Generic Assays for Preclinical Immunogenicity Assessment in Rodents

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Agenda

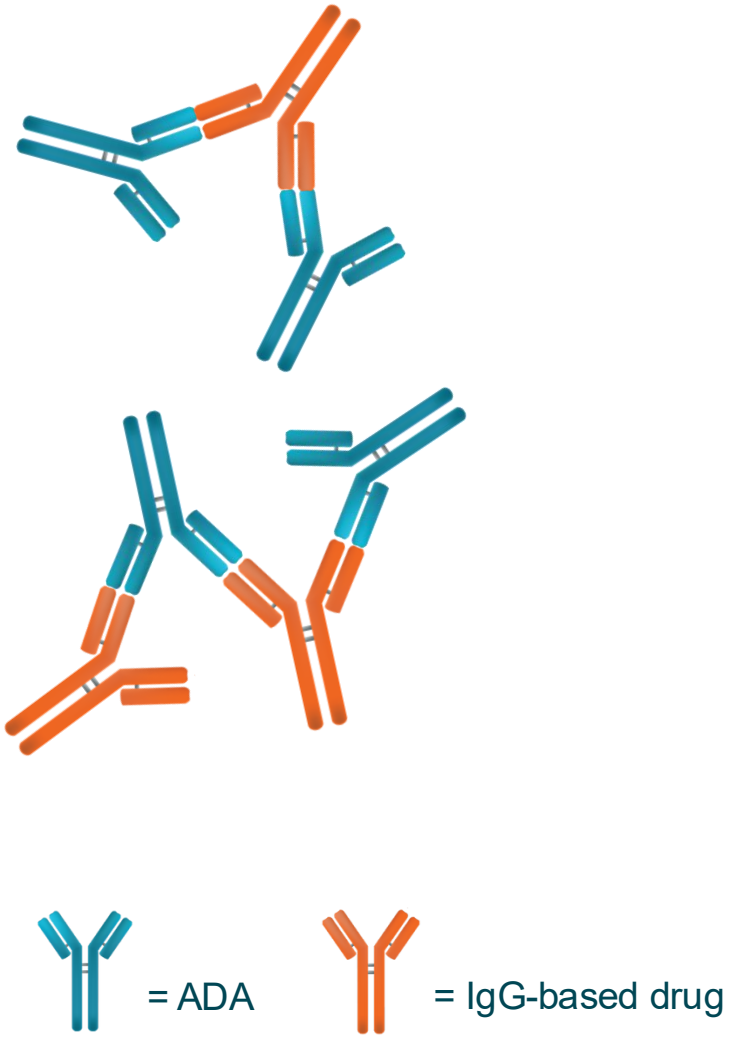
1 I Detection of immunogenicity in preclinical species

2 I Development process for a generic rodent ADA assay

3 I Impact of varying drug concentrations on the detection of ADA in preclinal species

Importance of immunogenicity assessment

- Any treatment with biotherapeutic drugs can lead to an immunogenic response in the form of Anti-Drug Antibodies (ADA)
- Effect of the drug may be hampered or even eliminated, and patients' symptoms can range from mild (skin reaction) to adverse (fatal allergic reaction)
- Detection of an immunogenic response in preclinical species is rarely predictive for clinical studies, but samples are banked for when needed



