

Improving Assay performance when complex sample pre-treatment is required – a CRO perspective

Laura Geary

Senior Scientist

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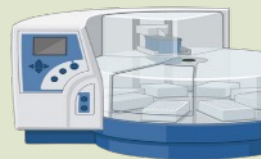
Case Studies



Improving assay performance
in a heat treatment assay



Improving analyst to analyst
variation in a PandA assay



Improving precision in BEAD
assays

Introduction

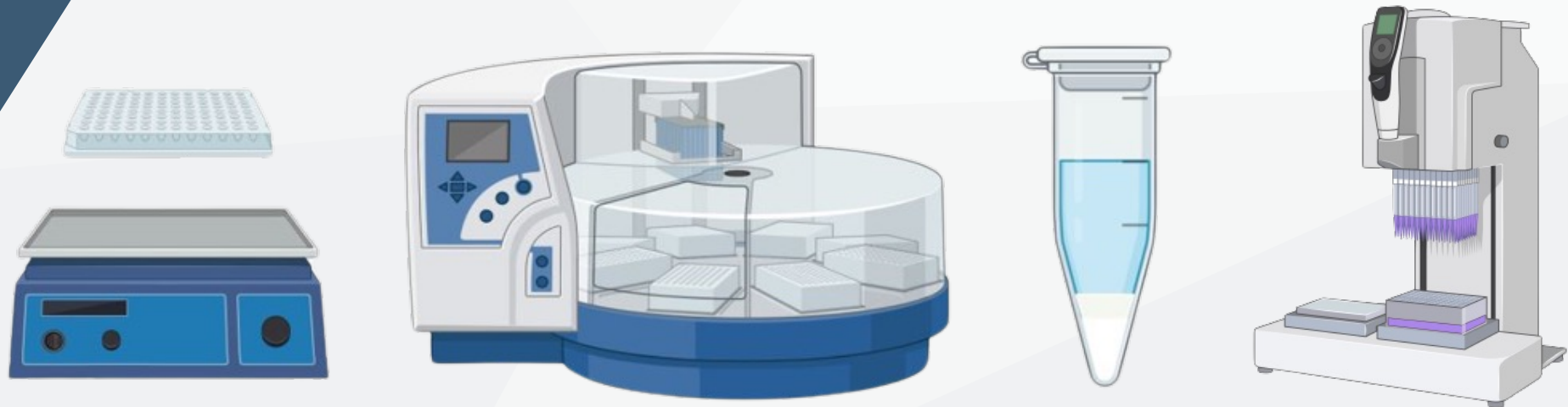
Complex sample pre-treatment methods are sometimes required to achieve the high levels of drug tolerance requested by sponsors

- ACE, precipitation, SPEAD, bead methods and heat treatment

These techniques can be:

- Time consuming
- Have poor precision
- Require specialized equipment

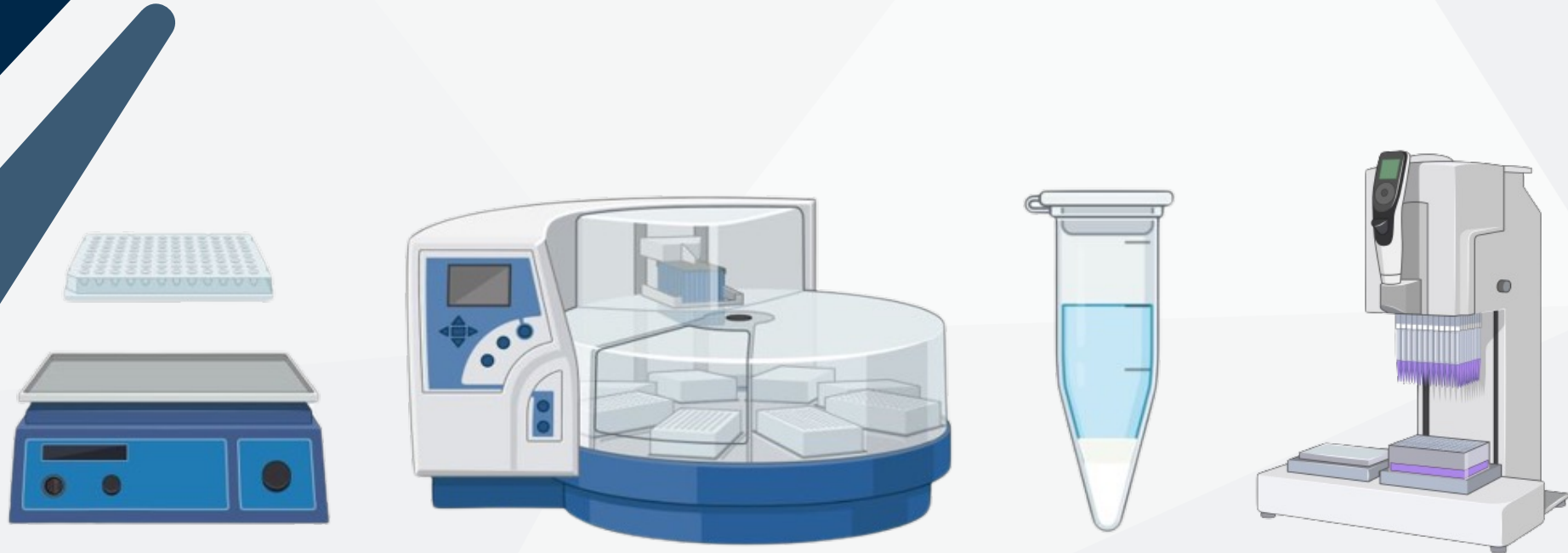
ARE WE DOING TOO MUCH?



