Adequate neutralization steps

Essential for the development of sensitive, robust and highly drug tolerant anti-drug antibody screening and confirmatory assays - Ardena case study

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Drug tolerance
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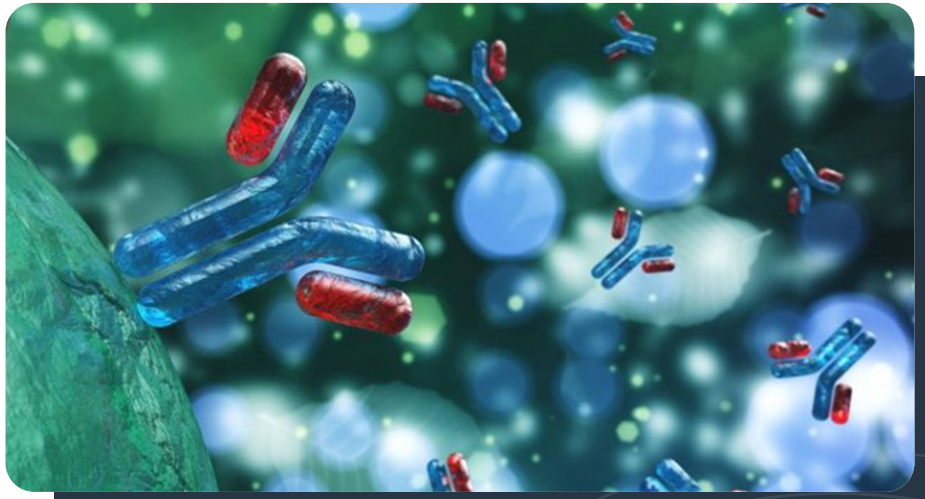
Introduction

- Immunogenicity testing for biopharmaceuticals is required for drug approval
- ADA Bridging assay susceptible to interference by residual drug
- Laboratory case study - Transfer of incorrect ADA assay to CRO
- Appropriate method for appropriate data interpretation
- Partnership between CRO and pharma could lead to cost reduction in drug development



Immunogenicity study

- Biopharmaceuticals:
  ADA (NAbs and non-NAbs) evaluation for patient safety and drug efficacy
- “Gold” standard is bridging assay
- 1. Labelled drug conjugates
  2. ADAs are captured
  3. Signal-giving complexes
- Pre-study method validation
  Recommendations of EMA (2015) and FDA (2019) - “true” CP

