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ADA and NAb Domain Characterization for Bi-specifics

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Principal Scientist

Bioanalytics. Analytical Sciences.

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- LC-MS/MS
- Immunoassay
- Immunogenicity (ADA, nAbs)
- DMPK
- Flow Cytometry, PCR
- Biomarkers
- Proteins, peptides, bi-specifics, oligonucleotides

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ANALYTICAL SCIENCES

- Analytical Chemistry
- Extractables & Leachables
- Elemental Analysis
- Impurity Identification
- Materials Characterization
- Foreign Matter Analysis

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Overview

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1. Introduction
2. Guideline
3. Resolian Strategy
4. ADA domain characterisation case study
5. Nabs assay strategy
6. Nabs domain characterisation case study

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Introduction

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- Bispecific refer to compounds that binds to two sites (epitopes)
- Introduced in 1960 by Nisonoff et al, but the idea had to wait until 1975 for the invention of hybridoma
- There are mainly two type:
  - ❑IgG Like
  - ❑Non-IgG Like
    - ➢Bites (bispecific T-cell engager)
    - ➢DART (Dual-affinity Re-targeting Antibody)

BsAbs potential

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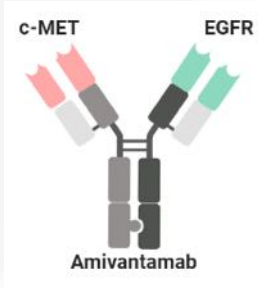
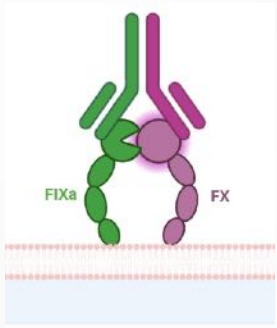
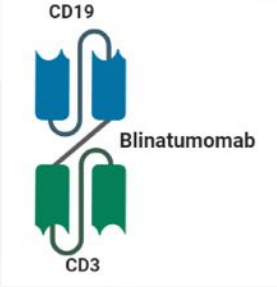
- Over 100 BsAbs at various stage of clinical trials
- BsAbs with different mode of action have been approved
- Cis (same cell)
  - ❑ Amivantamab used for non-small cell lung cancer
- Trans (two different cells)
  - ❑ Blinatumomab used for acute lymphoblastic leukemia
- Endogenous target
  - ❑ Emicizumab a FVIII replacement for Haemophilia A







Example of BsAbs half-life

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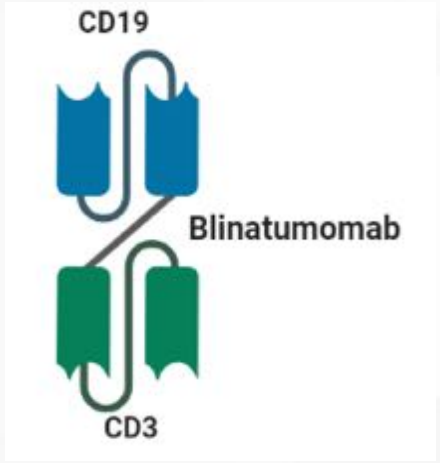
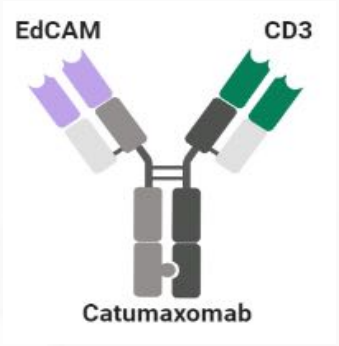
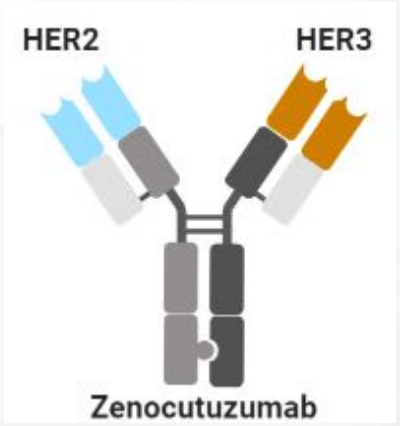


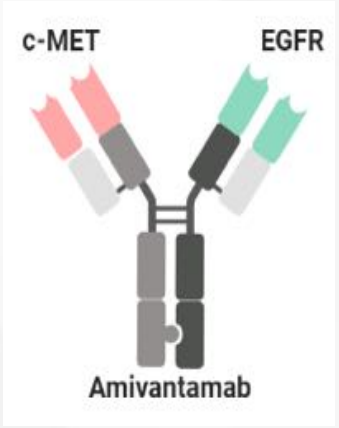
t½: 2.11 hrs



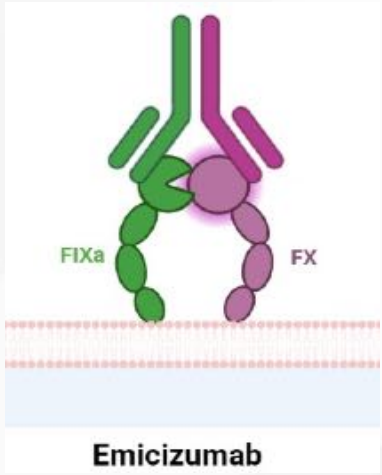
t½: 2.5 days



t½: 4.6 days



t½: 11.3 days



t½: 4-5 weeks

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Guideline

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- FDA 2019 Guidance

  ❖Section IV.A.3 states; 'An immune response to one domain may inhibit a specific function while leaving other intact. FDA recommends that sponsors direct initial screening and confirmatory tests against the whole therapeutic protein product.

  ❖Examination of immune responses to therapeutic protein products with multiple functional domains, such as bispecifics and ADCs may require development of multiple assays to measure immune responses to different domains of the molecules.

- EMA 2017 Guideline

  ❖Section 7.4; A strategy based on the competitive inhibition principle of the confirmatory assay to dissect the specificities of the antibodies to individual moieties may be used.

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Resolian Strategy

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- Domain characterisation is recommended to be performed in the ADA assay
- ADA assay are less complex compared to Nabs
- Nabs may even require the use of cell-based assay which may add to the complexity
- However, if the ADA assay is already developed and validated without the domain characterisation
- The Nabs assay may be developed to include domain characterisation

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Bispecific ADA tiers approach

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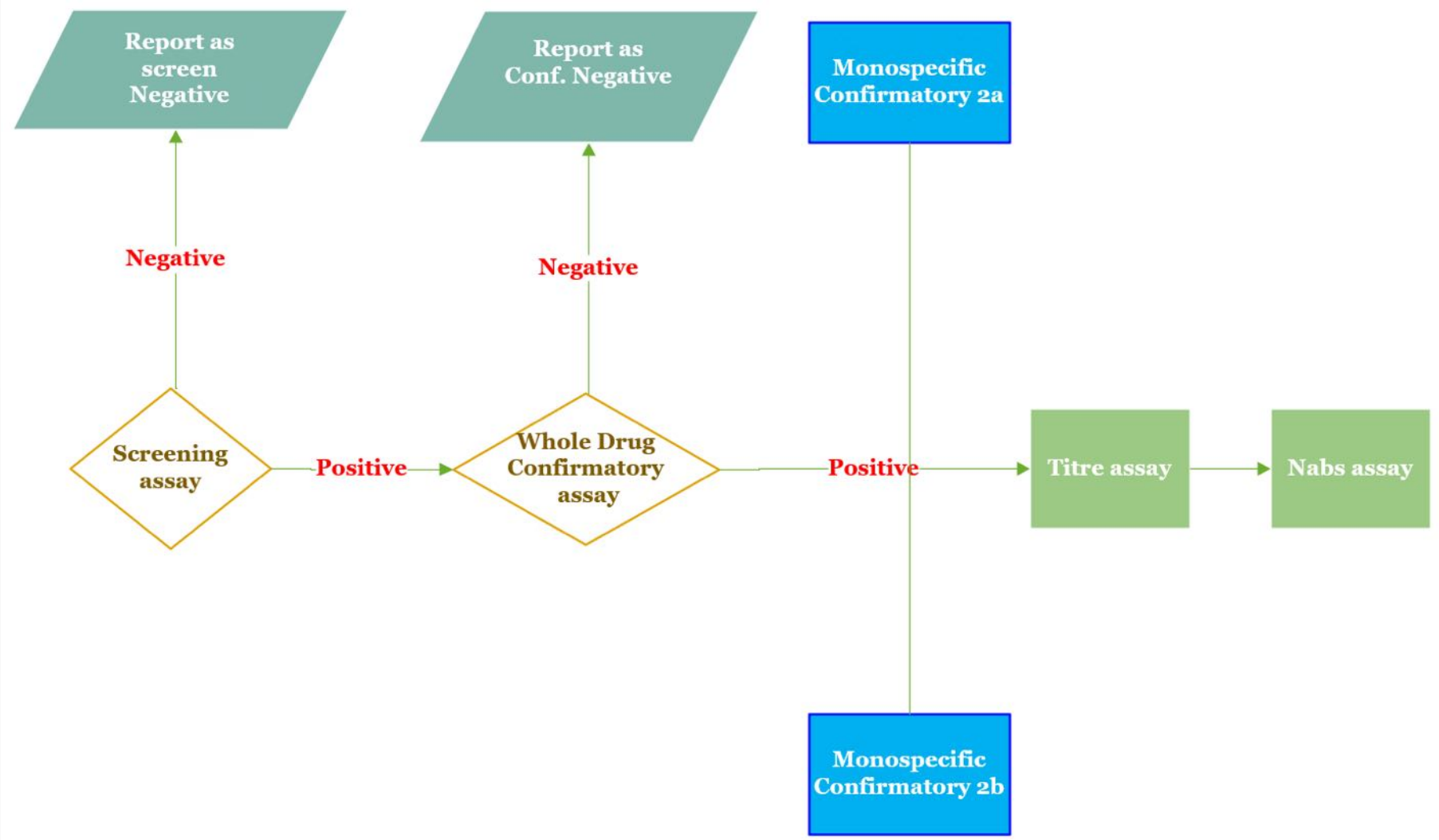