Introduction

ARE WE DOING TOO MUCH?

- A CRO needs to meet the requirements of the sponsor
- We need to know the level of drug expected in the ADA samples
- Complex sample pre-treatment is still required in some cases





Case Study 1: Heat treatment

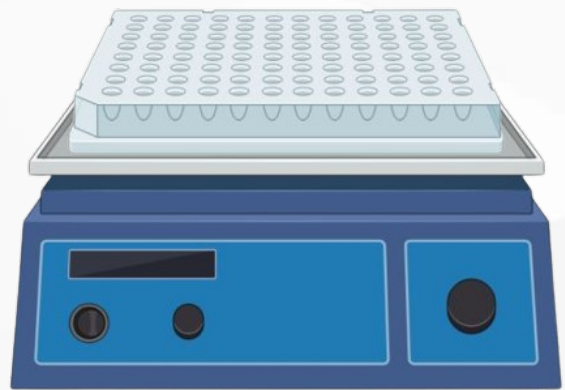
When it is required:

- Reduce matrix effects
- Improve drug tolerance to non-IgG therapeutics

Potential Assay problems:

- Changes to the matrix consistency leading to poor precision
- Evaporation of samples during heating leading to poor precision
- Denaturation of the PC
- Changes to pH due to the temperature change

Case Study 1: Heat treatment



Control	CV%	
	Intra Assay	Inter Assay
HPC	<10	<10
MPC	<10	<10
LPC	<10	<30
NC	<10	<50

Case Study 1: Heat treatment

CRO Solutions:

- Use specific tubes with screw cap lids
- Use heat treatment dimensions
- Set mirror sample volumes

