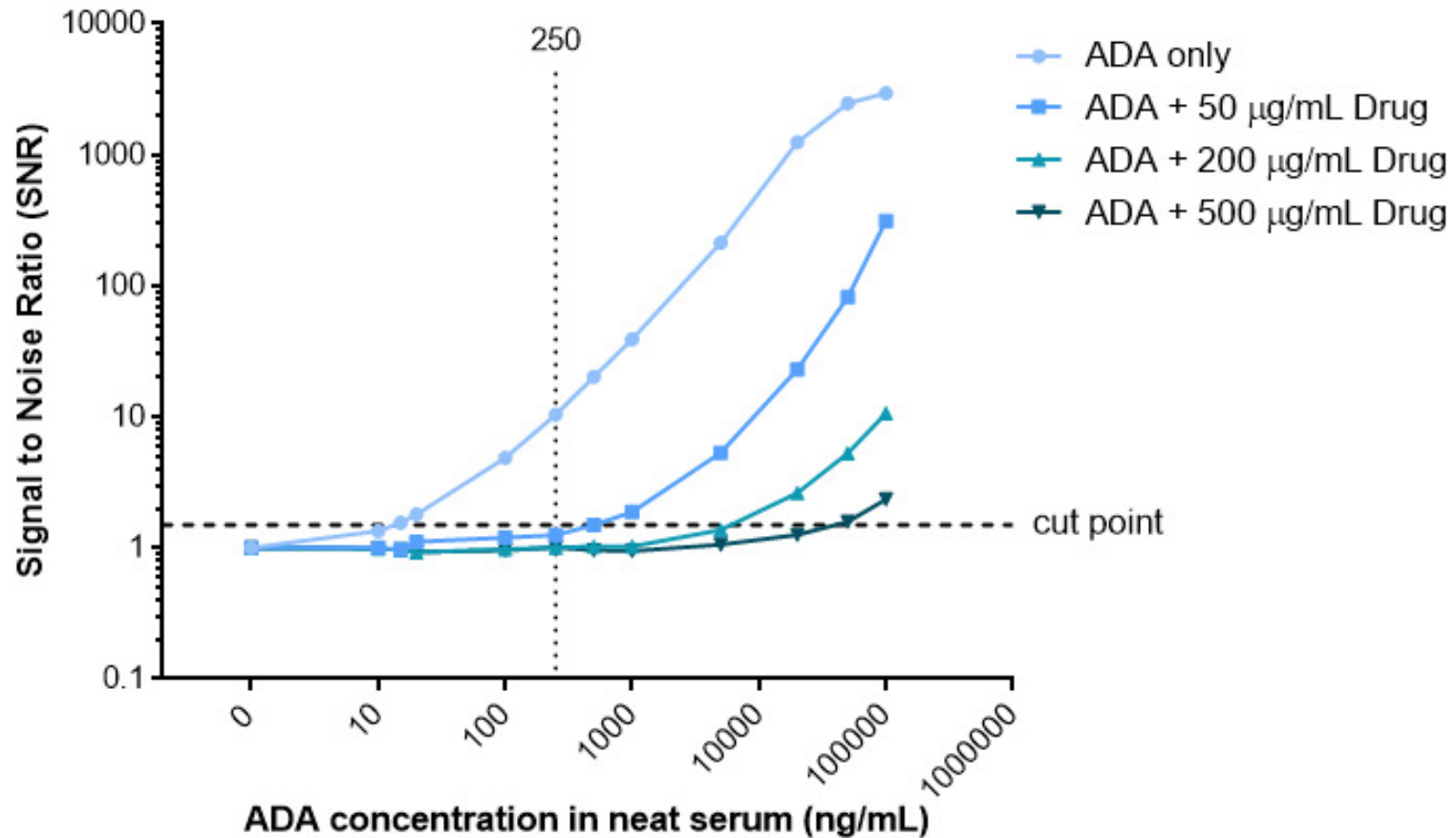
Research Article

Evaluation of Multiple Immunoassay Technology Platforms to Select the Anti-Drug Antibody Assay Exhibiting the Most Appropriate Drug and Target Tolerance