Assay development tool kit

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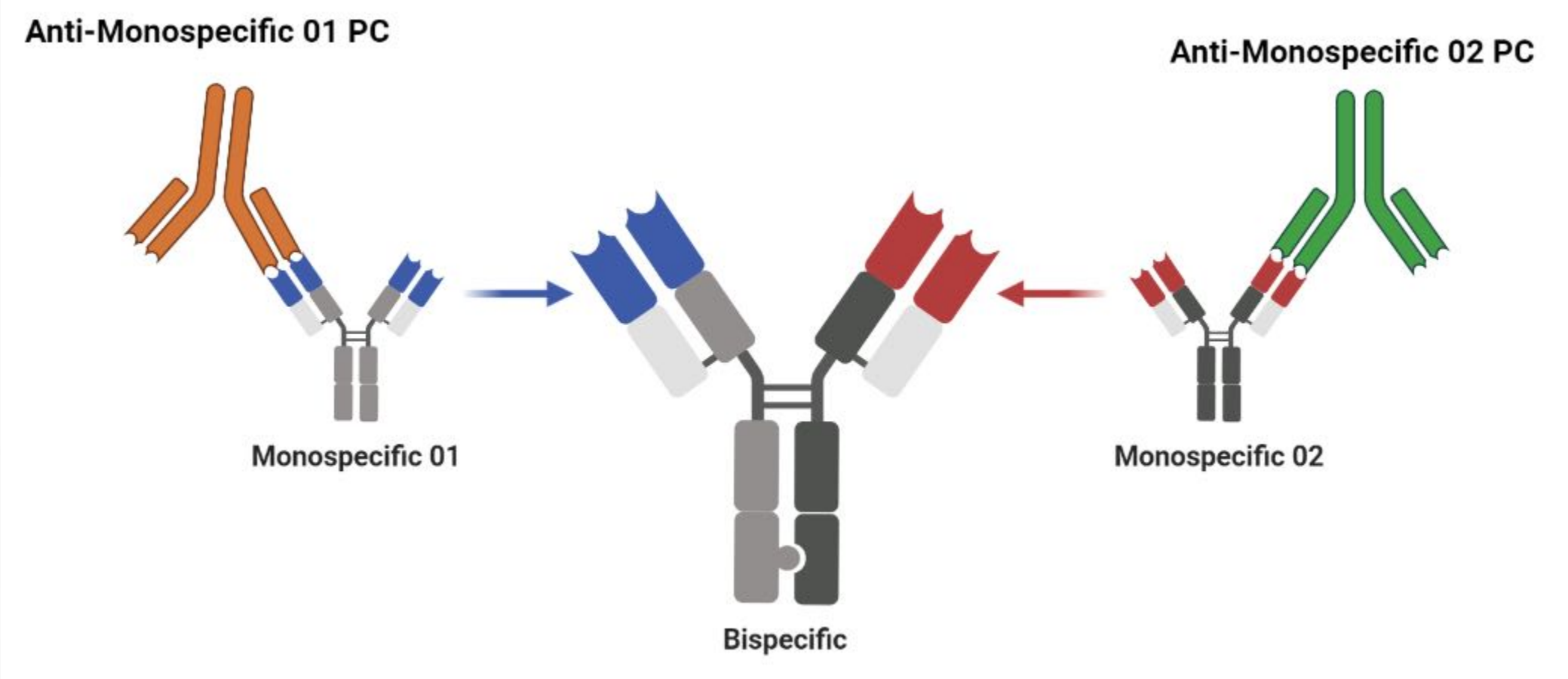
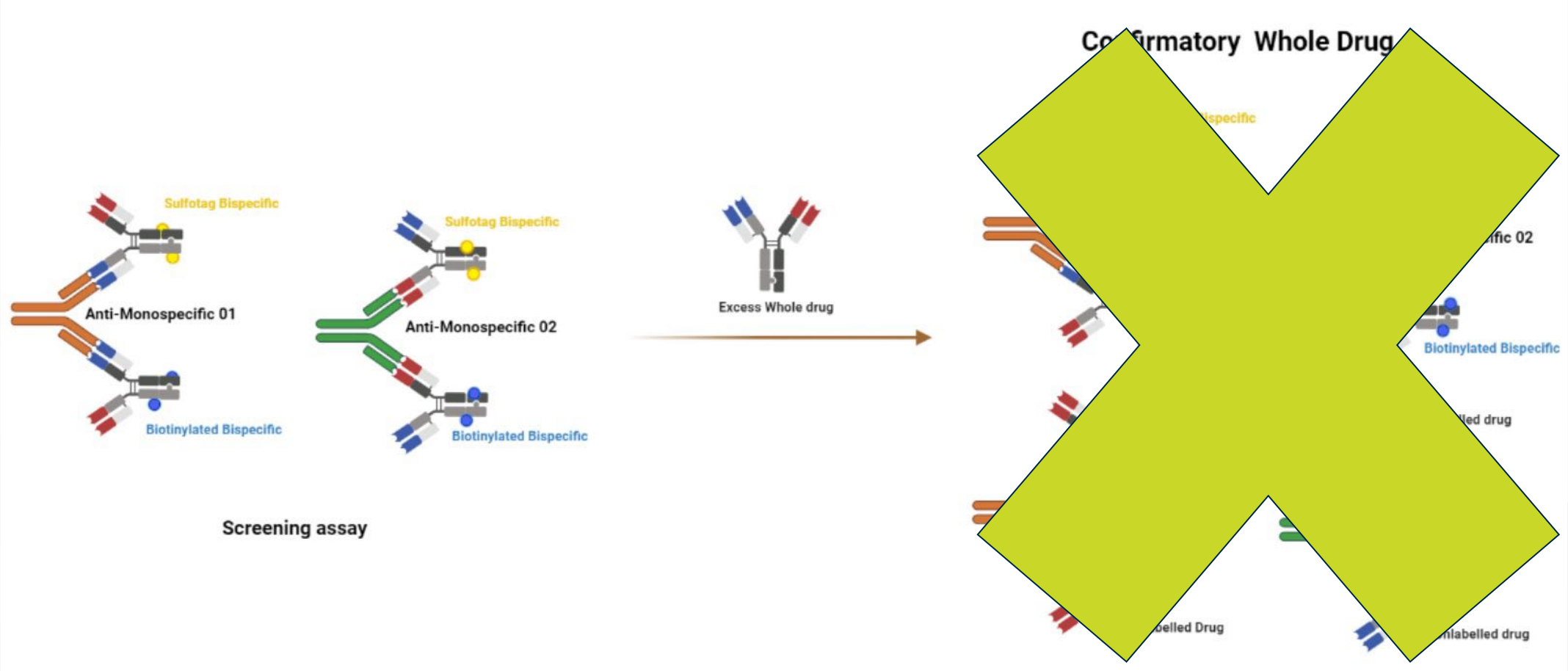


Screening and confirmatory assay format



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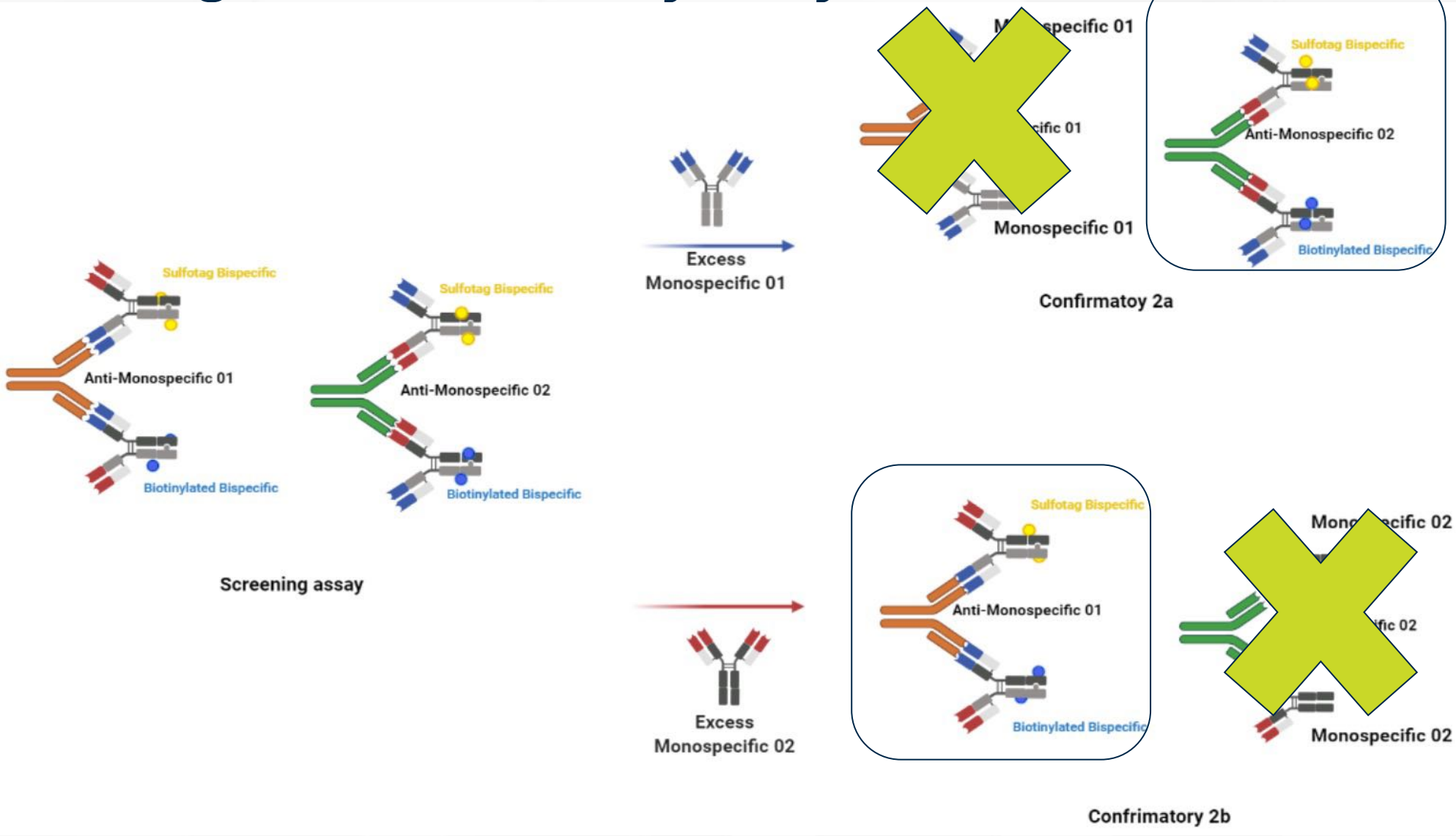