Control	CV%	
	Intra Assay	Inter Assay
HPC	<5	<5
MPC	<5	<5
LPC	<5	<10
NC	<5	<15



Case Study 2: Improving analyst to analyst variation in a PandaA assay

Day 1

Excess Therapeutic is added to the samples, forming complexes with the ADAs

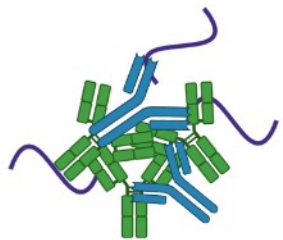


PEG is added to the wells, causing the ADA/therapeutic complexes to precipitate

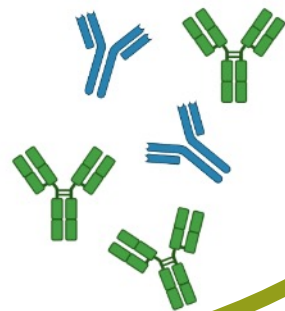







Day 2

The plate is centrifuged and excess liquid removed



The pellet is re-suspended in acid, causing dissociation

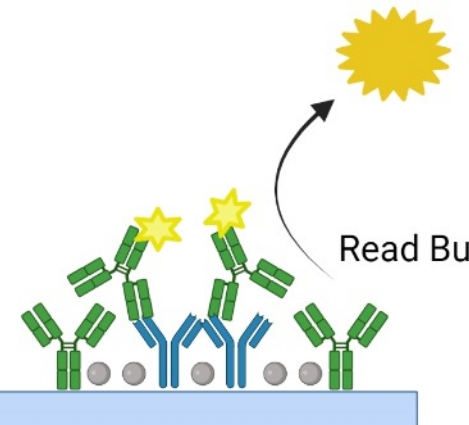


-  Therapeutic
-  ADA
-  PEG
-  Serum protein
-  Sulfotag conjugated therapeutic

Acidified solution is coated onto a MSD plate which is then blocked



Sulfotag conjugated therapeutic is added to the wells

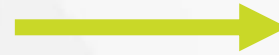


Read Buffer

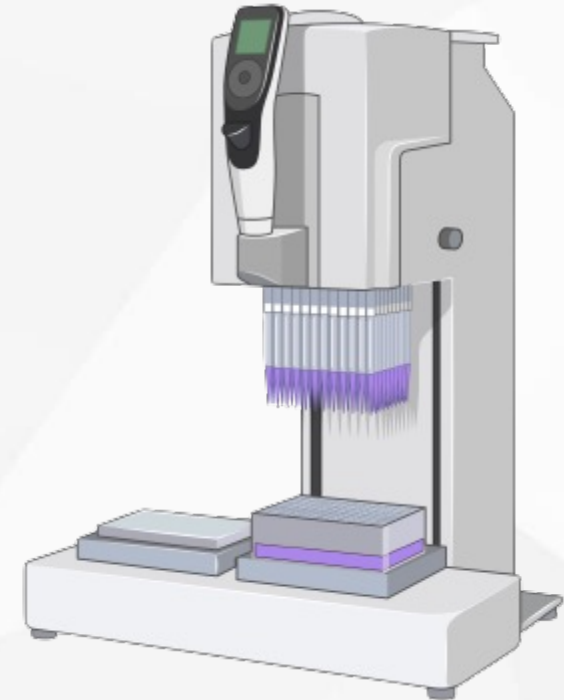
Case Study 2: Improving analyst to analyst variation in a PandaA assay

Manual pellet wash

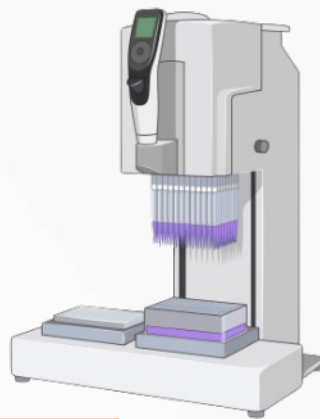
	Screen (S/N)			Screen RLU
	HPC	MPC	LPC	NC
Inter Mean	224.5	24.26	3.45	86
Inter %CV	28.8	30.4	20.3	5.2
Max Intra-assay % CV	10	9.7	7.2	4.2