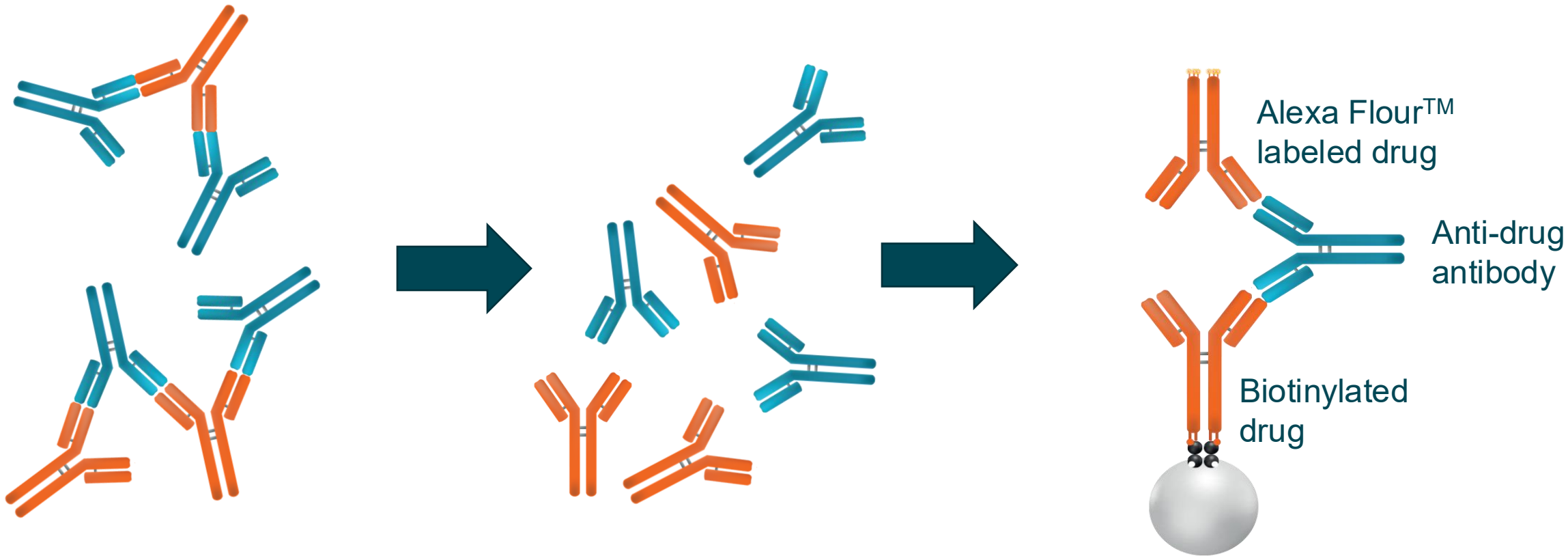
Preclinical assessment of immunogenicity

- Measurement of anti-drug antibodies (ADA) in nonclinical studies should be evaluated when there is
  1. Evidence of altered Pharmacodynamic (PD) activity
  2. Unexpected changes in exposure in the absence of a PD marker
  3. Evidence of immune-mediated reactions (immune complex disease, vasculitis, anaphylaxis, etc.)

- Since samples may not be evaluated for immunogenicity until called for by one of these, developing a drug specific assay for each biotherapeutic candidate can impact timelines.

ICH guideline S6 (R1) – preclinical safety evaluation of biotechnology-derived pharmaceuticals

A bridging assay for detecting ADA in samples



Circulating ADAs bound to the drug in the sample

Acidic conditions cause dissociation

Neutralization in presence of drug as capture and detection reagent
Requires dissociation of the ADA from any unlabeled drug

= ADA

= Human IgG-based drug

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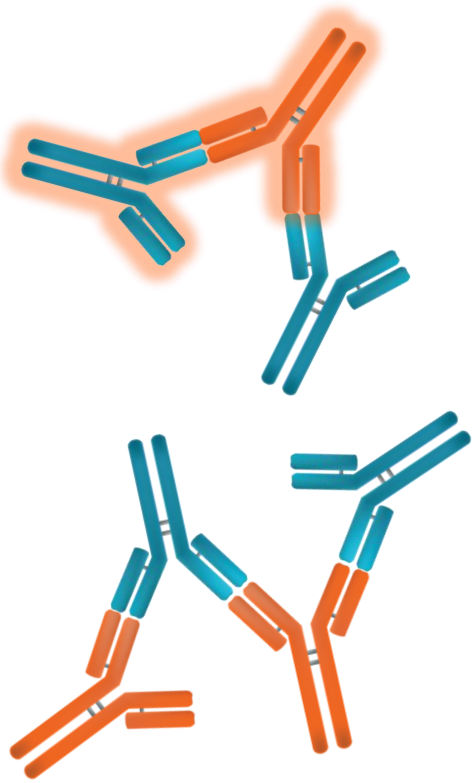
A generic assay for detecting ADA in rodents