Confirmatory assay sensitivity

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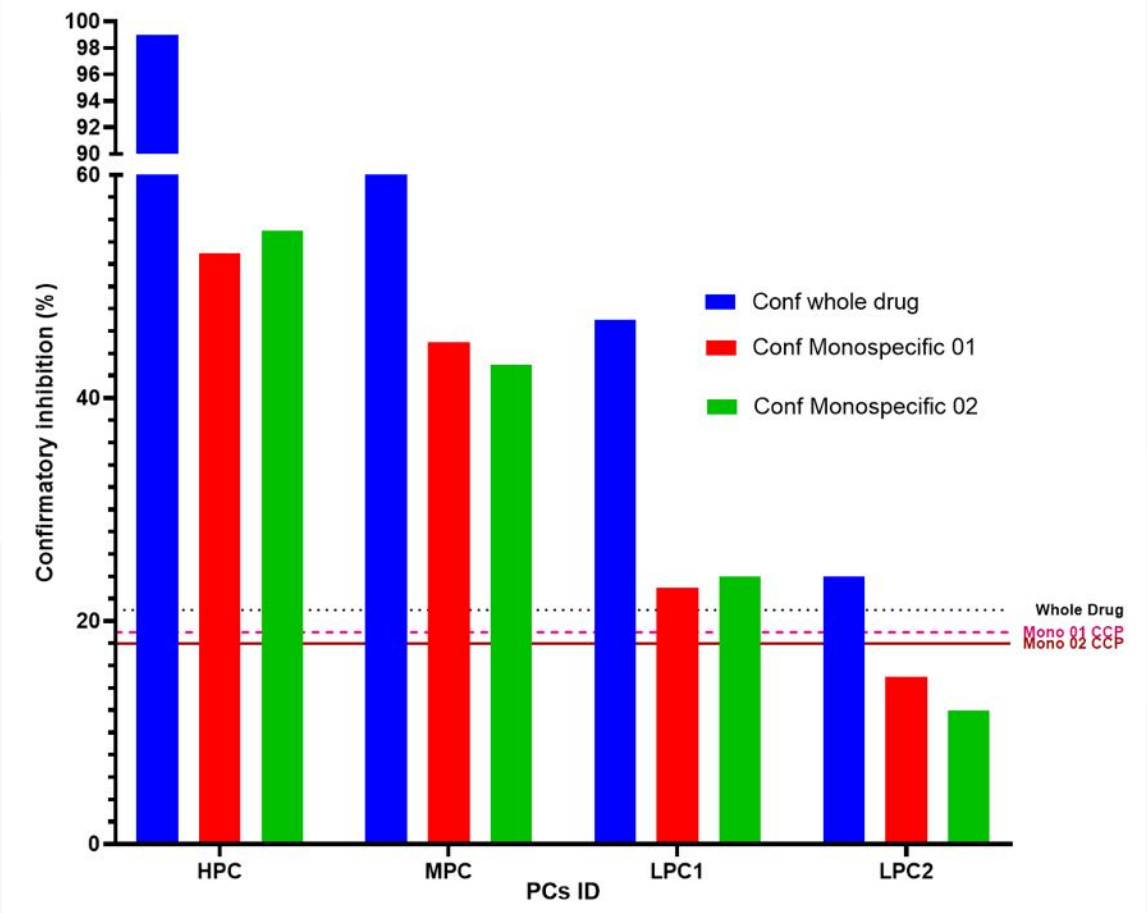

Single vs bispecific (Plates)

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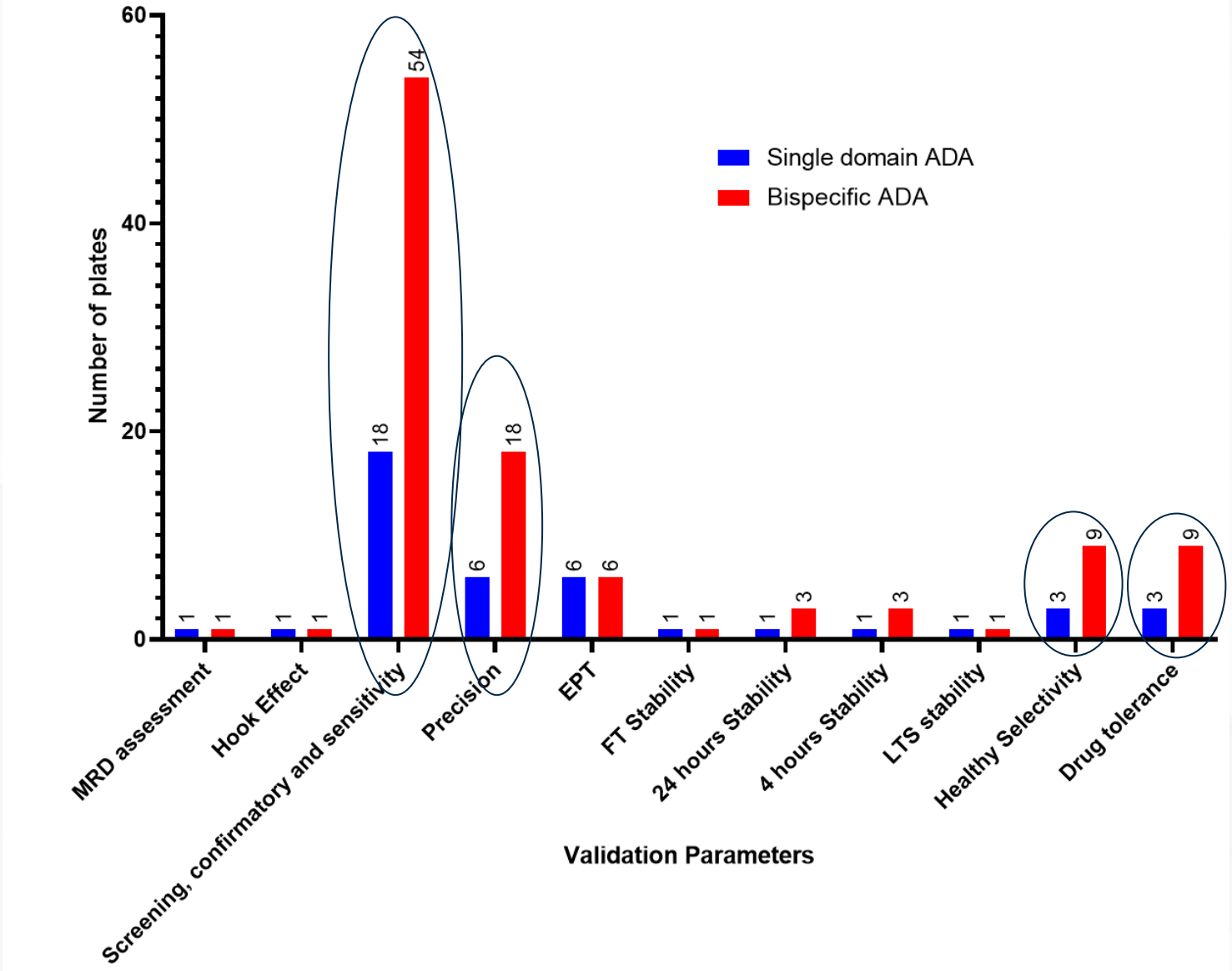
- Total number of plates:

❑ Single Domain assay:

~42 plates

❑ Bispecific Domain assay:

~106 plates



Single vs bispecific (Days)