The solution



Case Study 2: Improving analyst to analyst variation in a Panda assay



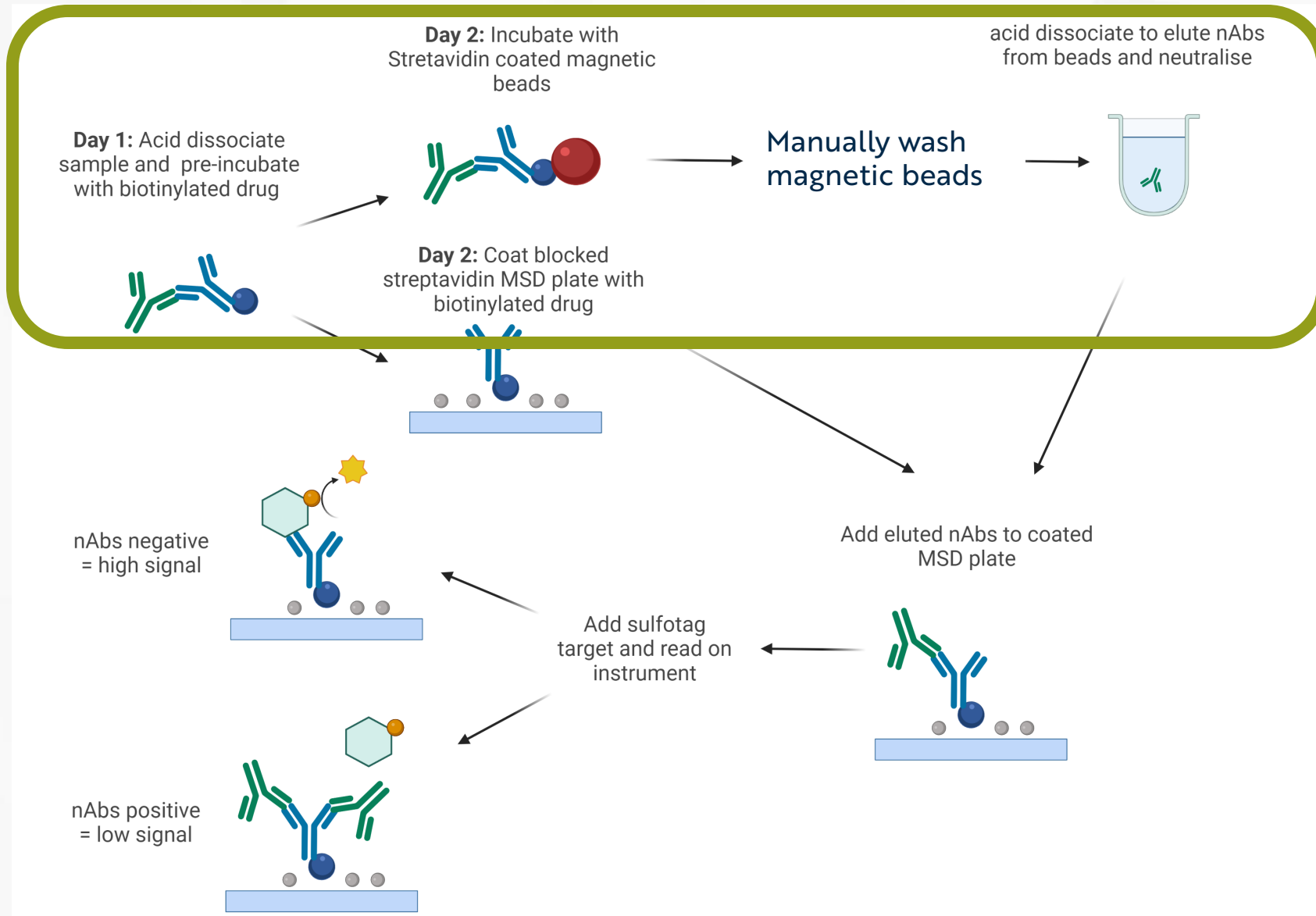
Manual pellet wash

Automated pellet wash

	Screen (S/N)			Screen RLU
	HPC	MPC	LPC	NC
Inter Mean	224.5	24.26	3.45	86
Inter %CV	28.8	30.4	20.3	5.2
Max Intra-assay % CV	10	9.7	7.2	4.2

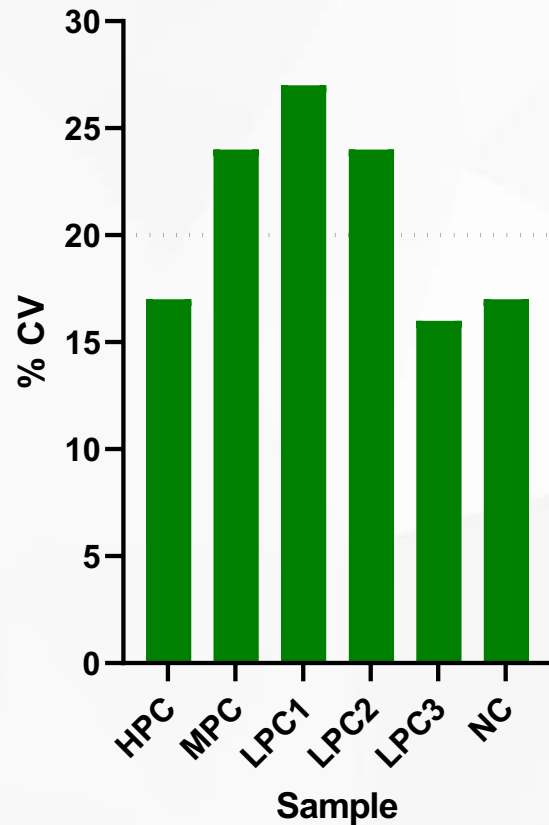
	Screen (S/N)			Screen RLU
	HPC	MPC	LPC	NC
Inter Mean	355.07	41.63	5.24	61
Inter %CV	7.1	6.9	6.7	8.3
Max Intra-assay % CV	9.5	8	7.9	10.1

Case Study 3: Improving precision in BEAD assays (nAbs)

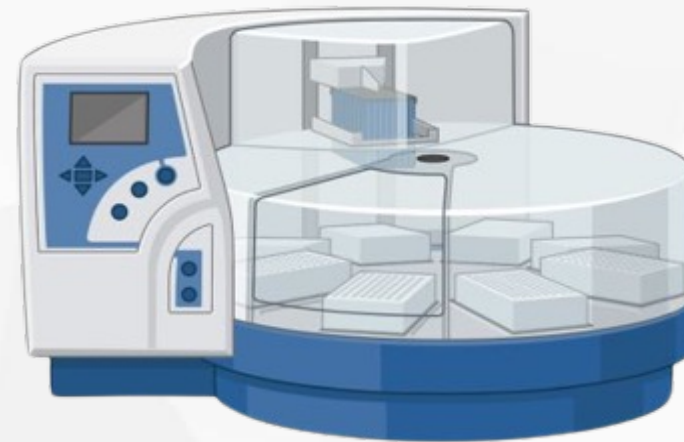


Case Study 3: Improving precision in BEAD assays (nAbs)