Justine Collet-Brose, Pierre-Jean Couble, Maureen R. Deehan, Robert J. Nelson, Walter G. Ferlin, and Sabrina Lory

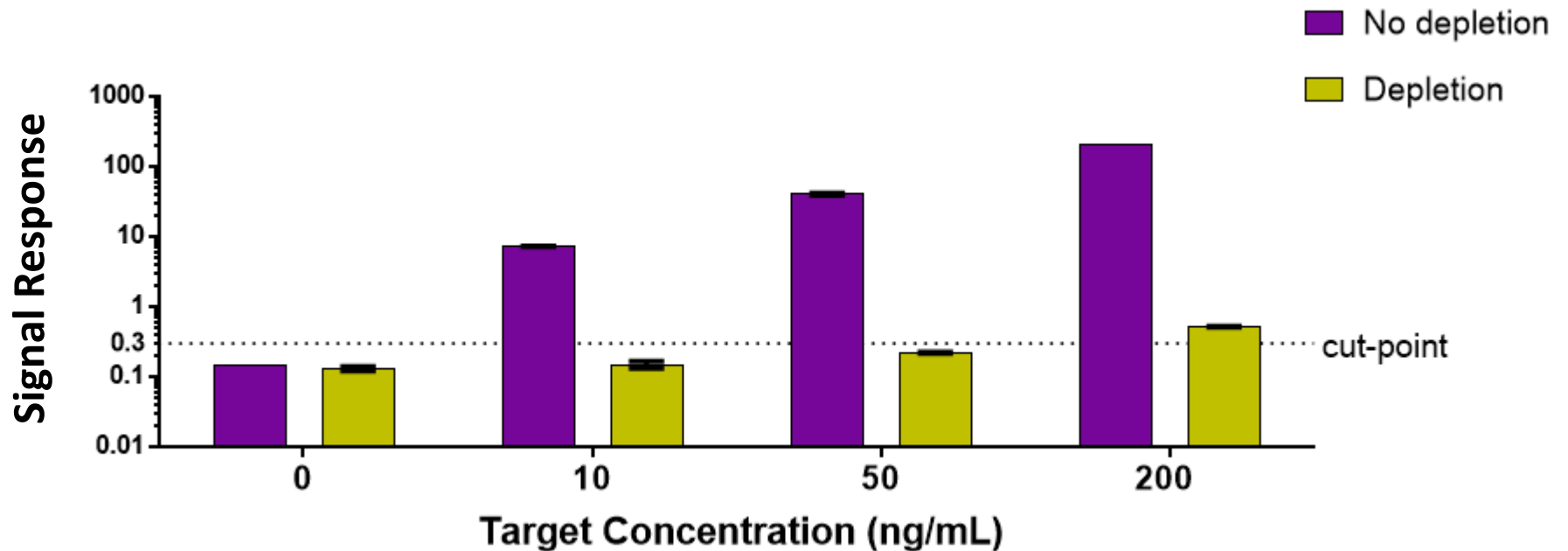
Novimmune SA, 14 Chemin des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland

Drug interference



- Loss of sensitivity with increasing free drug levels

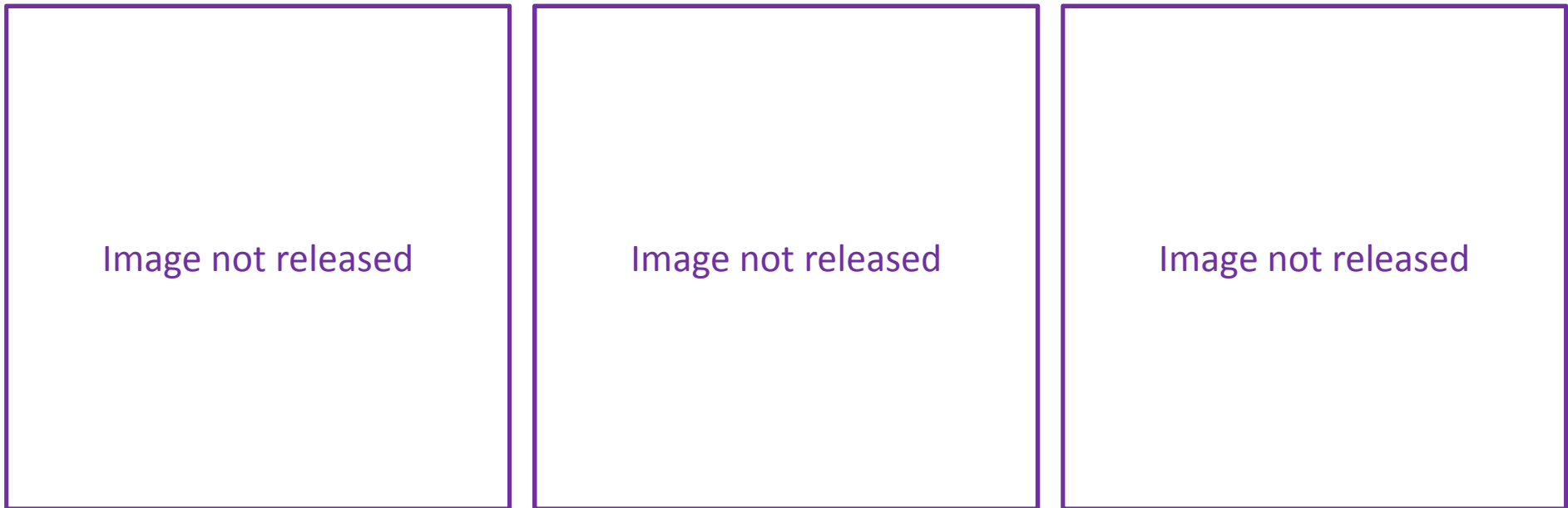
Target interference & depletion of target



- Target interference addressed effectively

When clinical assumptions go awry

PK & total target for Subjects #A, #B & #C



In study drug & target interference

Drug tolerance 20 µg/mL Target tolerance 50 ng/mL

Subject	Timepoint	Free Drug (µg/mL)	Total Target (ng/mL)
#A	Pre-infusion	0	<1
#A	SD8	<20	>100
#A	SD45	>40	>100
#B	Pre-infusion	0	<1
#B	SD9	>20	<10
#B	SD43	>40	<10

Free Drug greater than validated level of drug tolerance » potential false-negative

Total Target greater than validated level of target tolerance » potential false-positive

In study drug & target interference

Drug tolerance 20 µg/mL Target tolerance 50 ng/mL

Subject	Timepoint	Free Drug (µg/mL)	Total Target (ng/mL)	ADA Testing (Titer)
#A	Pre-infusion	0	<1	Negative
#A	SD8	<20	>100	Negative
#A	SD45	>40	>100	Negative
#B	Pre-infusion	0	<1	Negative
#B	SD9	>20	<10	Negative
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In study target tolerance better than we thought ?

In study drug & target interference

Drug tolerance 20 µg/mL Target tolerance 50 ng/mL

Subject	Timepoint	Free Drug (µg/mL)	Total Target (ng/mL)
#C	Pre-infusion	0	<1
#C	SD35	<20	<25
#C	SD62	<10	>500
#C	SD80	>40	<25

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In study drug & target interference

Drug tolerance 20 µg/mL Target tolerance 50 ng/mL

Subject	Timepoint	Free Drug (µg/mL)	Total Target (ng/mL)	ADA Testing (Titer)
#C	Pre-infusion	0	<1	Negative
#C	SD35	<20	<25	Positive (1.06)
#C	SD62	<10	>500	Positive (2.22)
#C	SD80	>40	<25	Positive (1.07)

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In study drug & target interference