Circulating ADAs bound to the drug in the sample

Direct detection of immune complexes

= ADA

= Human IgG-based drug

Alexa labeled anti-rodent IgG

Drug-ADA immune complex

Biotinylated anti-human IgG fragment

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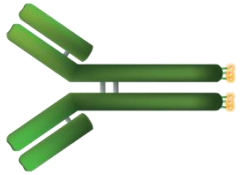
A generic assay for detecting ADA in rodents

- Detection of **immune complex** rather than targeting the unbound or free ADA
  - Anti-rodent IgG detection reagent
  - Anti-human IgG capture reagent
- Easy assay development
  - Generic assay conditions
  - Minimum Required Dilution (MRD) variable to population and species
- Universal for IgG based therapeutics
  - Requires IgG containing kappa (κ) light chain

Sample	Nominal conc. (ng/mL)	Intra-run CV %	Inter-run CV %
High PC	10,000	0.9 – 5.0	5.1
Mid PC	1,000	0.3 – 7.1	5.8
Low PC	100	0.3 – 8.0	7.2

Intra- and inter-run precision.
Positive Control (PC) samples covering the assay working range were run in triplicate in six separate runs by two different operators over two days.

Capture reagent
(Biotinylated anti-human IgG fragment)



Detection reagent
(Alexa labeled anti-rodent IgG)

Positive Control
Human IgG - Mouse IgG dimer

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Generic assay selectively detects circulating immune complexes

- Synthetic test system
  - Pooled mouse serum (1-in-10 MRD)
  - Human IgG biotherapeutic (golimumab, IgG1)
  - Commercially available mouse anti-human polyclonal antibody (pAb)
  - Commercially available PC (Human IgG - Mouse IgG dimer)

- Cut point of S/B ≥ 2 utilized to distinguish relevant signal from noise

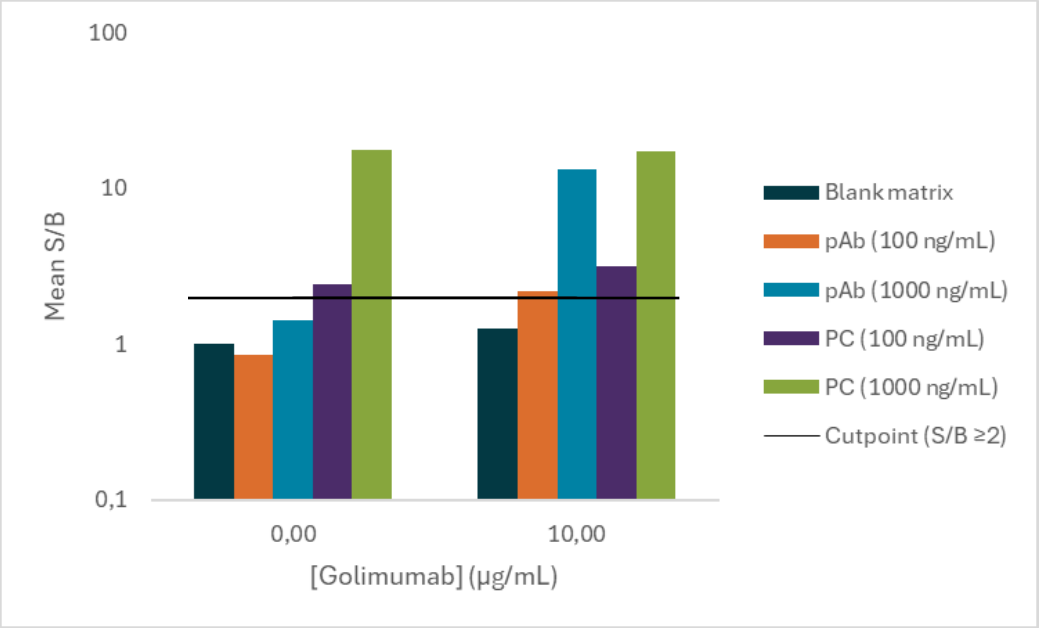
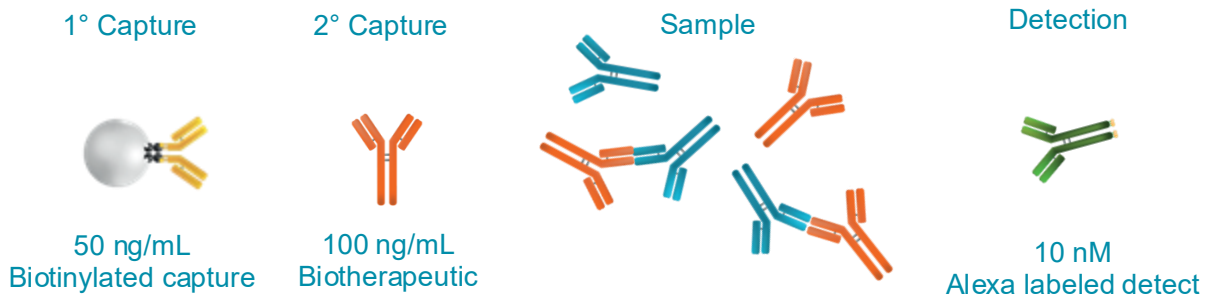