Validation Intra-Assay Precision
- manual bead steps

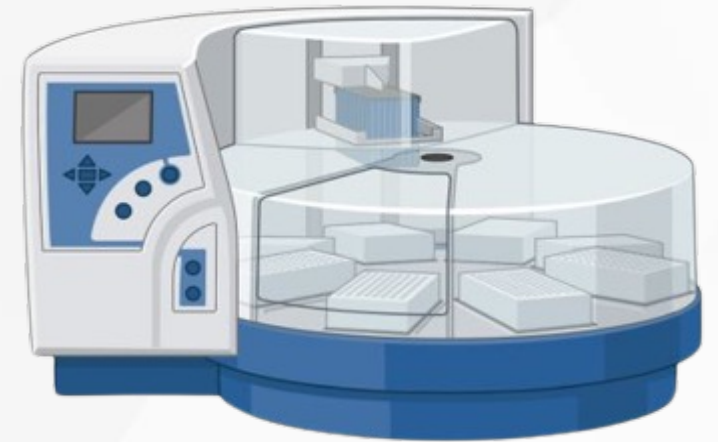
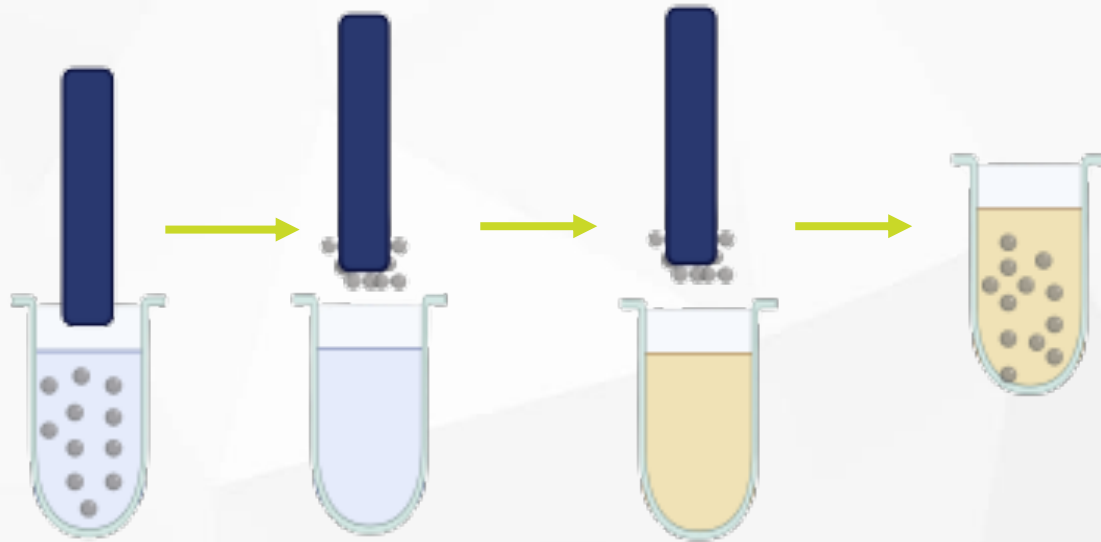


The solution



KingFisher Flex

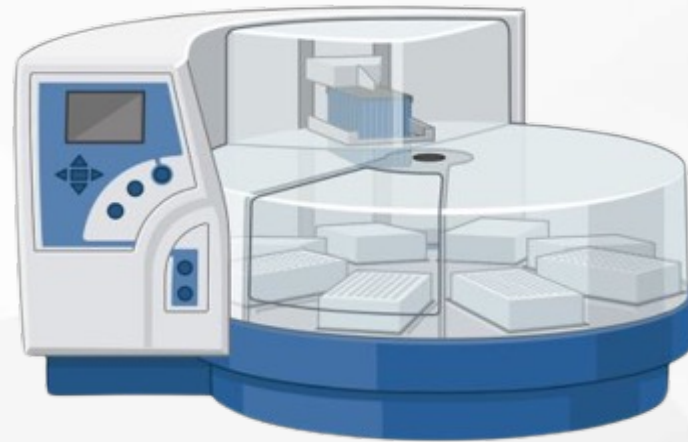
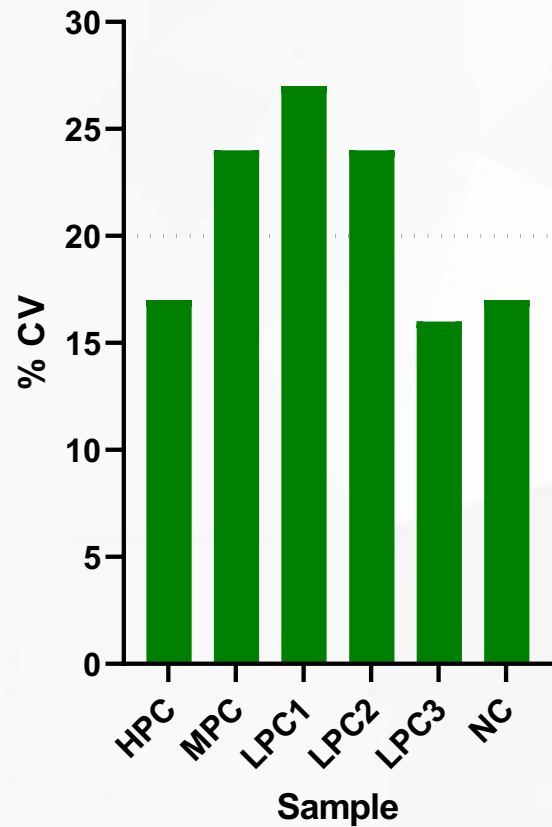
Case Study 3: Improving precision in BEAD assays (nAbs)