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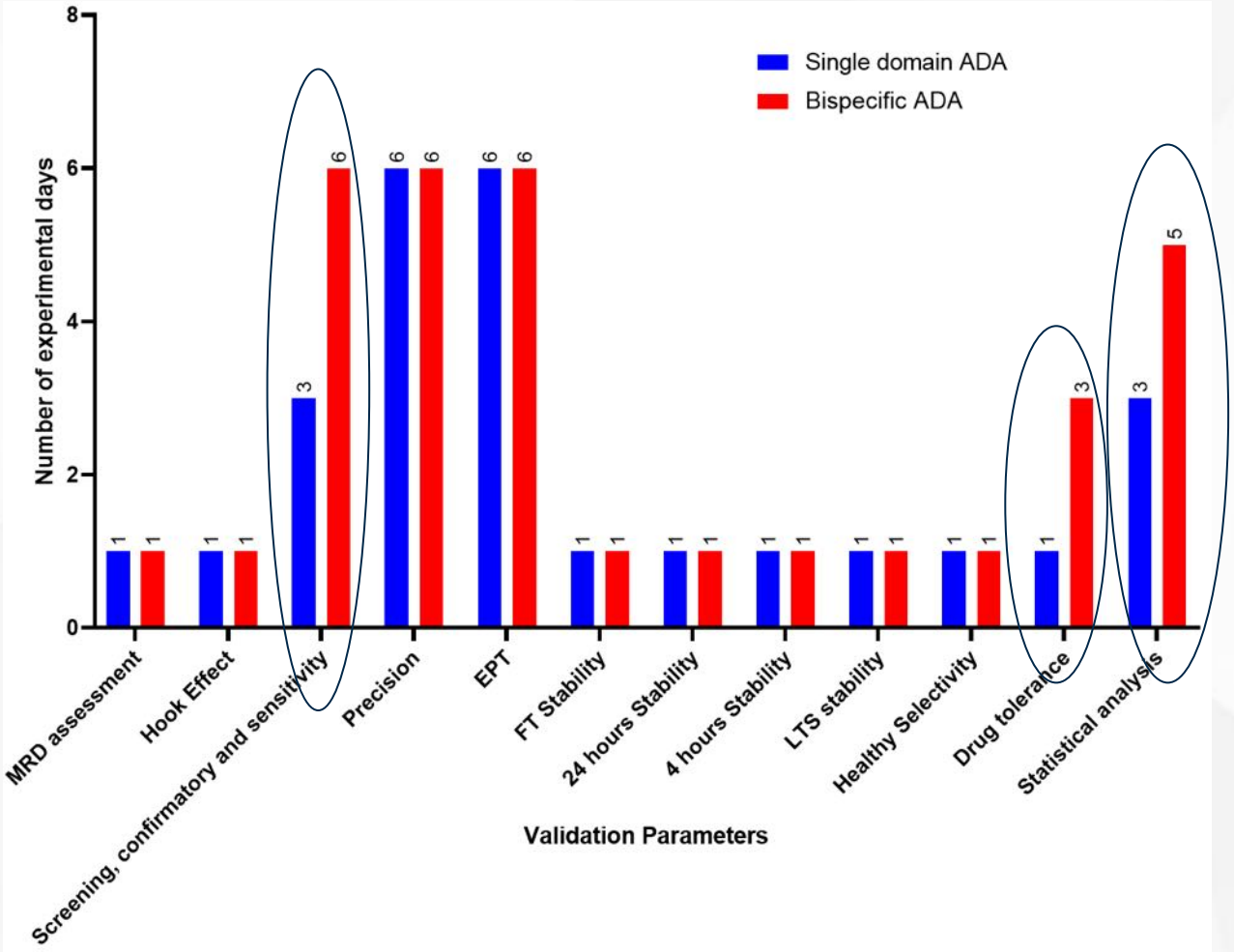
- Total number of days:

❑ Single Domain assay:

~26 days

❑ Bispecific Domain assay:

~33 days



Case study

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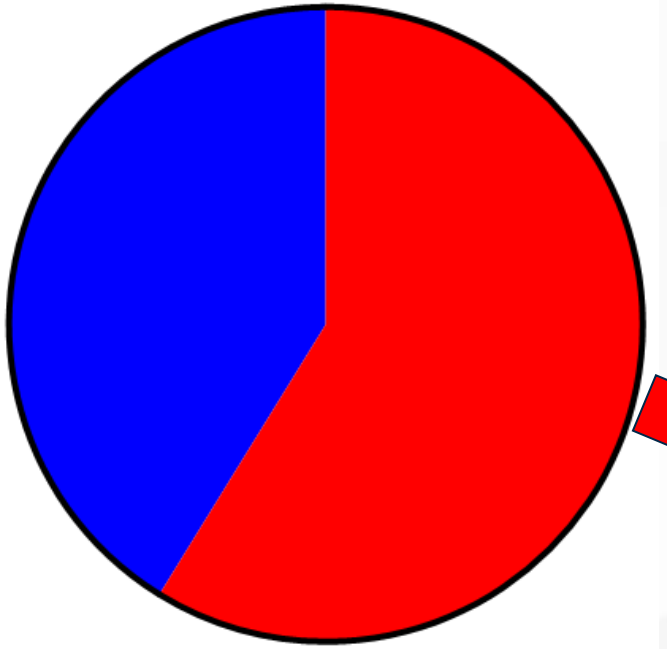
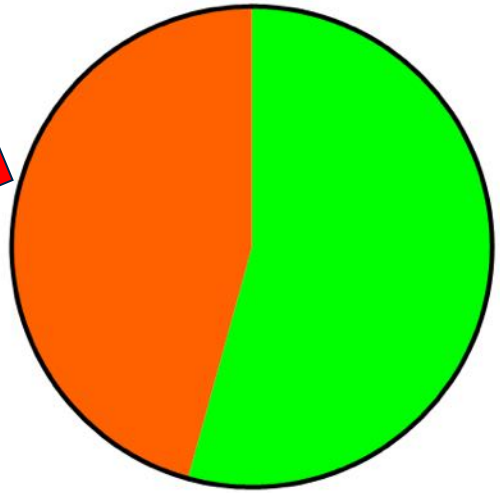
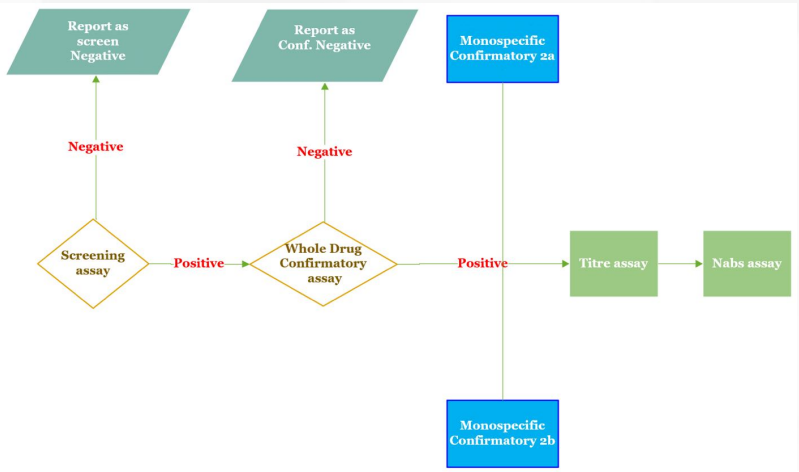
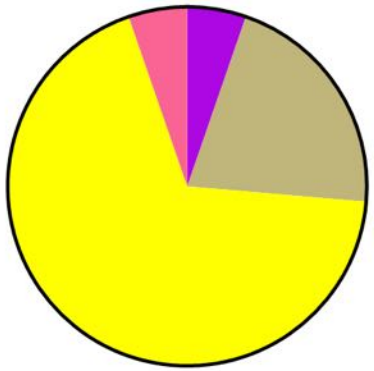


Screened Negative
Screened Positive



Confirmed positive against whole drug
Confirmed negative against whole drug





Confirmed Positive against both monospecifics
Confirmed Positive Monospecific 02
Confirmed Positive Monospecific 01
Confirmed Negative against both monospecifics

Summary

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Customer Need	• Bispecifics (Bs) are biopharmaceutical products that bind to two sites (epitopes) • The customer required an ADA assay against the whole Babs but also wanted to perform domain specific characterisation
Resolian Process	• Resolian suggest to perform domain characterisation in the ADA instead of Nabs • To successfully develop a domain characterisation. ADA against each domain are required from the Sponsor along with versions of mono-specific drugs for each of the arms of the BsAbs. • A bridging assay format with acid pre-treatment to improve drug tolerance is recommended • Resolian developed a screening assay against the whole drug and three different confirmatory assays. • The ADA response was characterised against the whole drug, and against each of the monospecific drug arms.
Customer Outcome	• The validated assay (screening, confirmatory tiers and titre) supported sample analysis throughout the method lifecycle. The customer is assured that the assay meets the regulatory requirements (both EMA and FDA).

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Resolian Strategy for Nabs

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- Cell-based assay is mainly advised for Nabs assay
- The mode of action of the drug is evaluated to assess suitable assay format: cell-based assay or ligand binding assay
- Sourcing functional cell line that respond to both domain of the bispecific can be challenging
- Initial attempts is made to purchase commercially available cells which can respond to each monospecific domain or both domains
- One vs two independent assays
- If no appropriate cell line can be sourced the ligand binding assay is taken forward