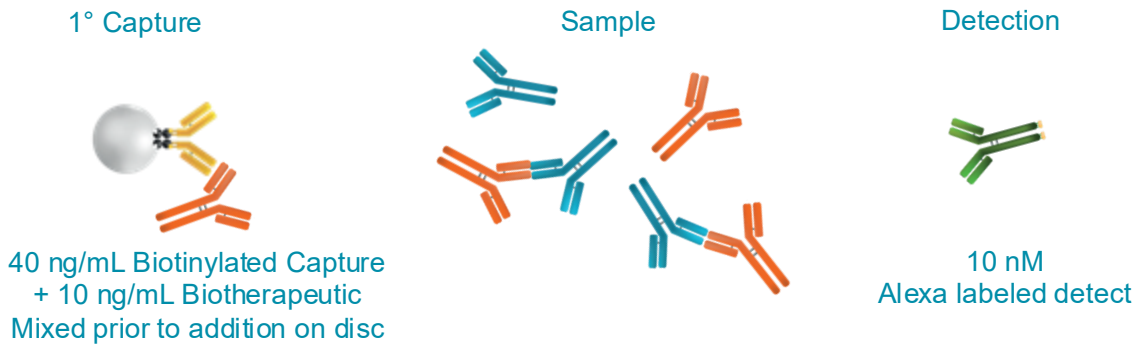
Figure 1. Signal to background (S/B) results obtained for select concentrations of pAb and PC in the presence or absence of biotherapeutic. All concentrations in neat serum

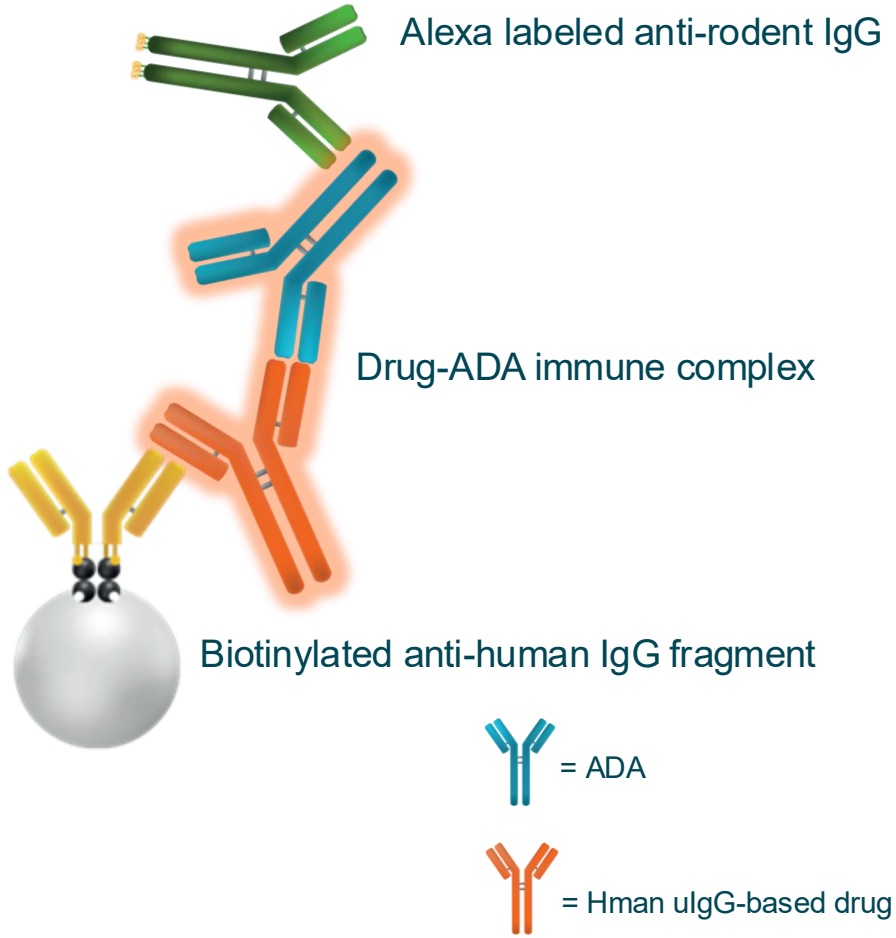
How to detect immunogenicity when no drug is present