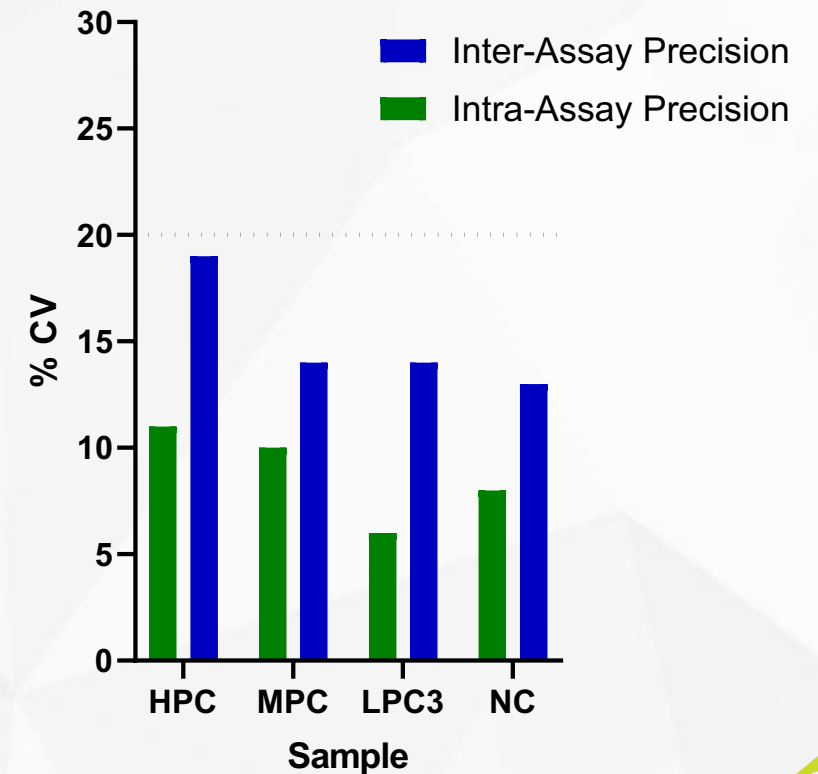
KingFisher Flex

Case Study 3: Improving precision in BEAD assays (nAbs)

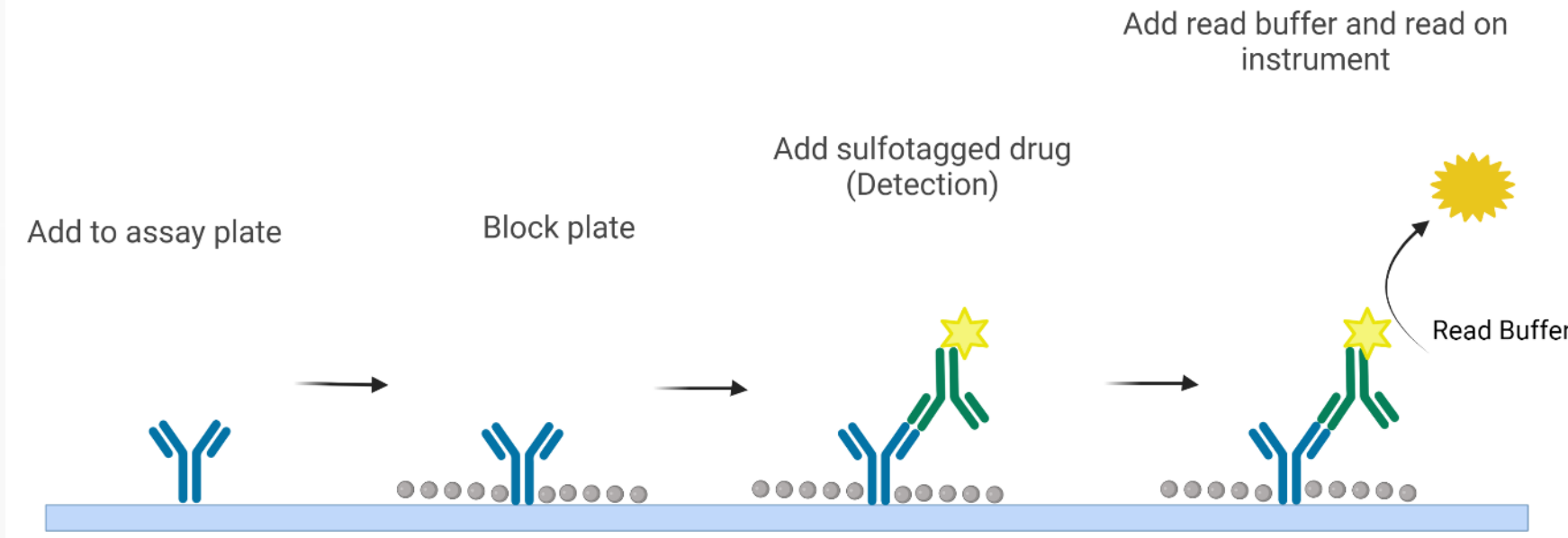
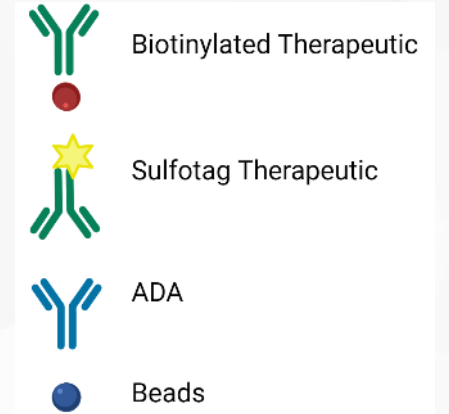
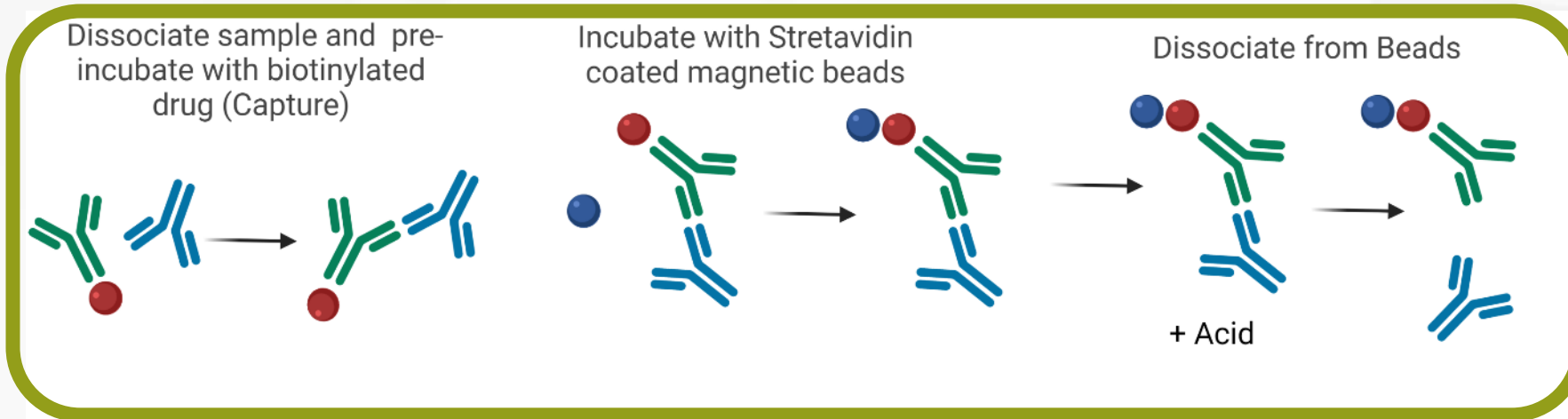
Validation Intra-Assay Precision
- manual bead steps



