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

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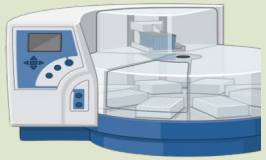
Case Studies

Improving assay performance in a heat treatment assay

Improving analyst to analyst variation in a PandA assay



Improving precision in BEAD assays

Introduction

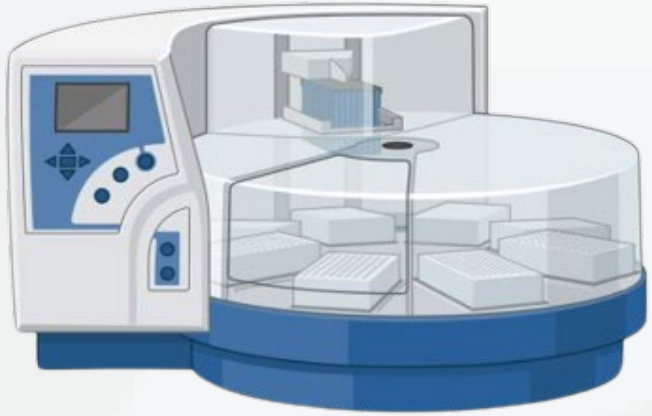
Complex sample pre-treatment methods are sometimes required to achieve the high levels of drug tolerance requested by sponsors

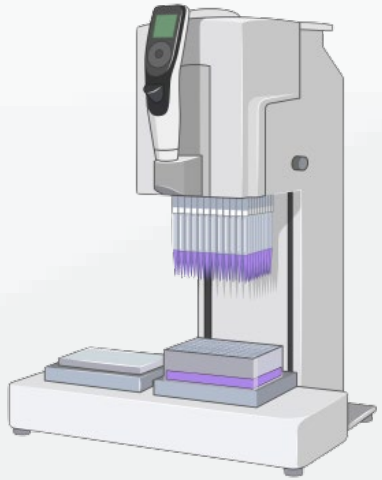
- ACE, Precipitation, SPEAD, Bead methods and heat treatment

These techniques can be:

- Time consuming
- Have poor precision
- Require specialized equipment

ARE WE DOING TOO MUCH?

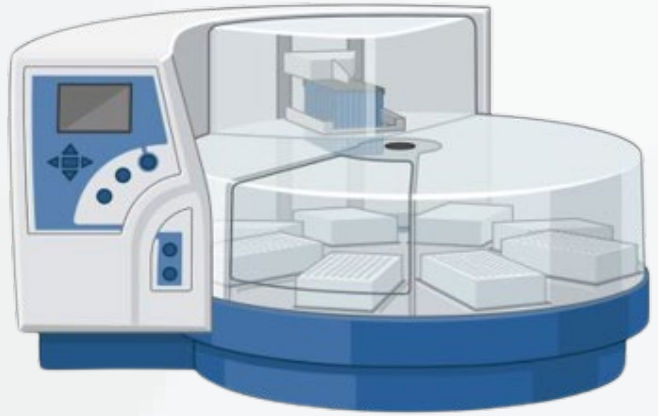


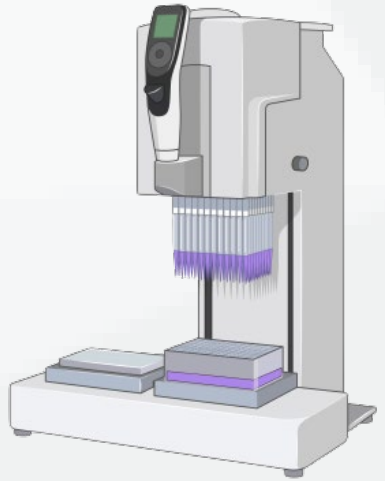


Introduction

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

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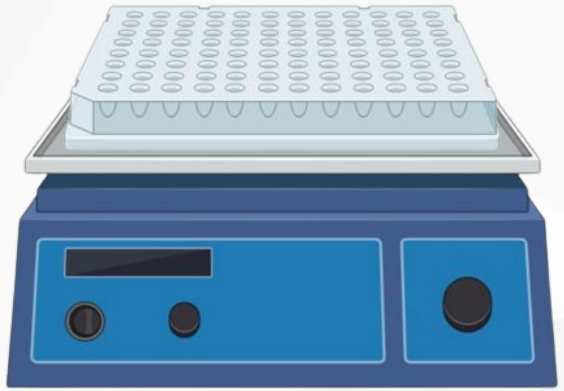
When it is required:

- 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

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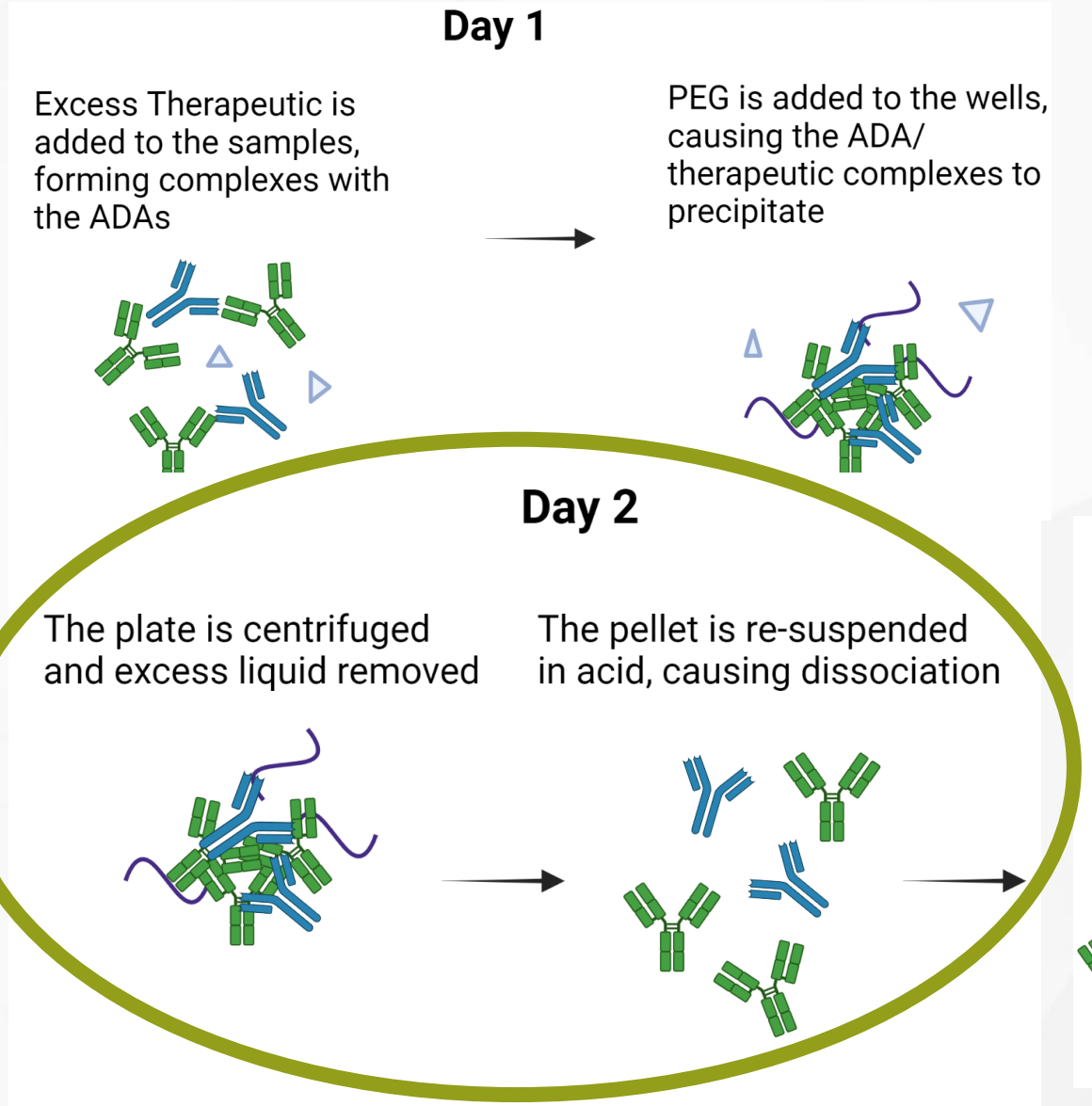
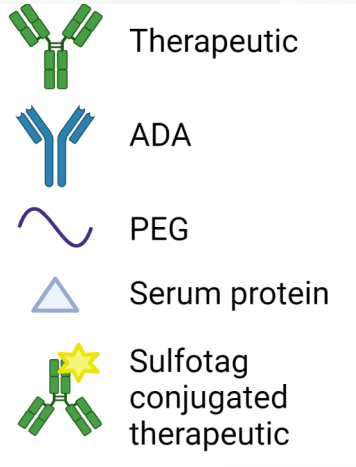
CRO Solutions:

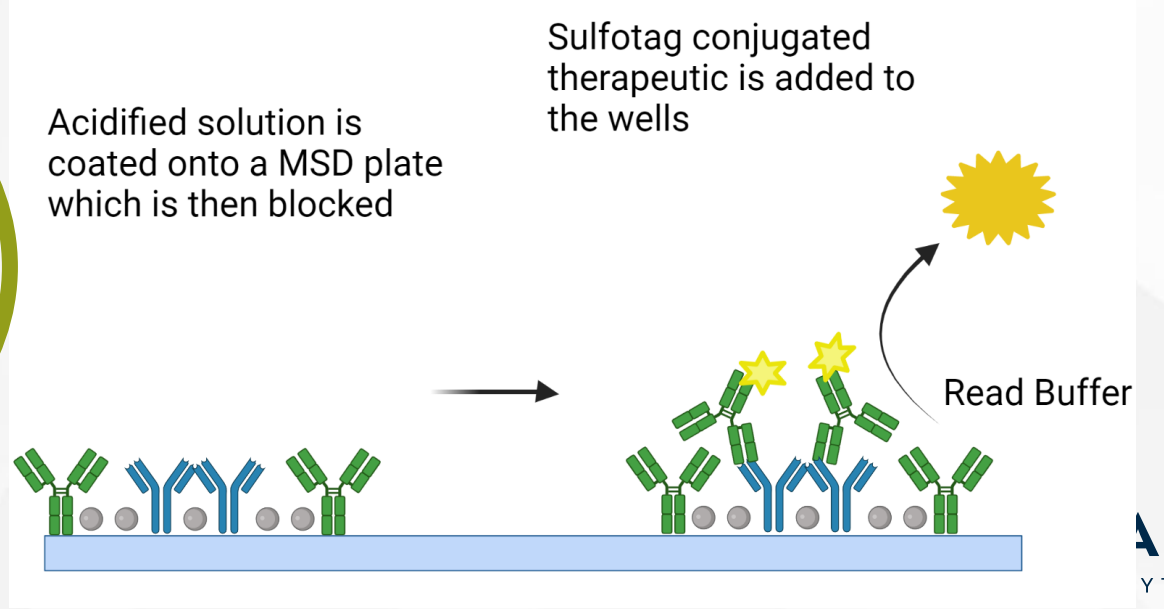
- Use specific tubes with screw cap lids
- Use heat block with specific dimensions
- Set minimum and maximum sample volumes

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 PandA assay





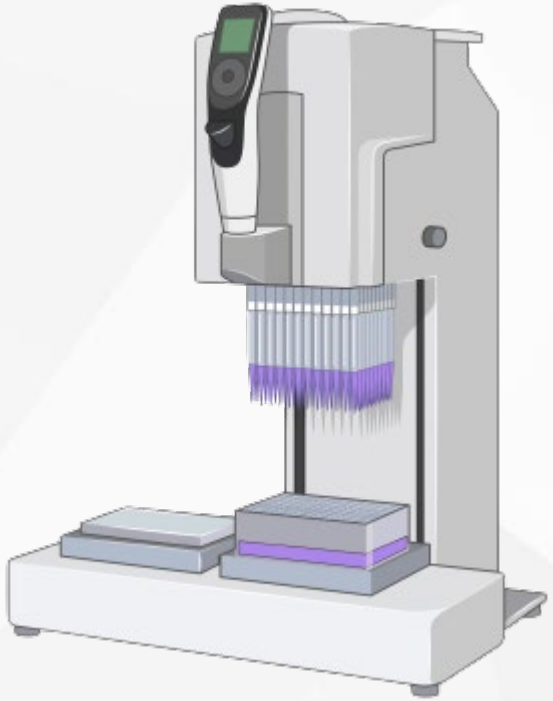


Case Study 2:
Improving analyst to analyst variation in a PandA assay

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



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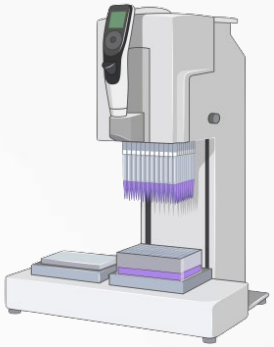