- Developed two new methods to achieve this goal
  - 4 Step method



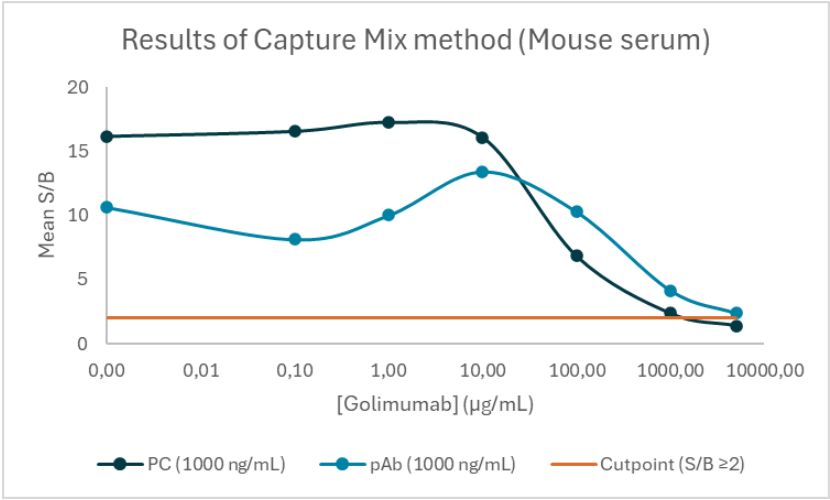
  - Capture Mix method





Important to select appropriate method

- Both methods show that pAb, which is analogous to unbound ADA, can be detected in the absence of biotherapeutic
- Inhibition of pAb signal observed in samples with ≥10 µg/mL of biotherapeutic on board. Each method demonstrates unique drug tolerance
- Addition of biotherapeutic prior to the sample in the 4-step method prevents detection of positive control.



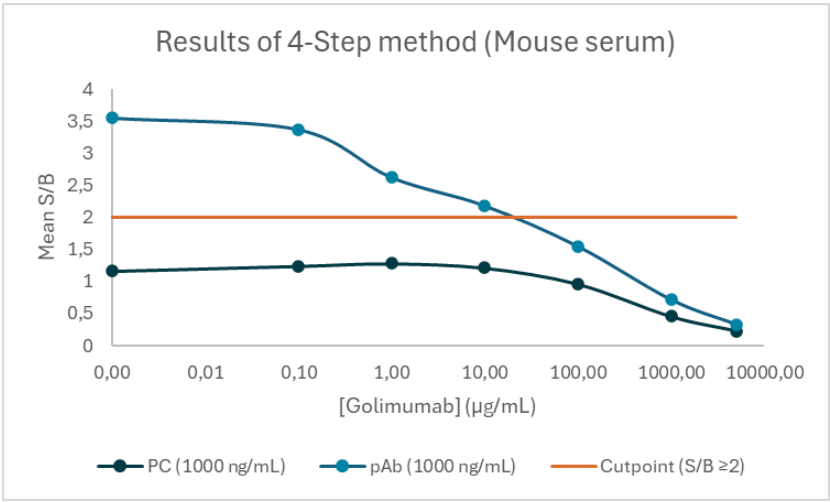
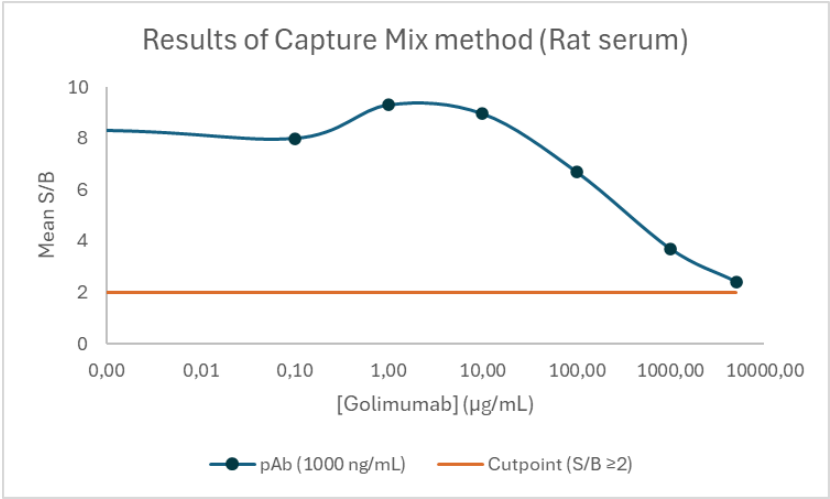