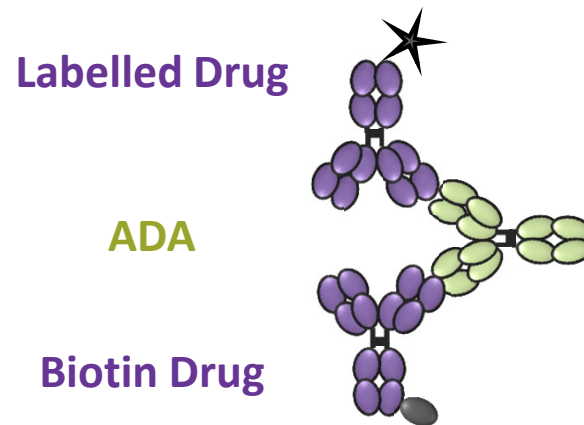
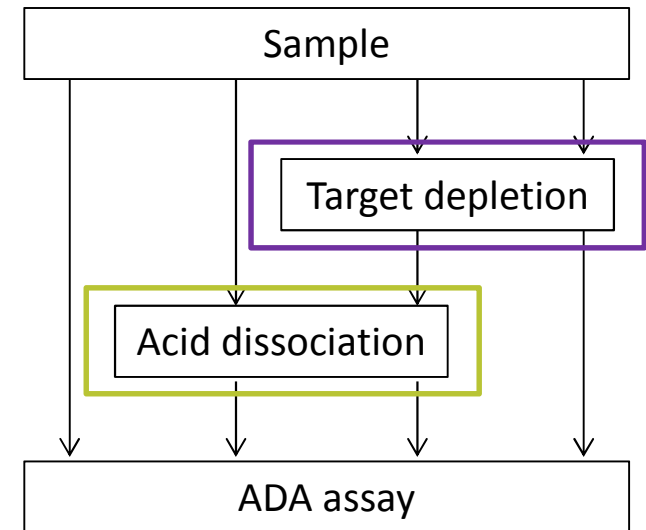
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In study target tolerance worse than we thought ?

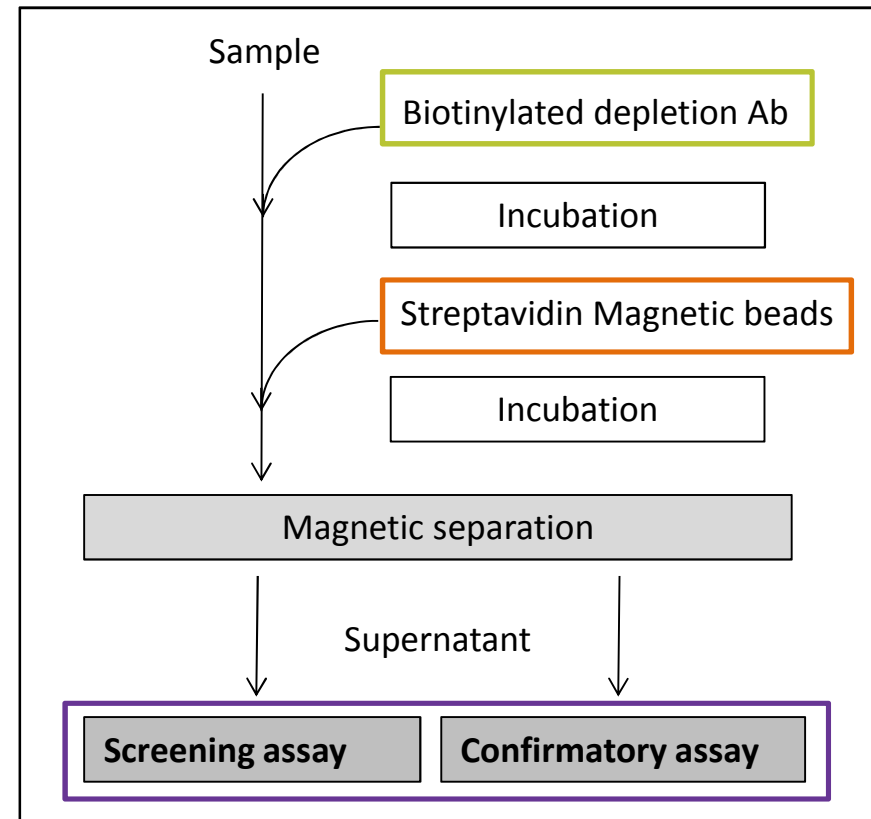
Improving drug & target tolerance

- **Bridging format**
- **Acid dissociation**
 - 0.5 M glycine pH 2.2
 - Drug tolerance of 200 µg/mL (250 ng/mL reference ADA)
- **Target depletion**
 - Target tolerance of 500-1000 ng/mL