Manual pellet wash

	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

Automated pellet wash

	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

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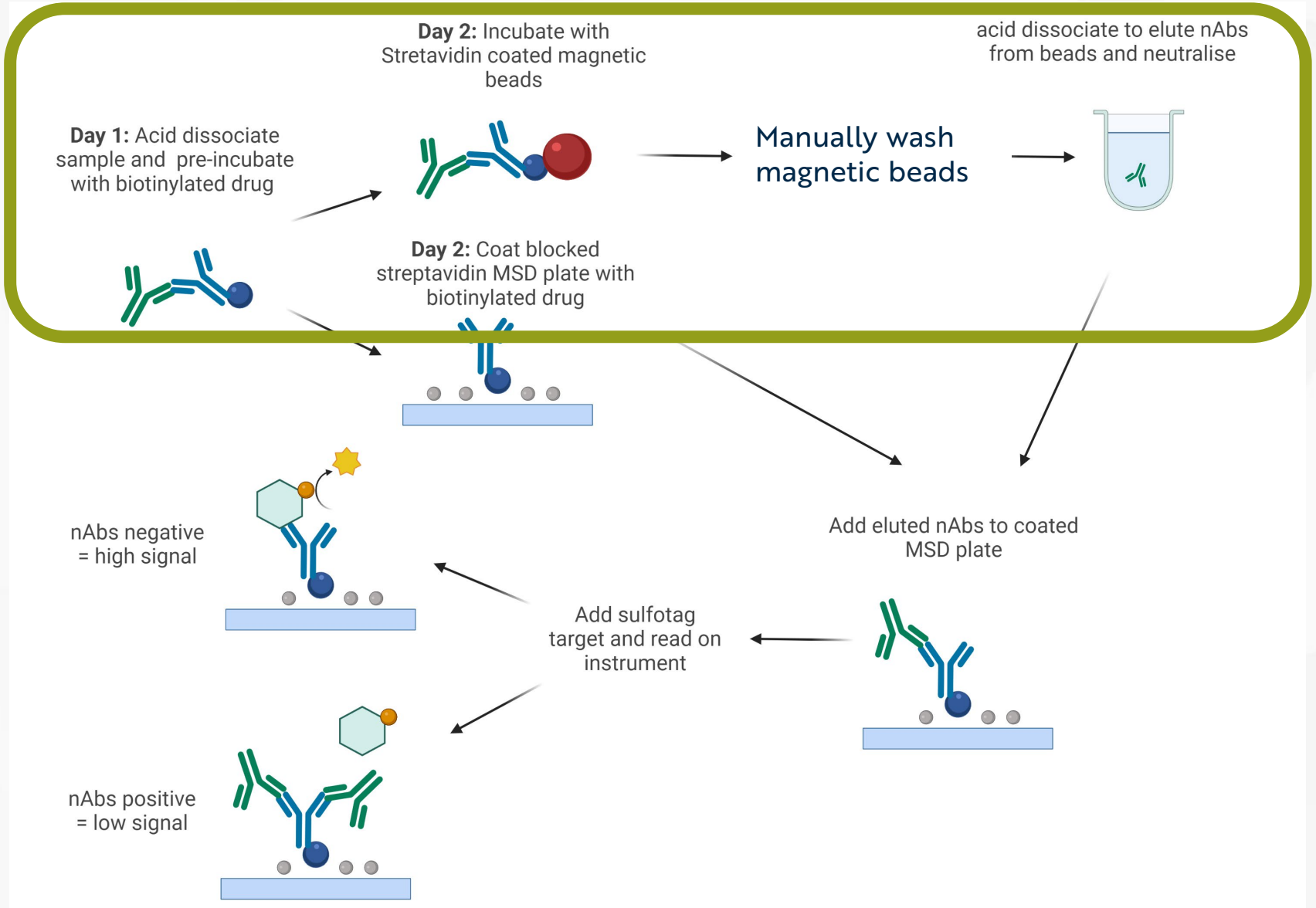
Case Study 3: Improving precision in BEAD assays (nAbs)