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Nabs case study

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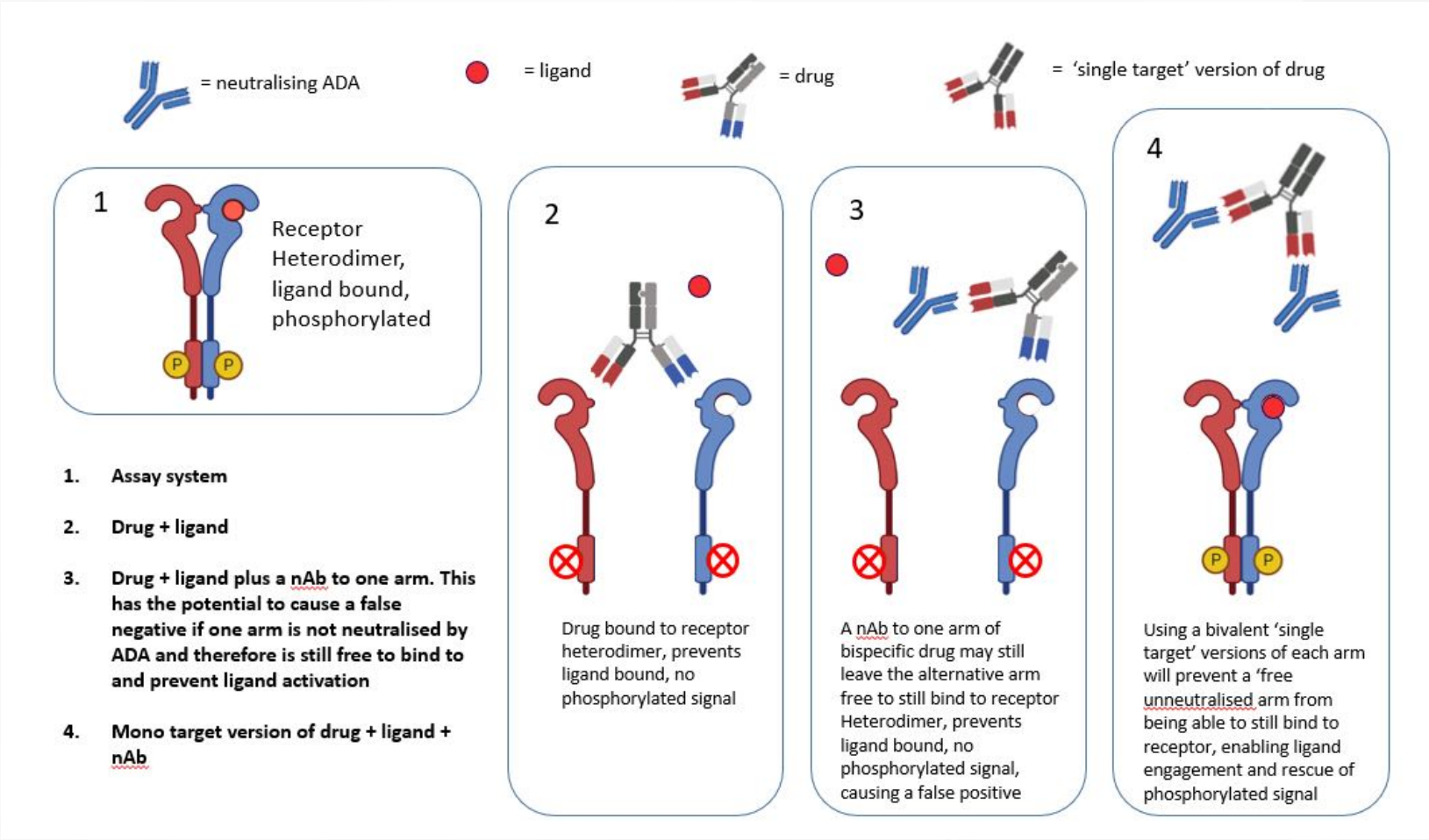
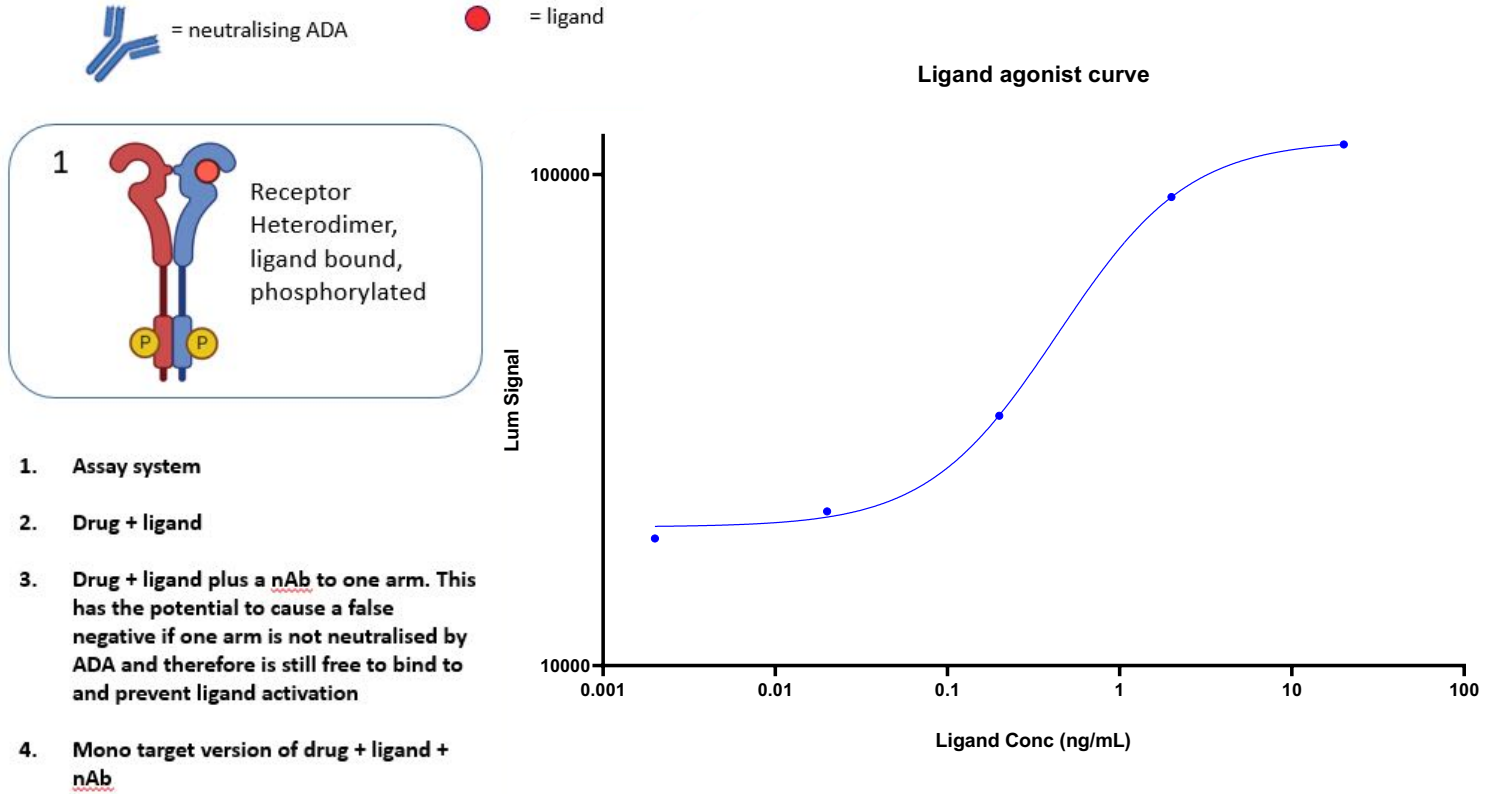
- The ADA developed did not include domain characterisation
- As such Nabs aimed to include domain characterisation
- Assessment of a cell-based assay nabs vs the ligand binding assay in parallel
- Challenges expected :
    - ❖ Sourcing a functional Cell based assay that respond to both bispecific domains
    - ❖ Sourcing the positive control containing neutralising antibodies against each of the domain
    - ❖ Achieving desirable sensitivity (250-500 ng/mL), for the whole drug and the monospecific domain
    - ❖ One assay format that allows for domain characterisation

Cell based assay format



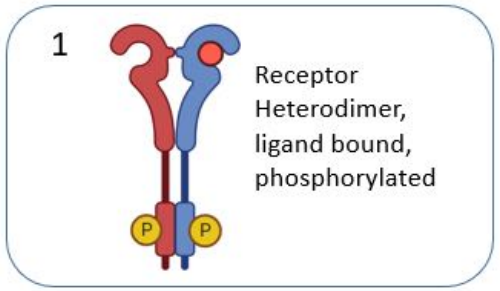
- Functional Cell based assay that respond to both bispecific domains?

Nabs against a bispecific therapeutic

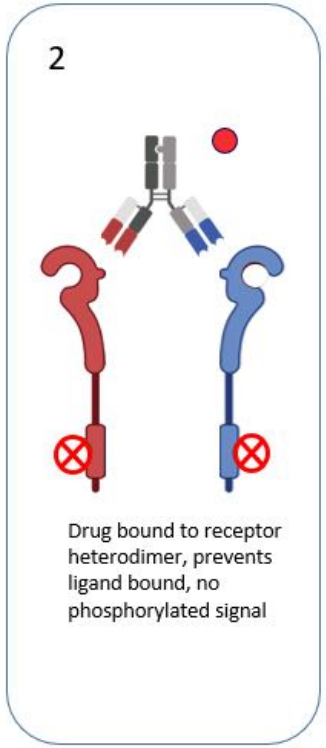


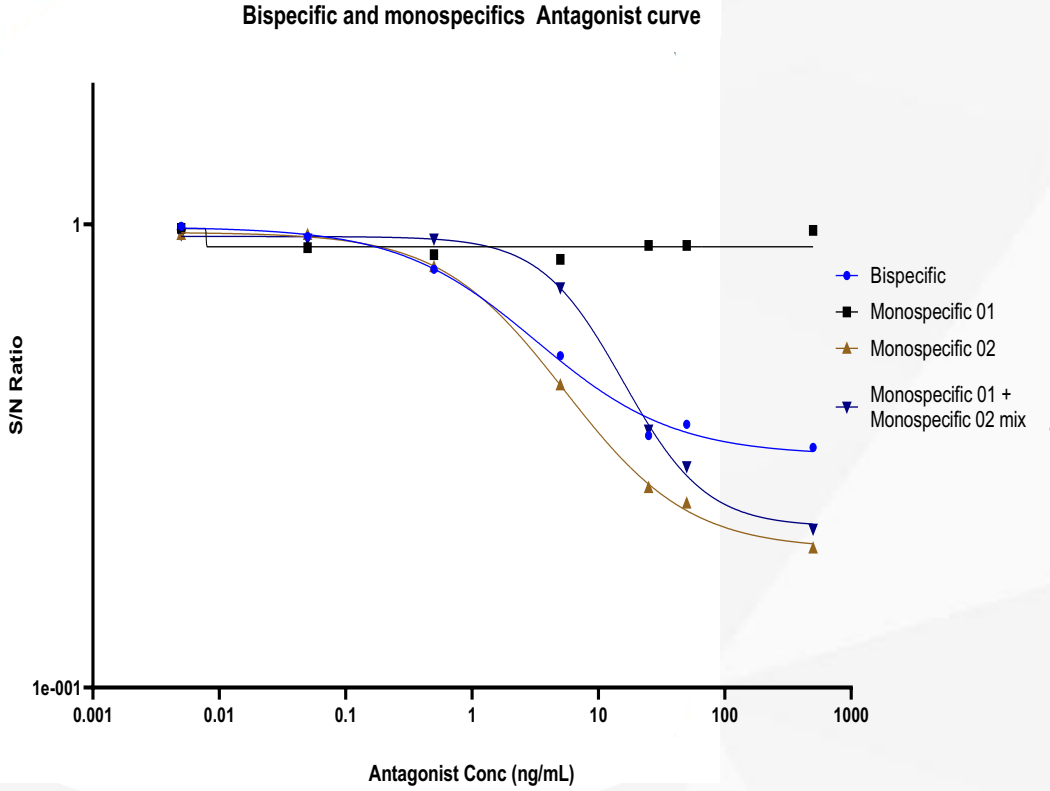
- Ligand induce receptor Heterodimerisation
- Leading to protein downstream phosphorylation and enzyme activation
- Enzyme then hydrolyse the substrate to generate chemiluminescent signal