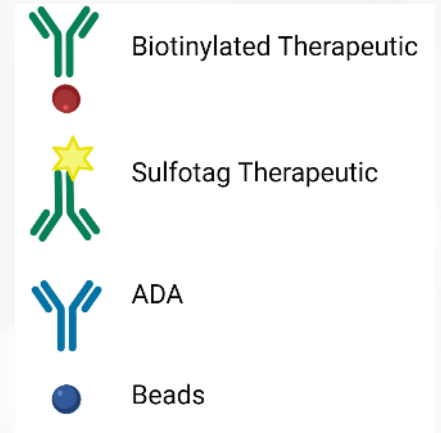
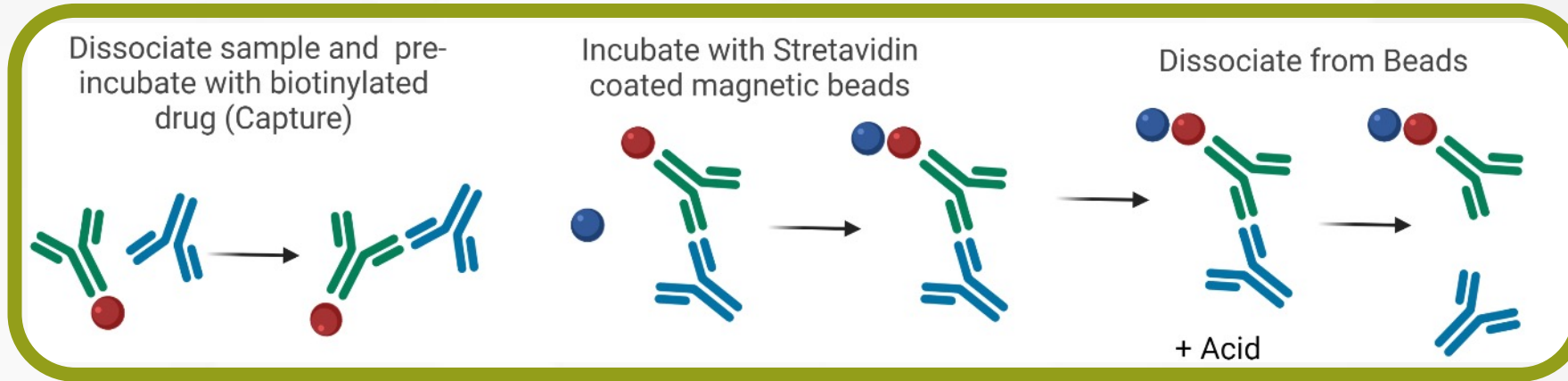
Validation Precision
- Automated bead steps



Case Study 3: Improving precision in BEAD assays (ADA)