- Appropriate ADA methods for appropriate data interpretation

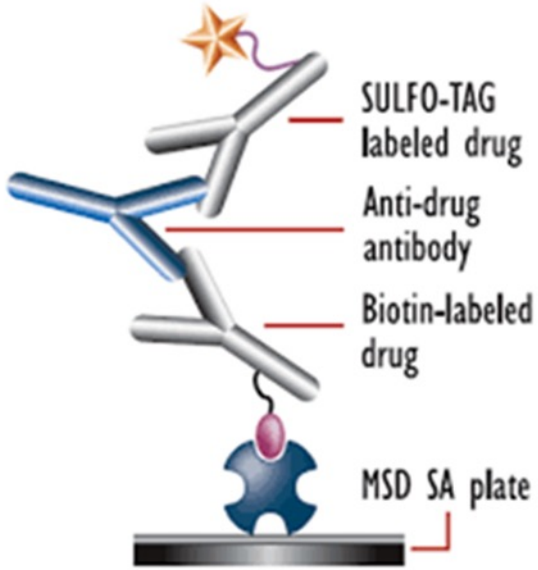
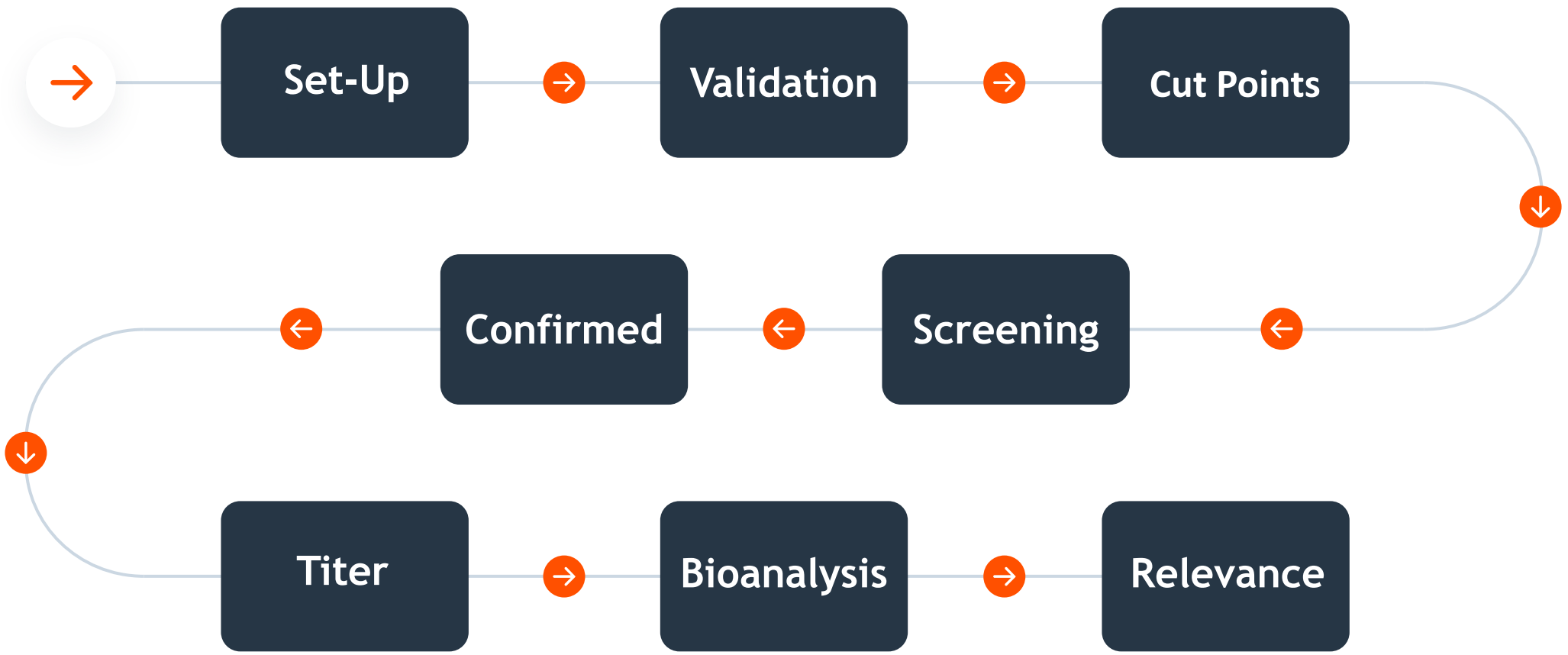