Cell based assay format



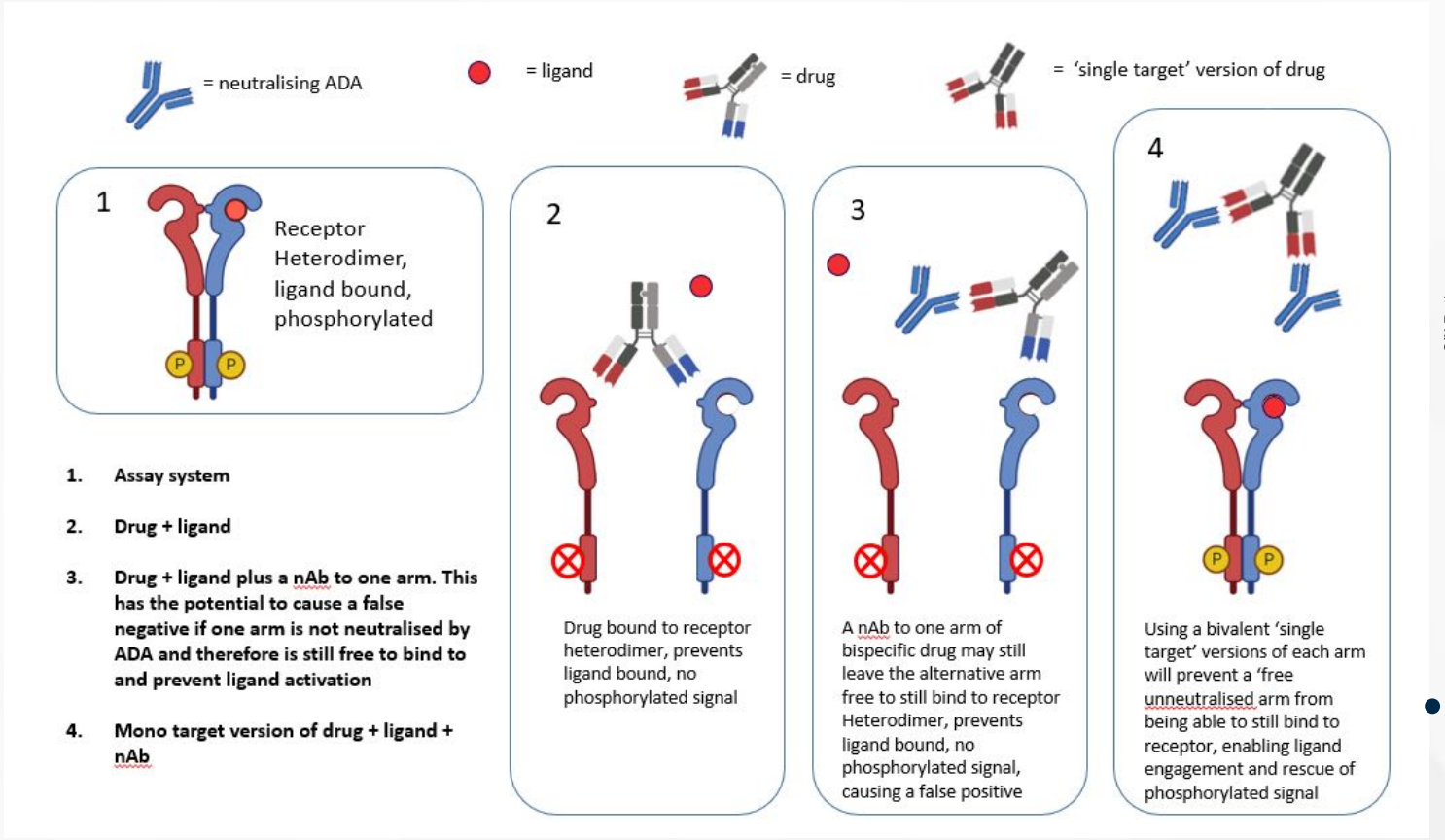
1. **Assay system**
2. **Drug + ligand**
3. **Drug + ligand plus a nAb to one arm. This has the potential to cause a false negative if one arm is not neutralised by ADA and therefore is still free to bind to and prevent ligand activation**
4. **Mono target version of drug + ligand + nAb**

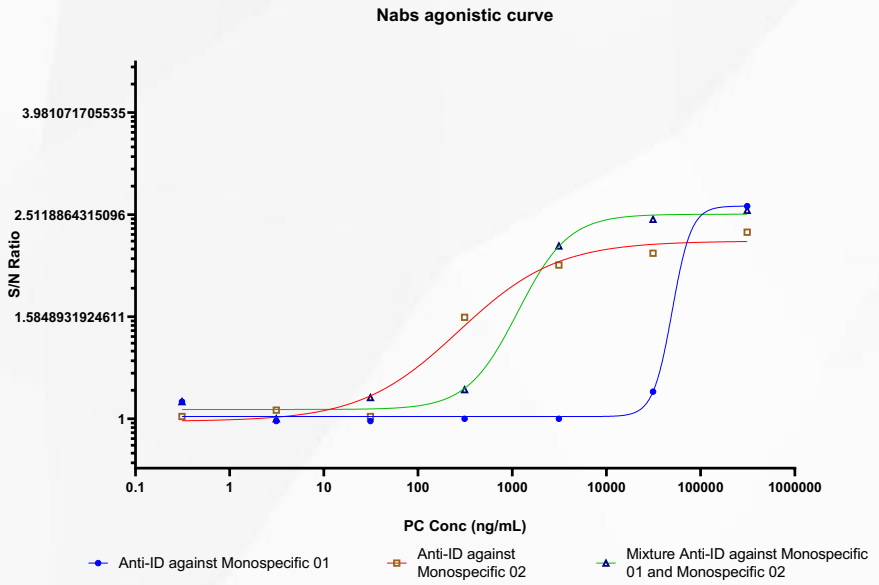




- The signal was also successfully inhibited in the presence of the Bispecific and one of the monospecific
- As such this cell line would have only been suitable for one domain not both

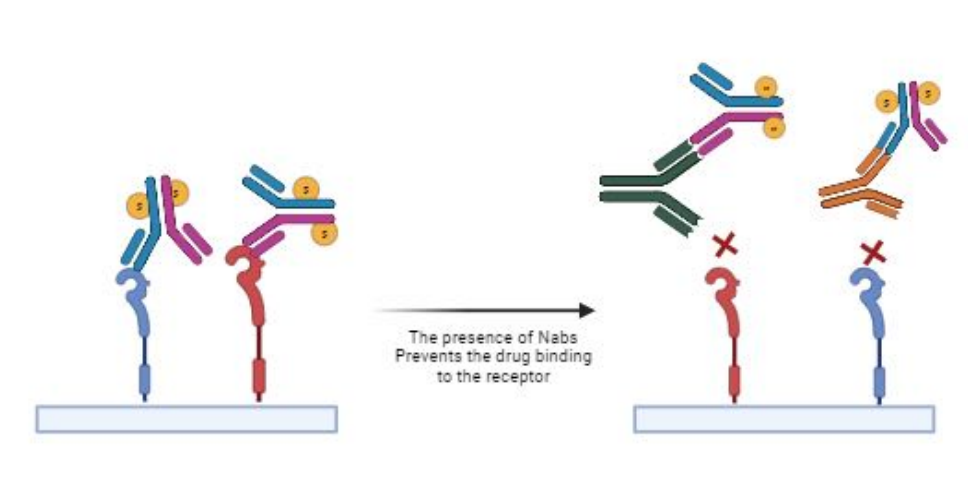
Cell based assay format



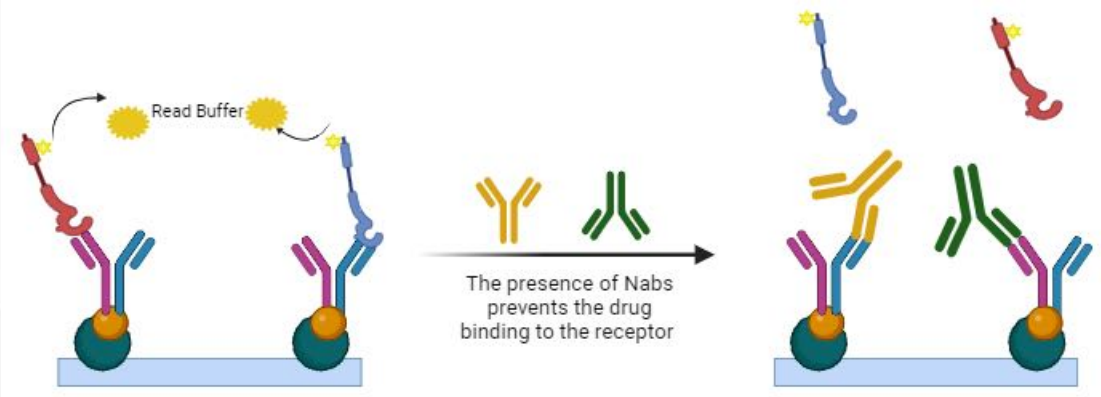


- Nabs signal restoration was demonstrated against the bispecific and against one domain

LBA Assay format



- **Option 1** for LBA assay was to use the receptor as the capture and then use the sulfotag conjugated drug as the detection.