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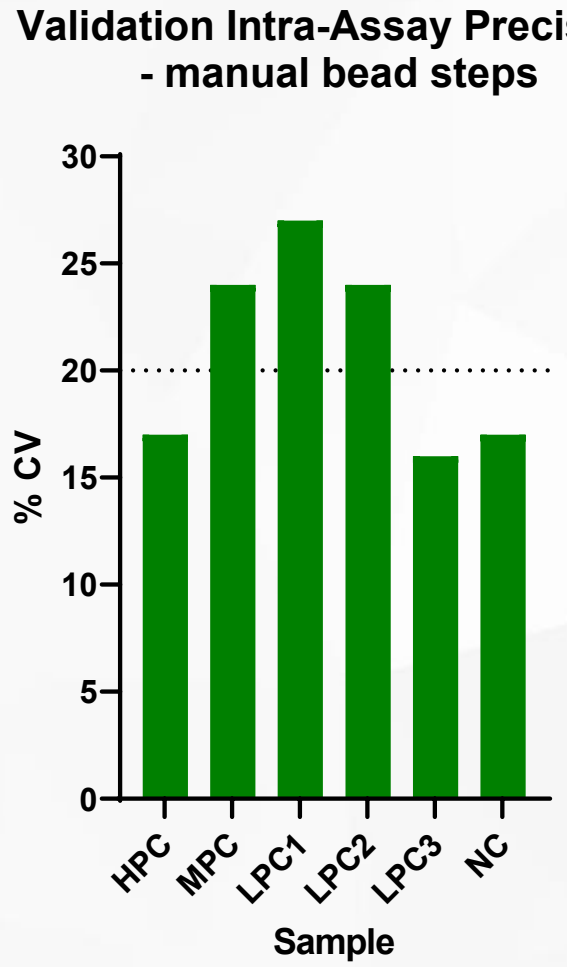
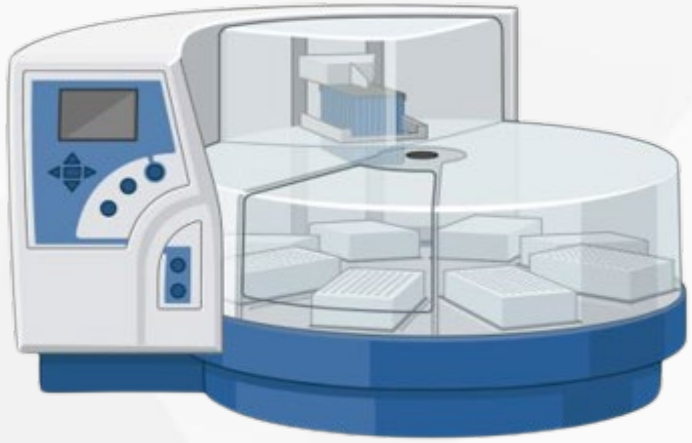


The solution



KingFisher

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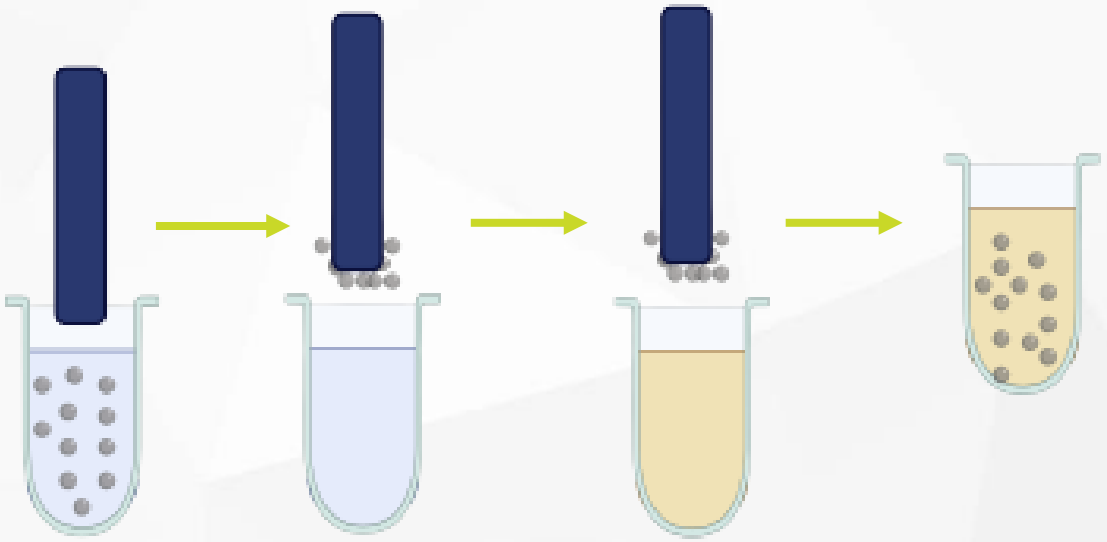