Plate bound drug
Detector-labelled drug

Immunogenicity assessment

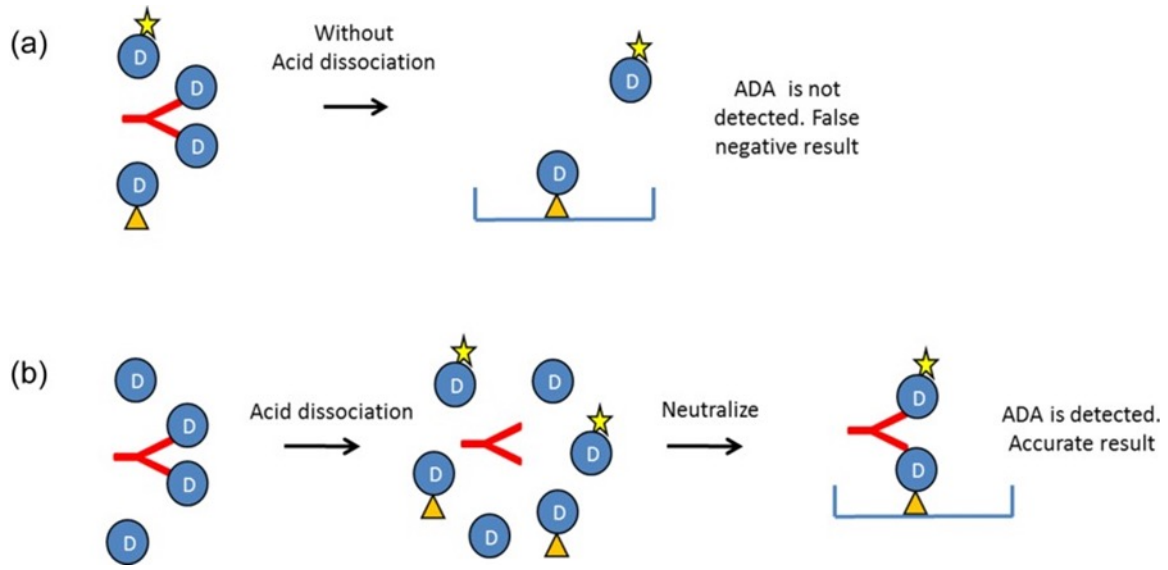


Bridging assay format
Susceptible to interference by residual drug

On-board drug forms complexes with ADA in sample

Preventing binding ADA to labelled assay reagents

False negative results - inaccurate outcome



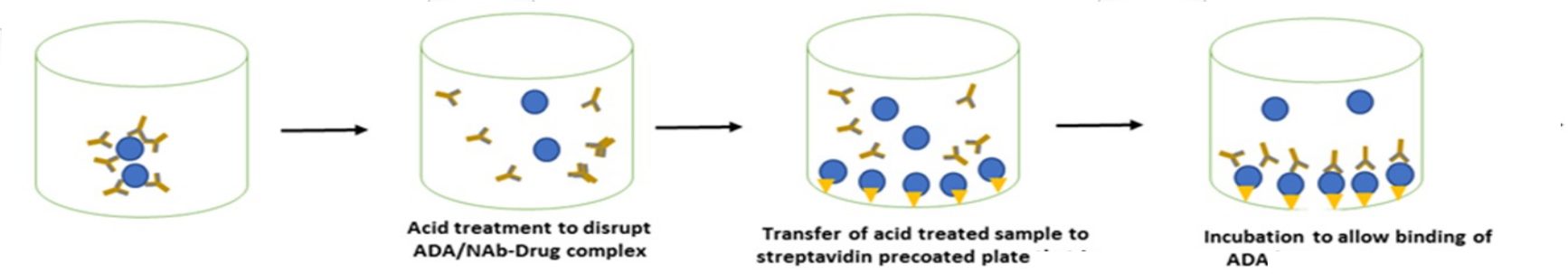
Acid pretreatment step

Method to dissociate the ADA residual-drug complexes

Molecular disruption by reducing pH

To achieve a higher assay drug tolerance

To reduce false negative results



Preclinical Case study - Assay Transfer

- Cynomolgus
- Rat plasma

- Transfer assay protocol Sponsor

- Sample 10.0 µL
- MRD 1

- Acid plate: acid dissociation step 300 mM Acetic Acid to samples
- Neutral plate: neutralization addition master-mix
- Blocked MSD plate

- Drug-Biotin
- Drug-Sulfo-Tag™
- Wash
- Read buffer

- Voltage applied
- Bound Sulfo-Tag™ to emit light
- Intensity measured

Neutralization:
Master-mix
- Sulfo-Tag™ labeled drug
- Biotin labeled drug

Bind to immobilized anti-drug antibodies

Case study Master-mix

- Screening Master-mix

- 0.150 µg/mL drug-Biotin
- 0.150 µg/mL drug-Sulfo-Tag
- Buffer
- Sample

- Confirmatory Master-mix

- 0.150 µg/mL drug-Biotin
- 0.150 µg/mL drug-Sulfo-Tag
- Buffer
- Sample
- 10.0 µg/mL drug

- Positive control dilution anti-drug antibodies 2000 - 1.95 ng/mL

PC DIL	Volume (µL)	Use solution	Cynomolgus plasma (µL)	Concentration (ng/mL)
Dil 1 (1)	60.0	HPC	N/A	2 000
Dil 2 (2)	30.0	HPC (Dil 1)	30.0	1 000
Dil 3 (4)	30.0	Dil 2	30.0	500
Dil 4 (8)	30.0	Dil 3	30.0	250
Dil 5 (16)	30.0	Dil 4	30.0	125
Dil 6 (32)	30.0	Dil 5	30.0	62.5
Dil 7 (64)	30.0	Dil 6	30.0	31.3
Dil 8 (128)	30.0	Dil 7	30.0	15.6
Dil 9 (256)	30.0	Dil 8	30.0	7.81
Dil 10 (512)	30.0	Dil 9	30.0	3.91
Dil 11 (1024)	30.0	Dil 10	30.0	1.95