- **Option 2** for LBA assay was to use the biotinylated drug as the capture and then use the sulfotag conjugated receptor as the detection.

LBA: Option 1

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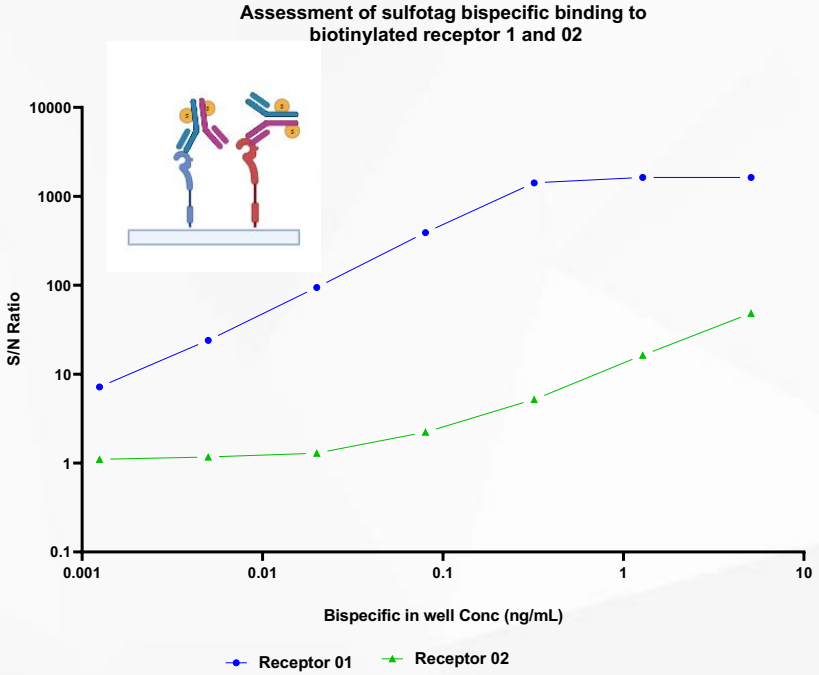
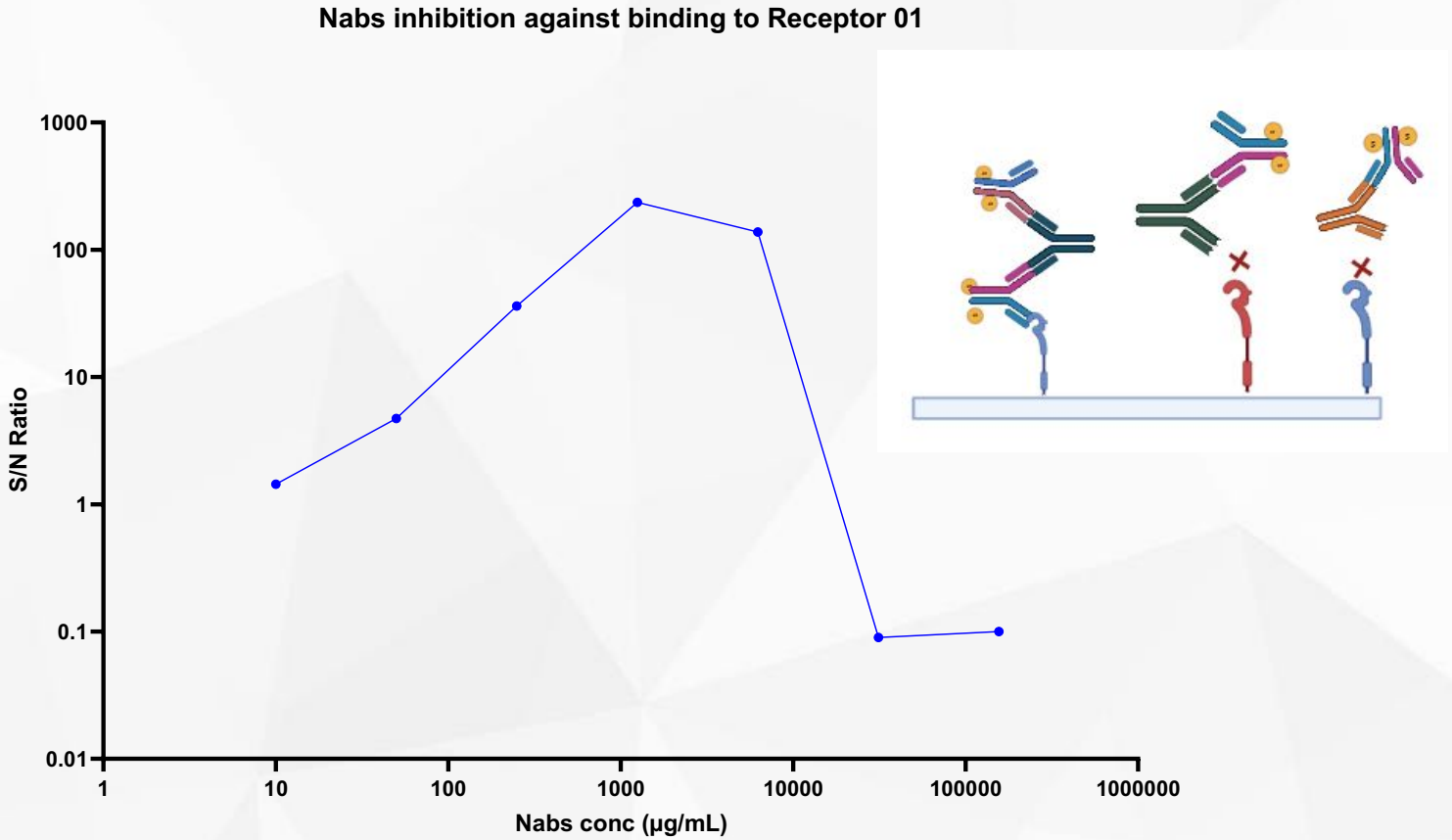
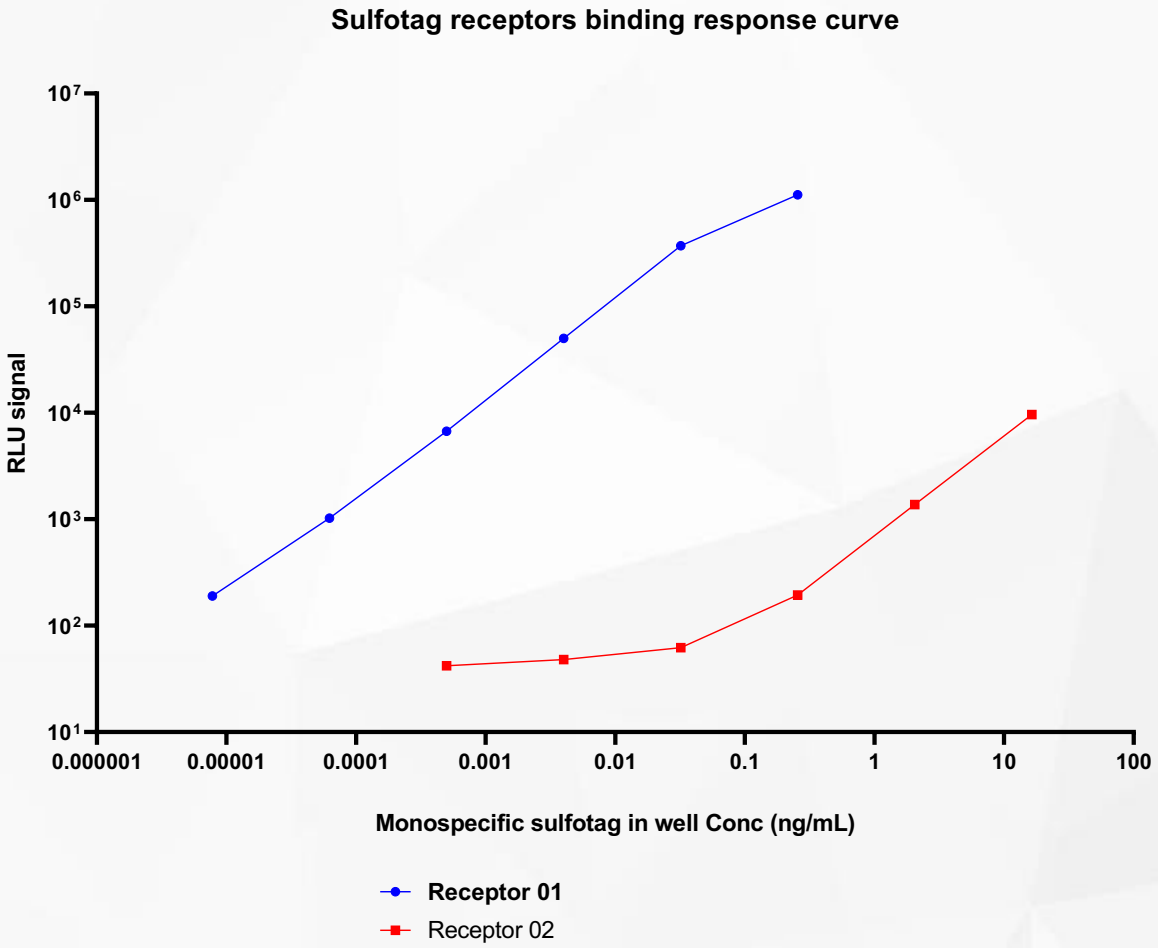
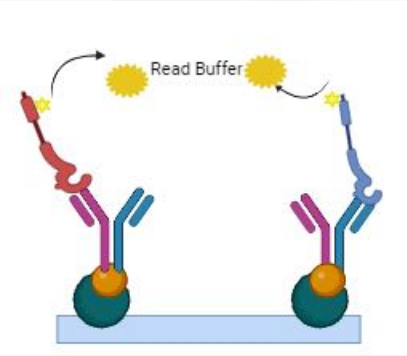