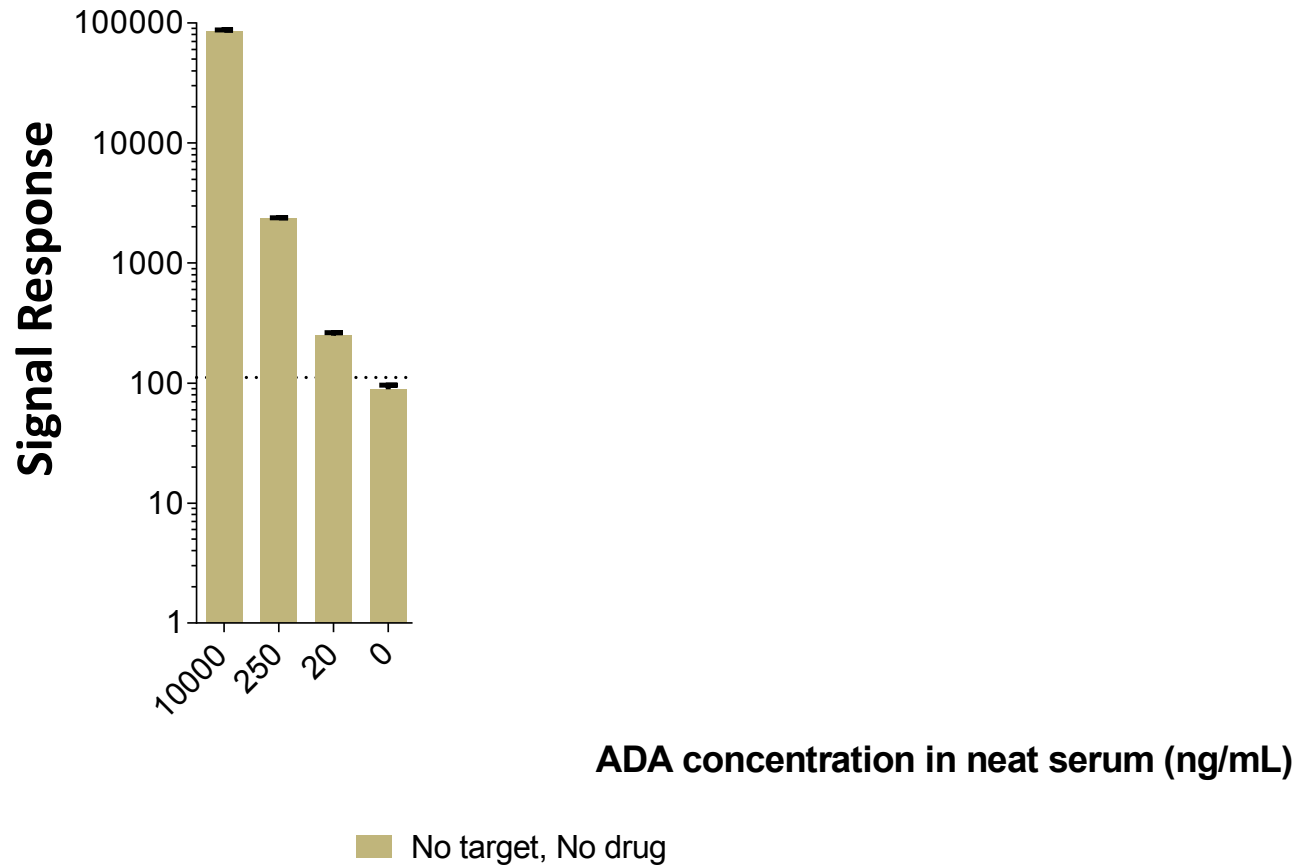


Optimising the target depletion protocol

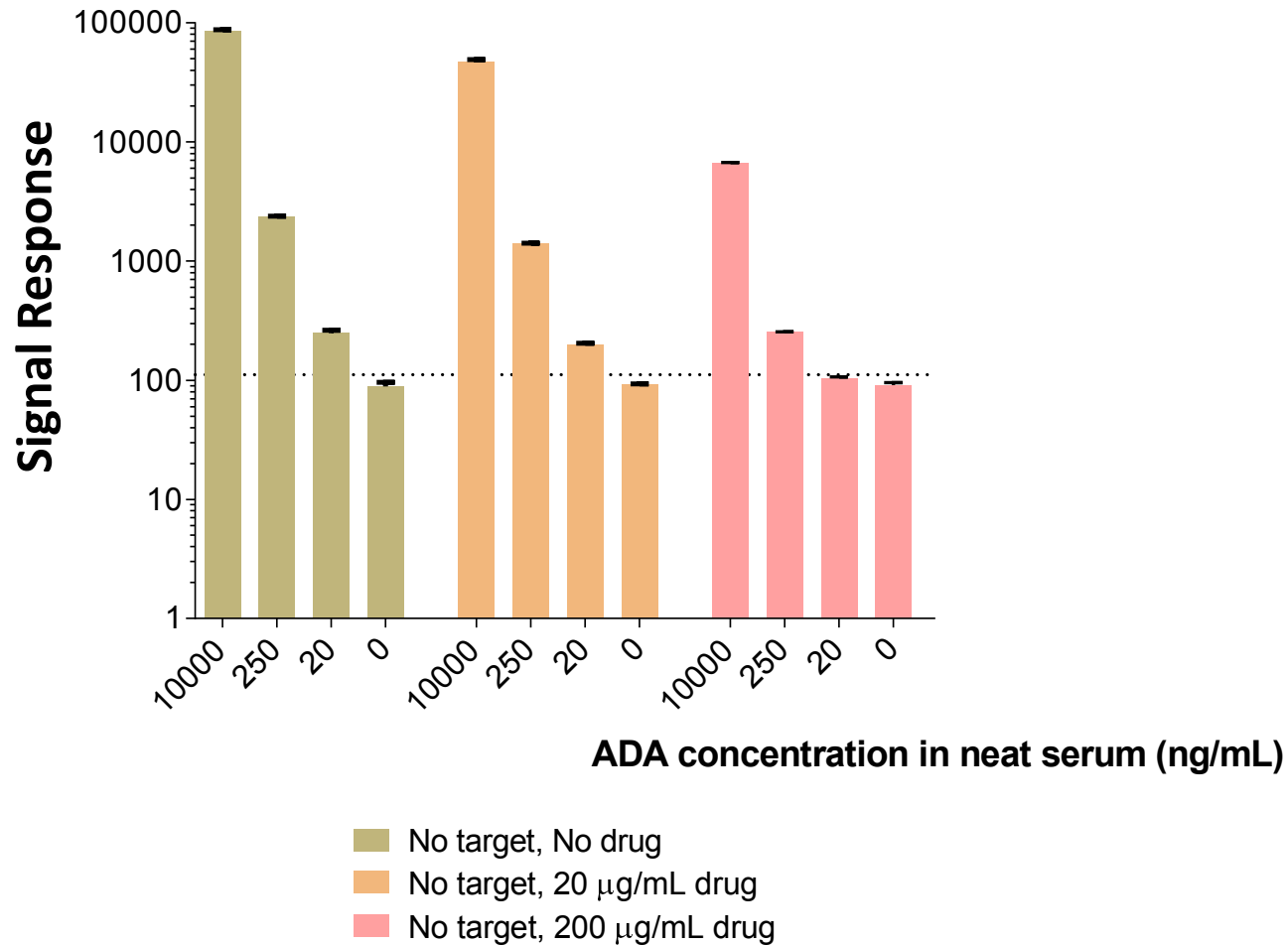
- Anti-target antibody **non-competitive** with drug
- **Solid phase extraction (SPE)** with magnetic beads
- Anti-target Ab **competitive** with drug added to capture-detection mixture