Method set-up

- Labelling drug with Sulfo-Tag™ and biotin

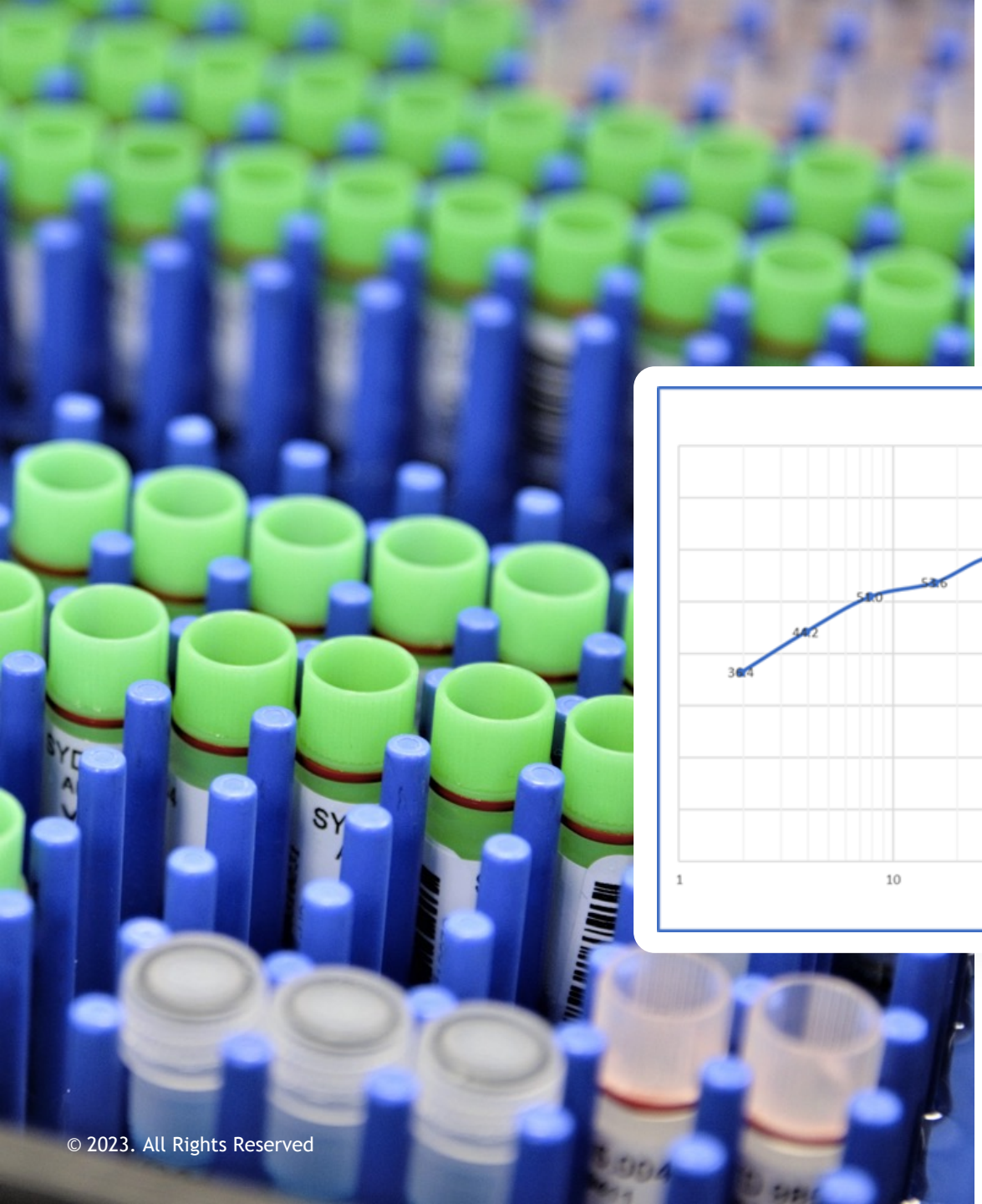
  Optimalisation of biotin-drug, Sulfo-TAG™-drug concentrations and ratio

- Screening sensitivity 1.22 ng/mL (transferred method 25.0 ng/mL)

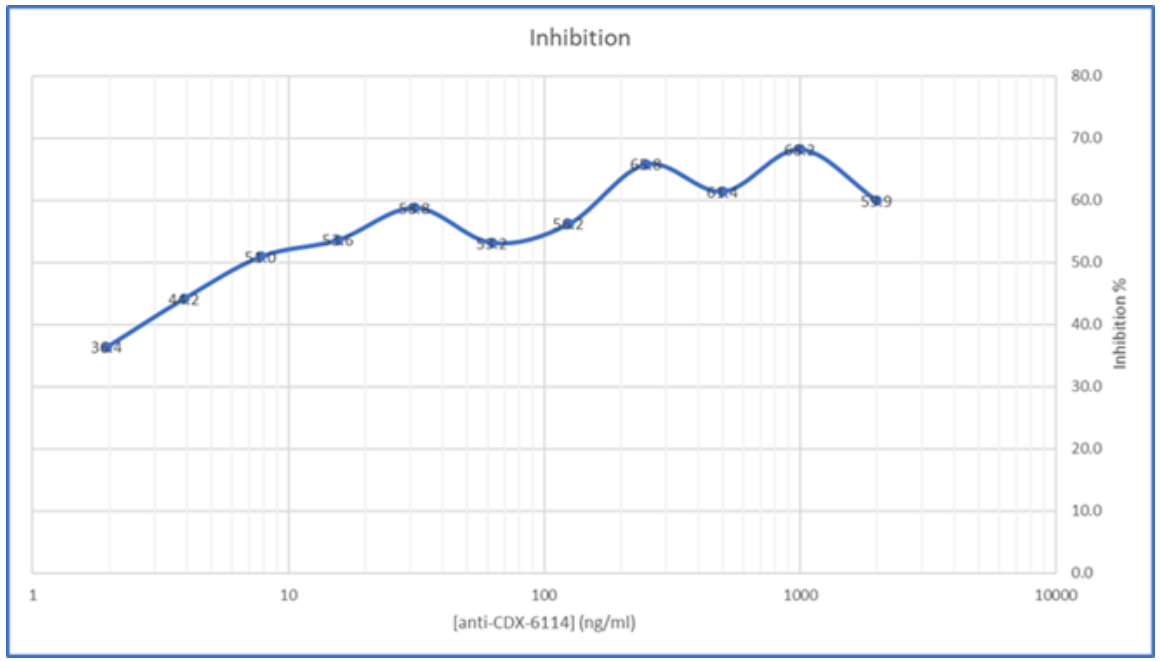
  Confirmatory assay issue: minor inhibition