KingFisher Flex

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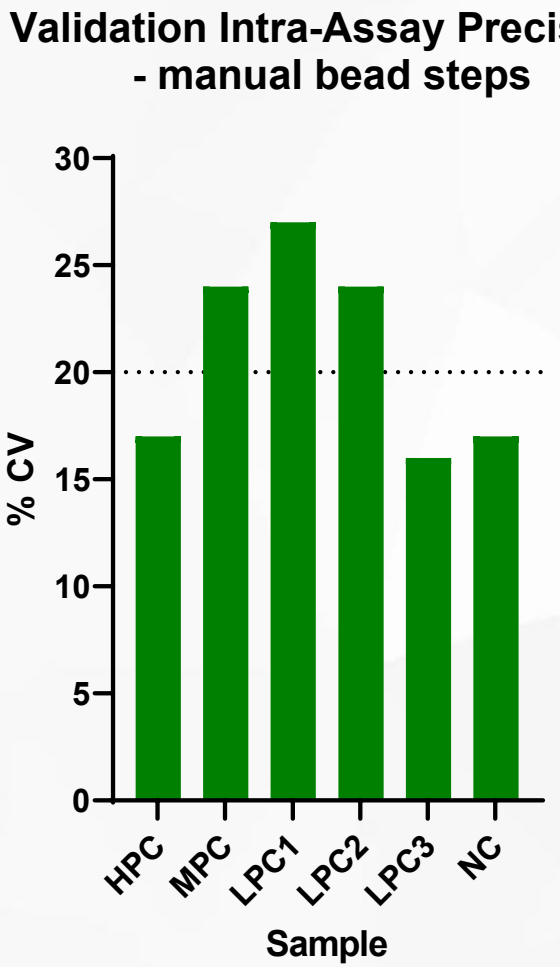