Figure 2 and 3. Signal to background (S/B) results from Capture Mix (Figure 2) and 4-step (Figure 3) methods obtained for constant concentrations of pAb and PC in the presence of variable concentrations of biotherapeutic. All concentrations in neat serum

Reproducible results in other rodent species

- Repeated assessment in rat serum
  - Pooled rat serum (1-in-10 MRD)
  - Human IgG biotherapeutic (golimumab, IgG1)
  - Commercially available rat anti-human polyclonal antibody (pAb)

- Drug tolerance increased over that seen in mice, potentially due to differences in affinity for commercially available pAbs used



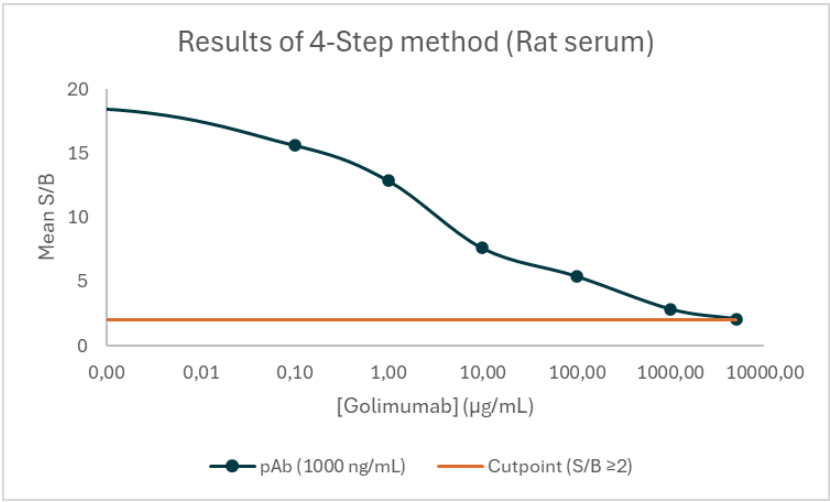


Figure 4 and 5. Signal to background (S/B) results from Capture Mix (Figure 4) and 4-step (Figure 5) methods obtained for constant concentration of pAb in the presence of variable concentrations of biotherapeutic. All concentrations in neat rat serum

Conclusions

- Automated detection of free or total ADA in rodent species demonstrated high drug tolerance due to high binding capacity of affinity column

- New Capture Mix (4:1) methodology able to detect free and complexed ADA in the absence of biotherapeutic
  - Drug tolerance in mouse serum = 1000 µg/mL
  - Drug tolerance in rat serum > 5000 µg/mL

- New 4-step method able to detect free ADA in the absence of biotherapeutic
  - Drug tolerance in mouse serum = 10 µg/mL
  - Drug tolerance in rat serum = 1000 µg/mL

- Assays now suitable for all timepoints in preclinical assessment

Unable to access samples from preclinical trials so all data from described synthetic samples

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QUESTIONS