- Sponsor procedure confirmatory drug added incorrect incubation step

HPC DIL	Conc (ng/mL)	Inhibition (%)
HPC_Dil 1	2000	59.9
HPC_Dil 2	1000	68.2
HPC_Dil 3	500	61.4
HPC_Dil 4	250	65.8
HPC_Dil 5	125	56.2
HPC_Dil 6	62.5	53.2
HPC_Dil 7	31.3	58.8
HPC_Dil 8	15.6	53.6
HPC_Dil 9	7.81	51.0
HPC_Dil 10	3.91	44.2
HPC_Dil 11	1.95	36.4



Confirmatory Minor Inhibition



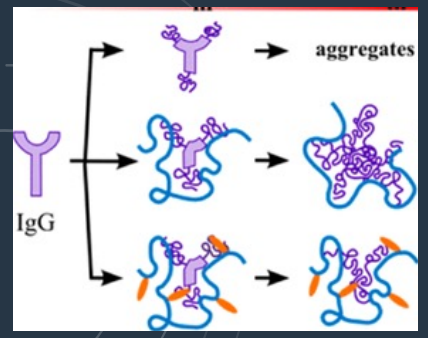
Transfer of Incorrect confirmatory assay

Acid degraded not only the ADA/onboard drug-complex

Degradation onboard drug in complex highly desirable for screening assay

Degradation of confirmatory drug unwanted for confirmatory assay

No degradation of ADA PC

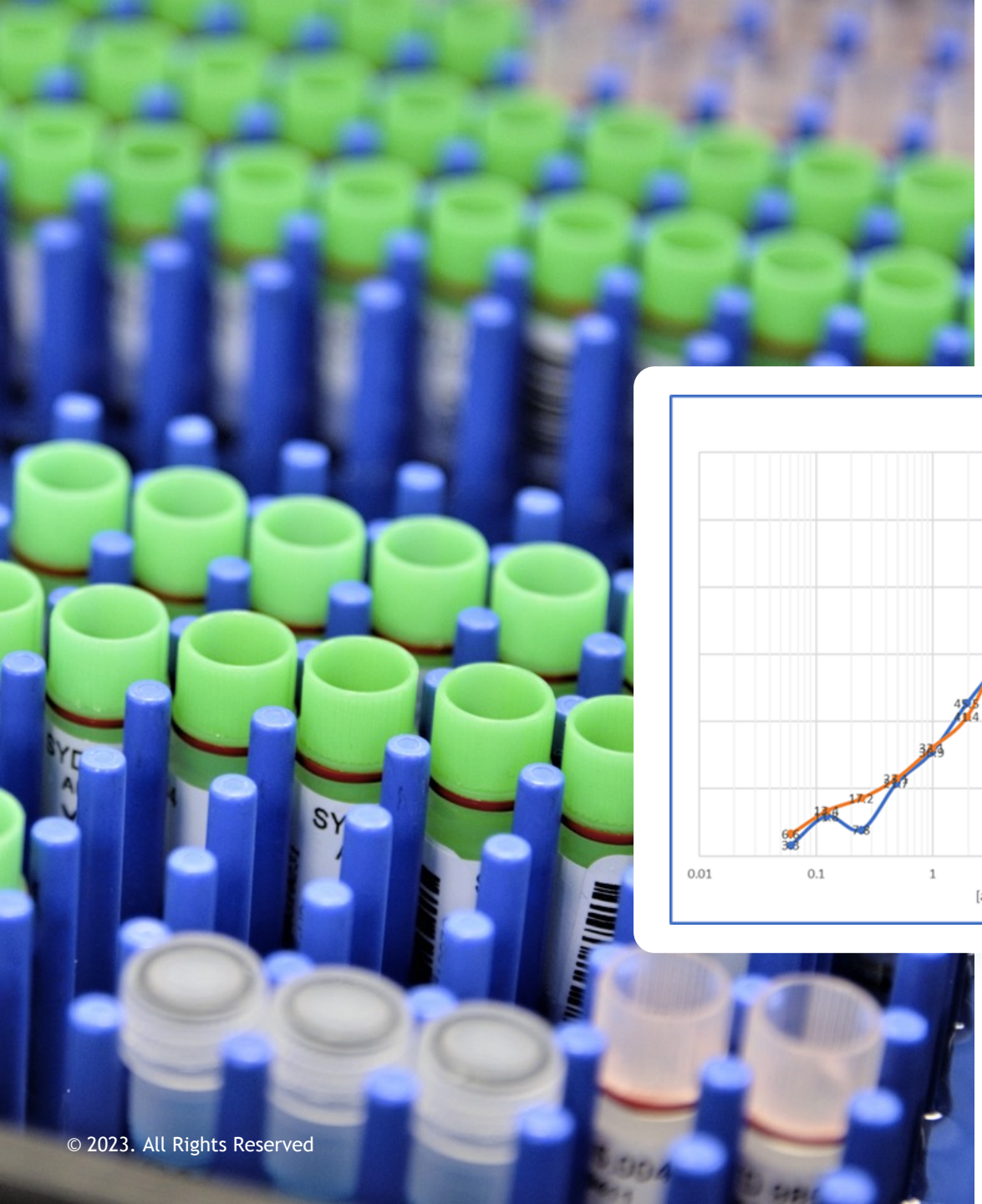


Method set-up continued

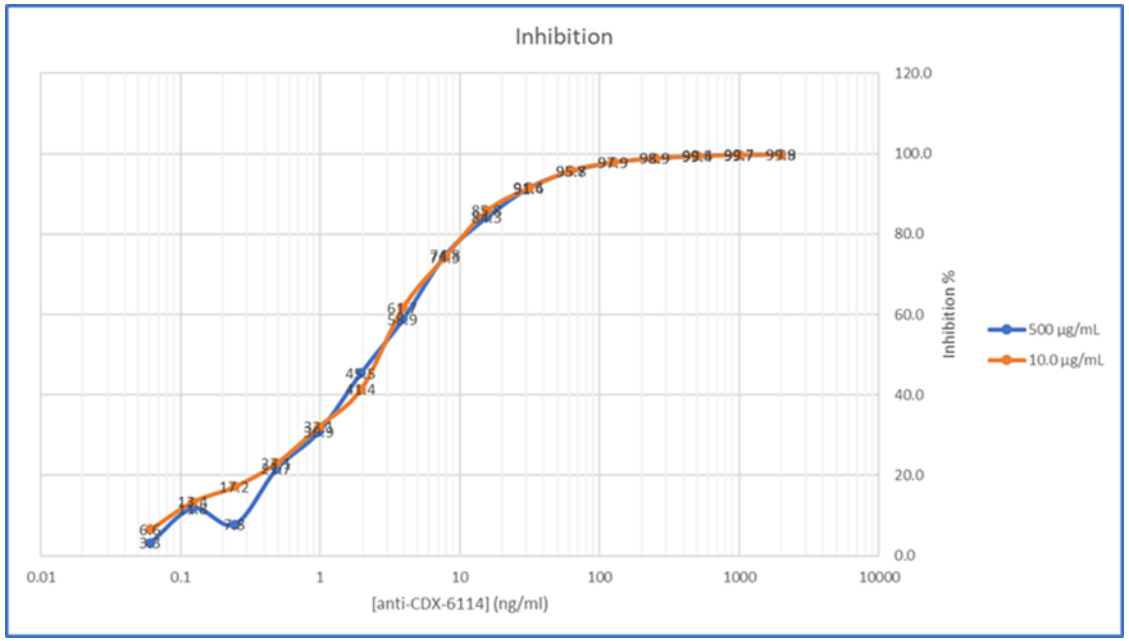
- Screening assay sensitivity 1.22 ng/mL
  Resulting in very low LPCs and LPCc