Case Study 3: Improving precision in BEAD assays (ADA)

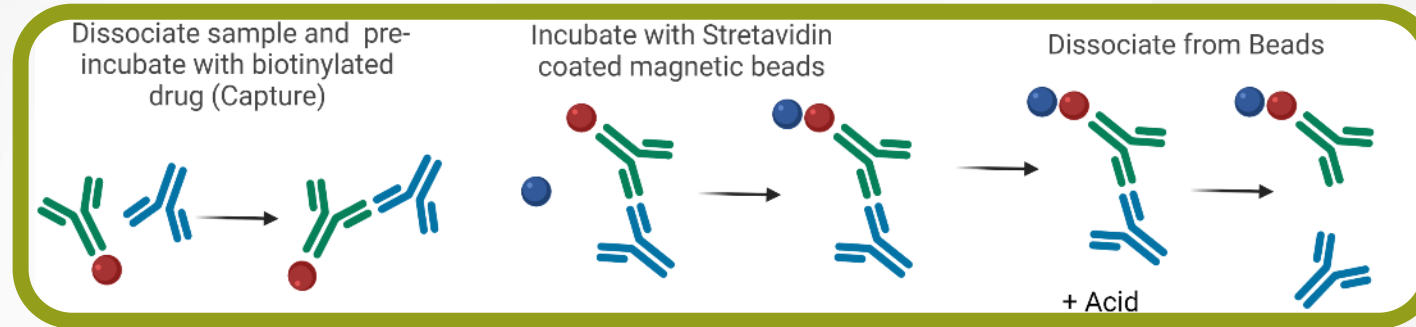






- Poor precision seen, particularly in the NC with manual bead processing method

	1-2	5-6
A	NC	NC
B	HPC	Blank individual

	1-2	5-6
A	15000	46
B	120000	55

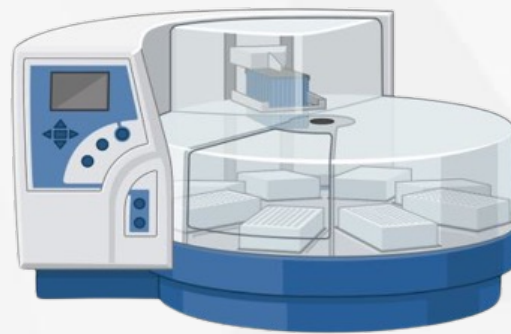
Case Study 3: Improving precision in BEAD assays (ADA)



-  Biotinylated Therapeutic
-  Sulfotag Therapeutic
-  ADA
-  Beads

Inter-assay precision using automated bead processing

Control	CV%	
	Screen S/N	Confirmatory
HPC	<10	<1
MPC	<15	<1
LPC	<19	<10
NC	<15 (RLU)	<10



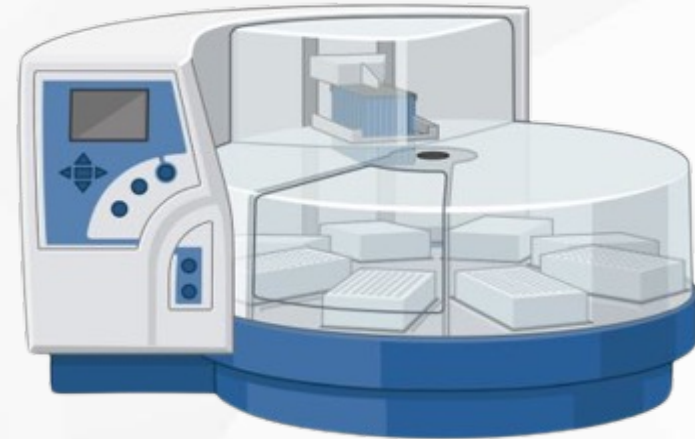
Intra-assay precision using automated bead processing

Control	CV%	
	Screen S/N	Confirmatory
HPC	<3	<1
MPC	<5	<1
LPC	<5	<3
NC	<5 (RLU)	<10

Case Study 3: Improving precision in BEAD assays (ADA)

KingFisher Protocol optimisation:

- Incubation times
- Shaking times and speeds
- Buffers
 - Inclusion of detergent



Summary

- ▶ Complex sample pre-treatment is often required for immunogenicity assays
 - ▶ They can have poor precision and poor assay performance
 - ▶ The simple assay formats should be assessed first
 - ▶ Accurate drug tolerance levels are required
- ▶ There are methods to eliminate the assay variability
 - ▶ Ensuring consumables remain consistent e.g. screw cap tubes to heat samples
 - ▶ Use automation and electronic equipment where possible
- ▶ Our recommendations
 - ▶ Heat treatment can only be used to improve drug tolerance with a non-IgG therapeutic
 - ▶ You can achieve high levels of drug tolerance with PandA, but you may encounter the licensing problems
 - ▶ Automated bead-based methods are simple and achieve high levels of drug tolerance

Acknowledgements

Resolian IA Department colleagues

References

Images Created on BioRender.com



Thank you for listening,

Any questions?