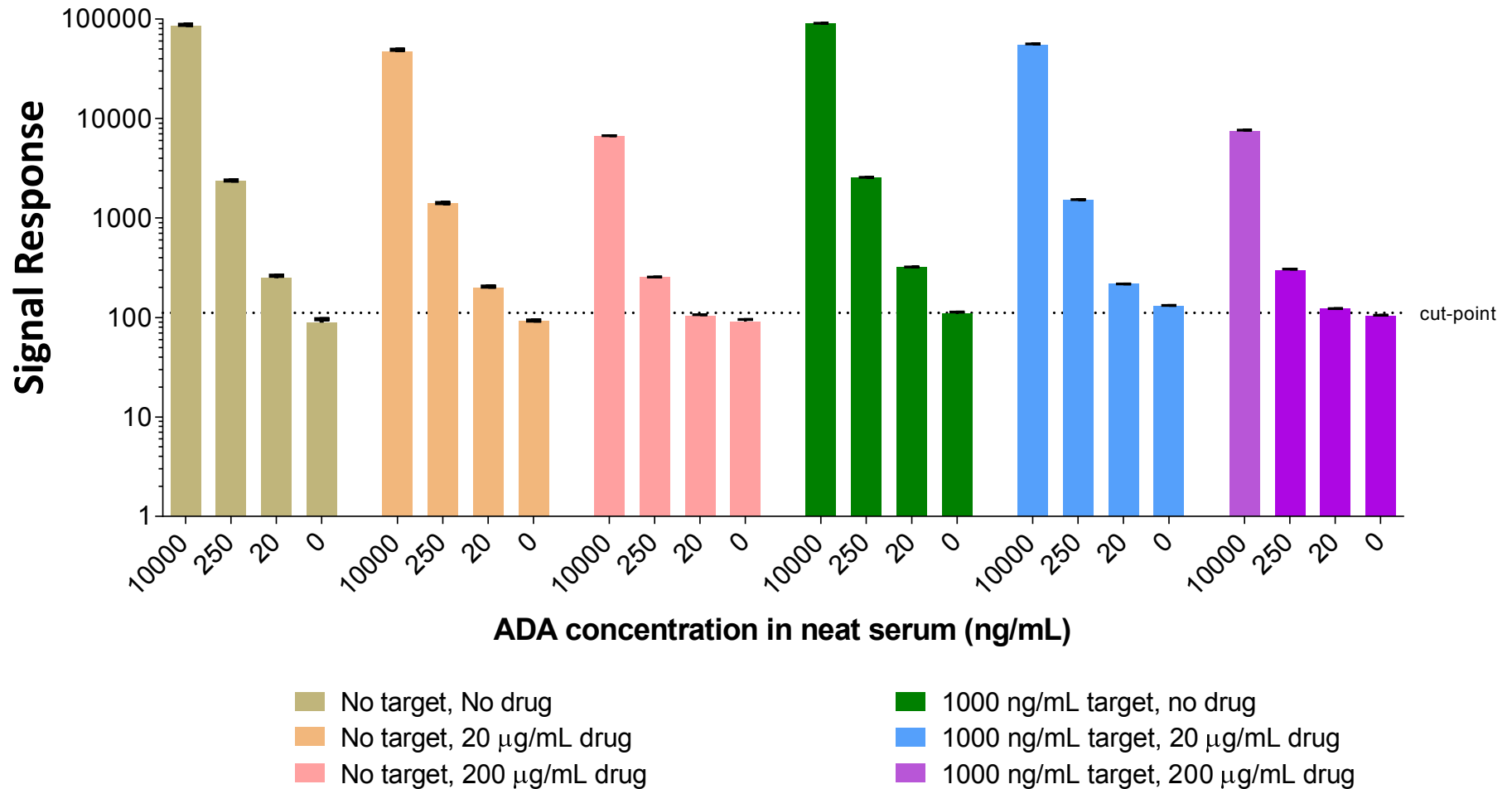
Improved drug & target tolerance



Improved drug & target tolerance



Improved drug & target tolerance



Summary

- **Best-laid schemes**
 - Assay developed with tolerance targets based on available evidence
 - Anticipated trough levels for drug around 10-20 µg/mL
 - Estimated target levels up to 50 ng/mL
- **When clinical assumption go awry**
 - Actual drug levels around 20-50 µg/mL, but higher levels also observed (100-200 µg/mL)
 - Target levels much higher than anticipated, many samples above 50 ng/mL, 500-1000 ng/mL observed
 - Assay methodology adapted rapidly

Thank you for your attention!

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