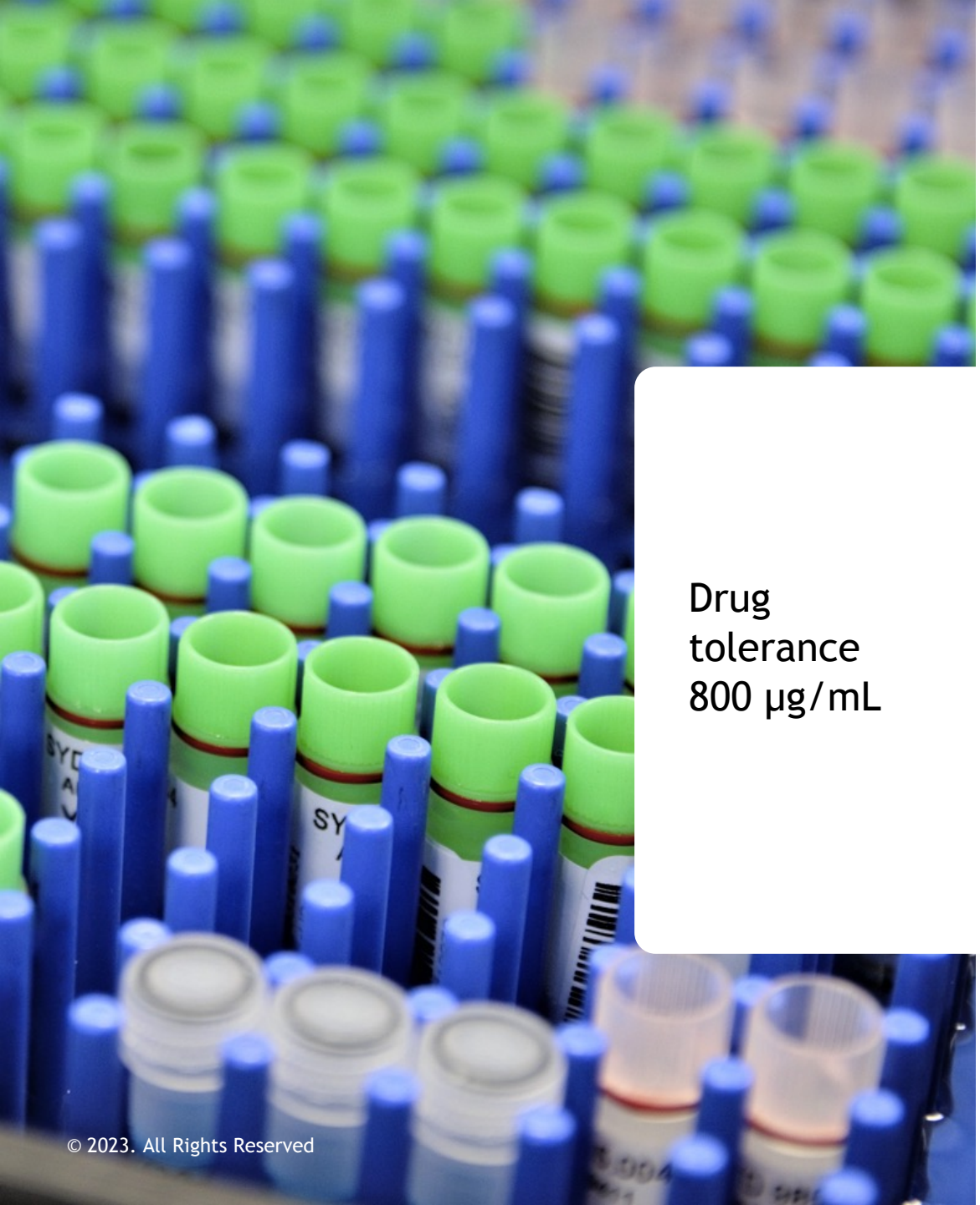
- For confirmatory assay - Master-mix
  Including 10.0 µg/mL drug
  Sensitivity confirmatory assay improved

HPC DIL	Conc (ng/mL)	Screening	500 µg (%)	10.0 µg (%)
HPC_Dil 1	2000	40424	99.9	99.8
HPC_Dil 2	1000	22090	99.7	99.7
HPC_Dil 3	500	10753	99.5	99.4
HPC_Dil 4	250	5432	98.9	98.9
HPC_Dil 5	125	2760	97.9	97.9
HPC_Dil 6	62.5	1356	95.7	95.8
HPC_Dil 7	31.3	690	91.6	91.4
HPC_Dil 8	15.6	382	84.3	85.8
HPC_Dil 9	7.81	222	74.8	74.3
HPC_Dil 10	3.91	141	58.9	61.7
HPC_Dil 11	1.95	99	45.5	41.4
HPC_Dil 12	0.977	81	30.9	32.1
HPC_Dil 13	0.488	72	21.7	23.1
HPC_Dil 14	0.244	64	7.8	17.2
HPC_Dil 15	0.122	64	11.8	13.4
HPC_Dil 16	0.0610	61	3.3	6.6
NC	0.00	62	3.3	3.4