LBA: Option 02

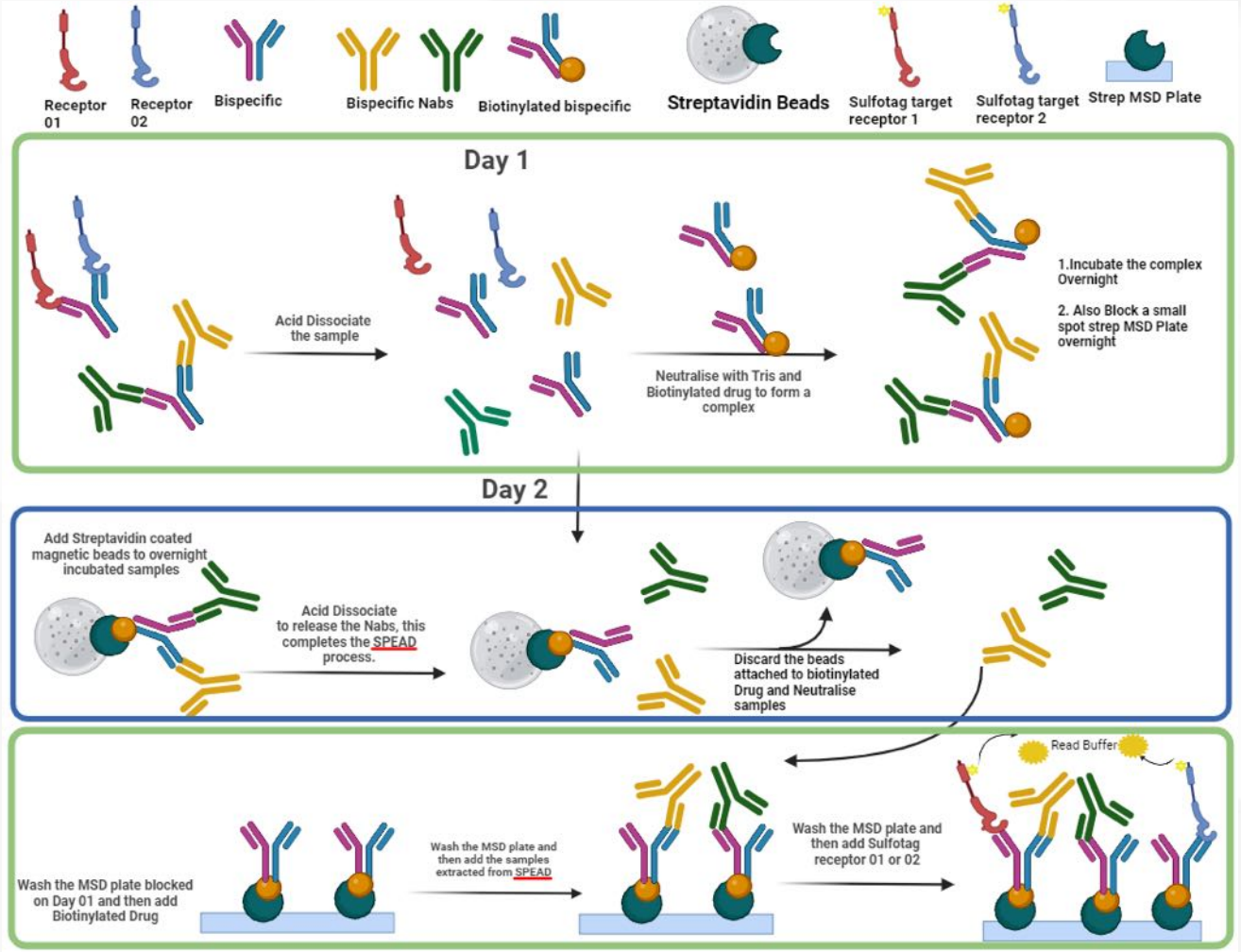




- **Option 2** did not have any evidence of hook effect
- This format was therefore appropriate to take forward
- This format did not have sufficient drug tolerance
- Hence, the requirement to use the bead method.

BEAD assay for Nabs domain characterization

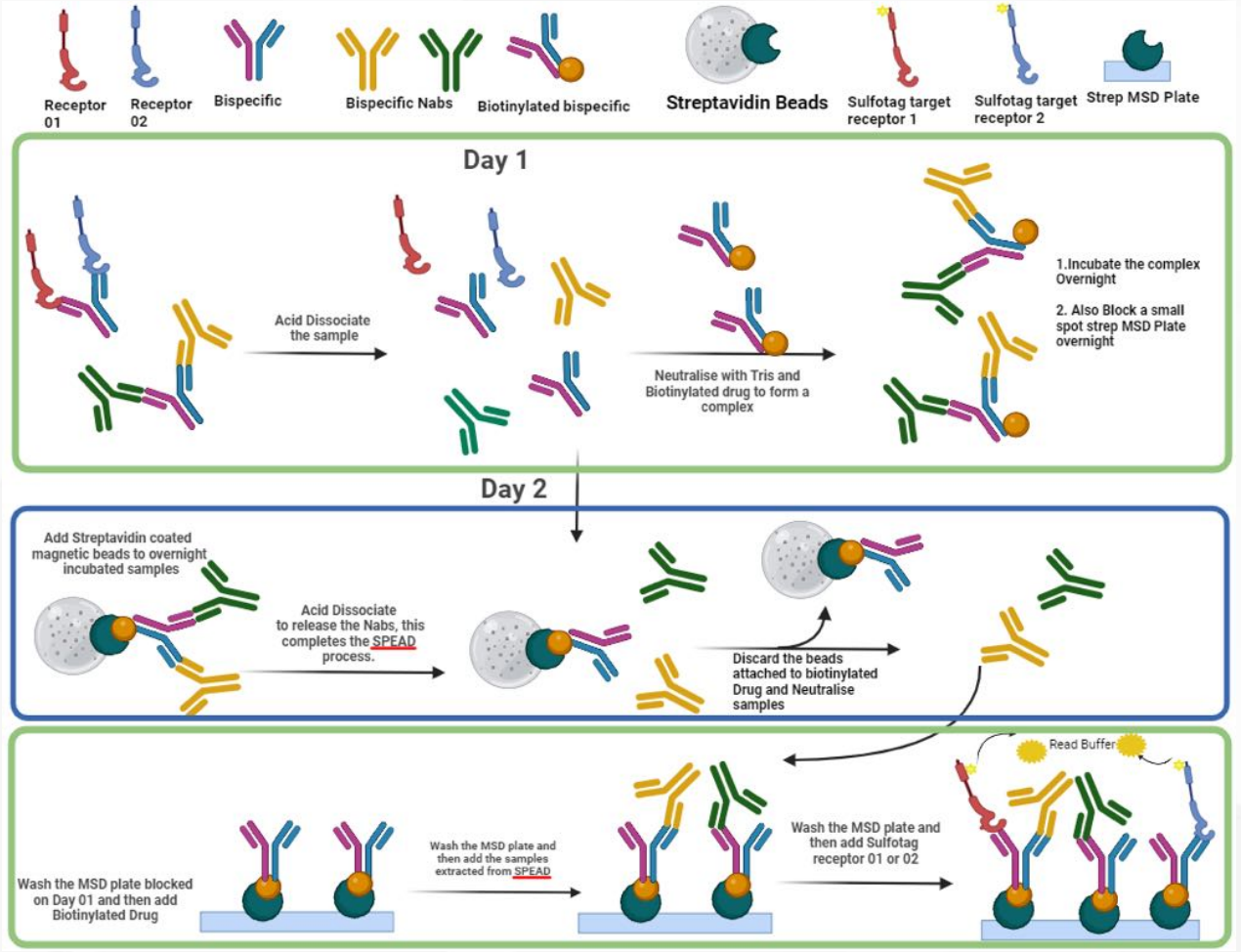
- BEAD method using the kingfisher to achieve desired drug tolerance
- Eluted Nabs were added to an MSD plate coated with bispecific drug
- Both Sulfotag receptors were used to detect Nabs against whole drug



BEAD assay for Nabs domain characterization

- Each sulfotag receptor added for domain specific characterisation
- Assay was developed and validated in singlicate (<10% CV across the plate).
- Sensitivity at 200 ng/mL for the whole drug and monospecific characterisation

	PC	Concentration (ng/mL)	Combination detection	Detection Receptor 1	Detection Receptor 2
Intra-assay CV (%) (S/NC for PC, RLU for NC)	10,000	10,000	4.9	3	11.6
	200	200	6.8	2.4	2.4
	0	0	6.5	2.7	3.9
Inter-assay CV (%) (S/NC for PC, RLU for NC)	10,000	10,000	12.3	9.5	13.4
	200	200	7.8	2.8	12.5
	0	0	10.7	6.2	14.7



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Resolian Process	• Resolian suggest to perform domain characterisation in the ADA instead of Nabs but in this case there was no option • To successfully develop a domain characterisation. Nabs against each domain were required from the Sponsor. Anti-IDs against each domain were pooled to make the PC. • Resolian developed a screening assay against the whole drug and the option for domain characterisation. • The Nabs response was characterised against the whole drug, and against each of the monospecific drug arms.
Customer Outcome	• The validated assay supported sample analysis throughout the method lifecycle. The customer is assured that the assay meets the regulatory requirements (both EMA and FDA).

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