Confirmatory assay Inhibition by drug





Screening assay Drug tolerance

Drug tolerance 800 µg/mL

Concentration drug (µg/mL)	ILPC (25.0 ng/mL)
0.00	1087
10.0	962
20.0	856
40.0	697
60.0	573
80.0	527
100	467
200	311
400	204
600	157
800	136
Plate specific Cutpoint (counts)	90

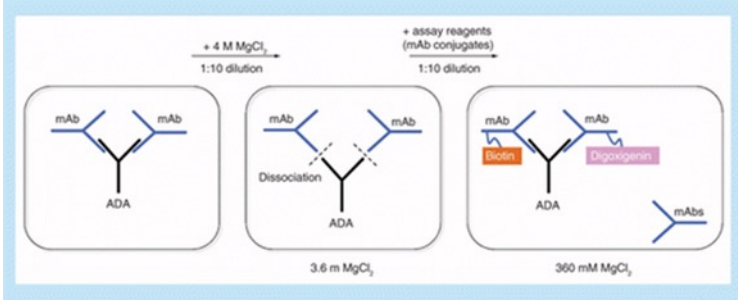
Method set-up outcome

- Timing and performance neutralization step crucial for:
  1. Adequate inhibition confirmatory assay
  2. Sensitivity confirmatory assay

- Screening Drug tolerance (detection 25 ng/mL PC in presence 800 µg/mL drug); acid dissociation similar to PandA and HISDA (laborious)

- Denaturation confirmatory drug by low pH could lead to ADA underestimation

  ADA ‘incidence’ rates can vary due to bioanalytical methods that are not fully understood



High Ionic strength dissociation method (HISDA)

Interference in ADA method

- Expected onboard-drug concentration-time profiles and required assay drug tolerance should be carefully assessed

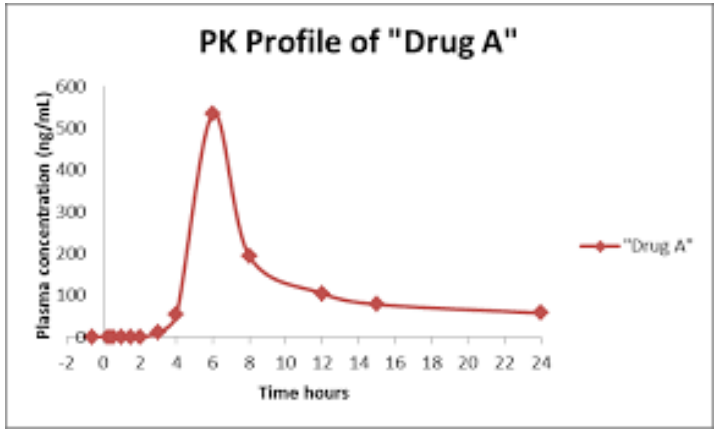
- Study design - Sampling time-points – not under report ADA incidence

Undetected ADA-positive samples

1. Interference by drug (drug-ADA complex)
2. Interference by drug soluble target

(new problem: drug-target complex=target-mediated bridging)

- Inaccurate interpretation - clinical consequences



Method set-up Conclusion

- Adequate neutralization steps essential for sensitive, robust and highly drug tolerant ADA screening and confirmatory assays

- Context of use of ADA assay

  determine the required drug tolerance

  consequently, the level of assay improvement

Context of Use - Drug tolerance

- Type of study (Pre-clinical GLP toxicity, Clinical)
  Drug levels known

- Q: Full validation > drug tolerance validated at PC 100 or 250 ng/ml only?
  A: Flexibility needed; PC and drug concentration open
  A: Evaluation of drug tolerance needed

- Drug tolerance - Scientific needs (add value, not: cutting corners)

Responsibility of CRO or Sponsor

- Partnership

  Survey CROs and biotech companies: Fine-tuning the partnership

  Mc Kinsey & Company June 09, 2022

- Opportunity for CRO to be good strategic partner for pharma companies

  Providing strategic input and accommodate pharma needs

- CRO to work in appropriate way and to scientific needs

  Long-term contract arrangements

CRO to partner with customer

- Appropriate methods for appropriate data interpretation
  - Fit-for-purpose method
  - Scientific needs
  - Communication with Sponsor
  - Reduce costs
- Sponsor to compare immunogenicity data to previous and future studies

Conclusions

- Appropriate methods for appropriate data interpretation
- Transfer of correct ADA assay to CRO
- CRO prefers to be good strategic partner for pharma companies
- Drive development speed with fit-for-purpose quality
- Partnership between CRO and pharma could lead to cost reduction in drug development

Thank You

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