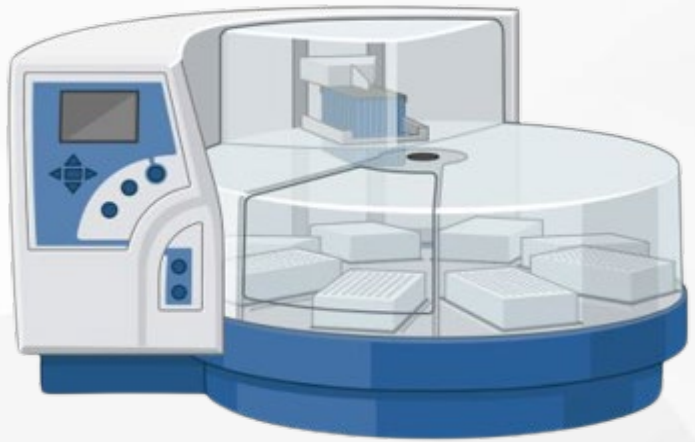

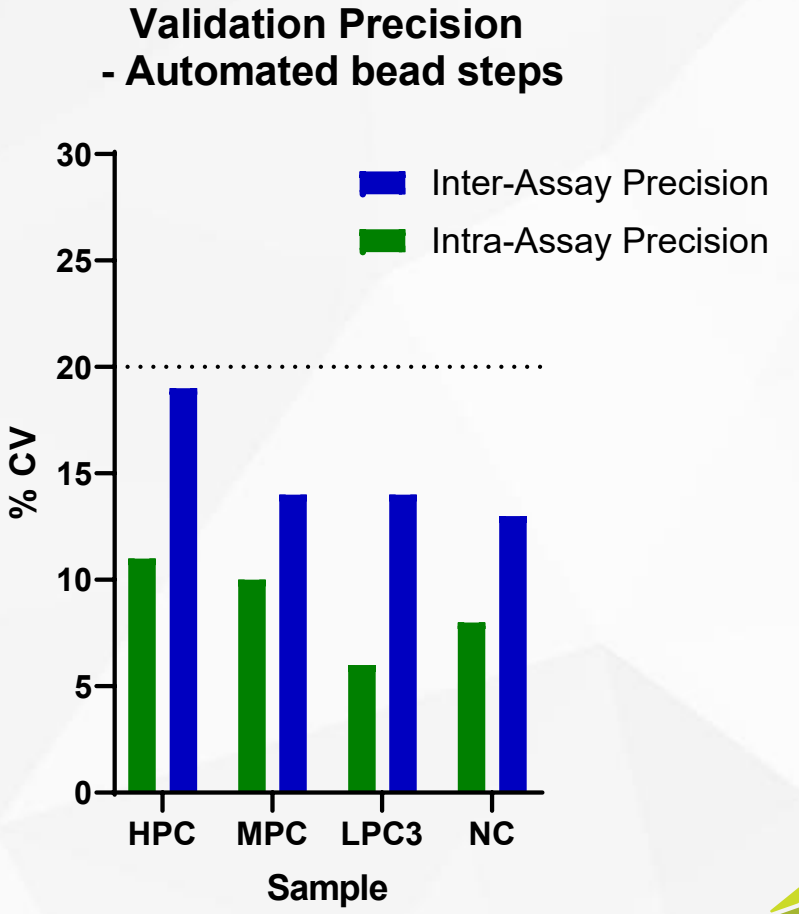

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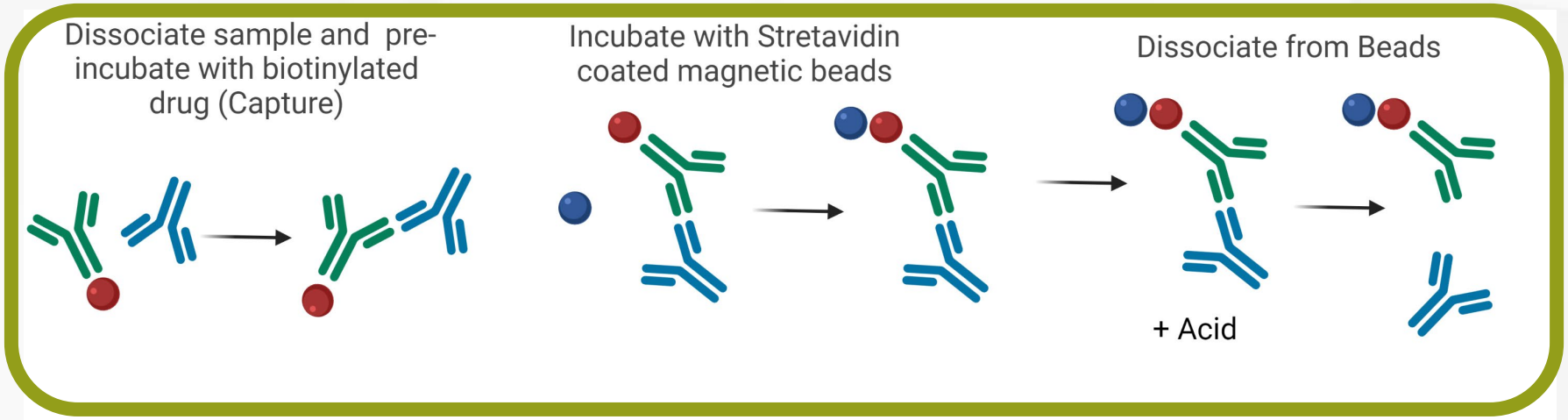
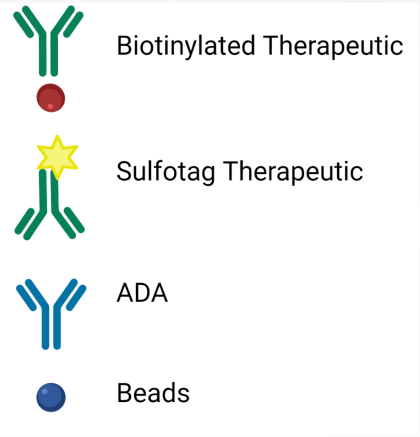
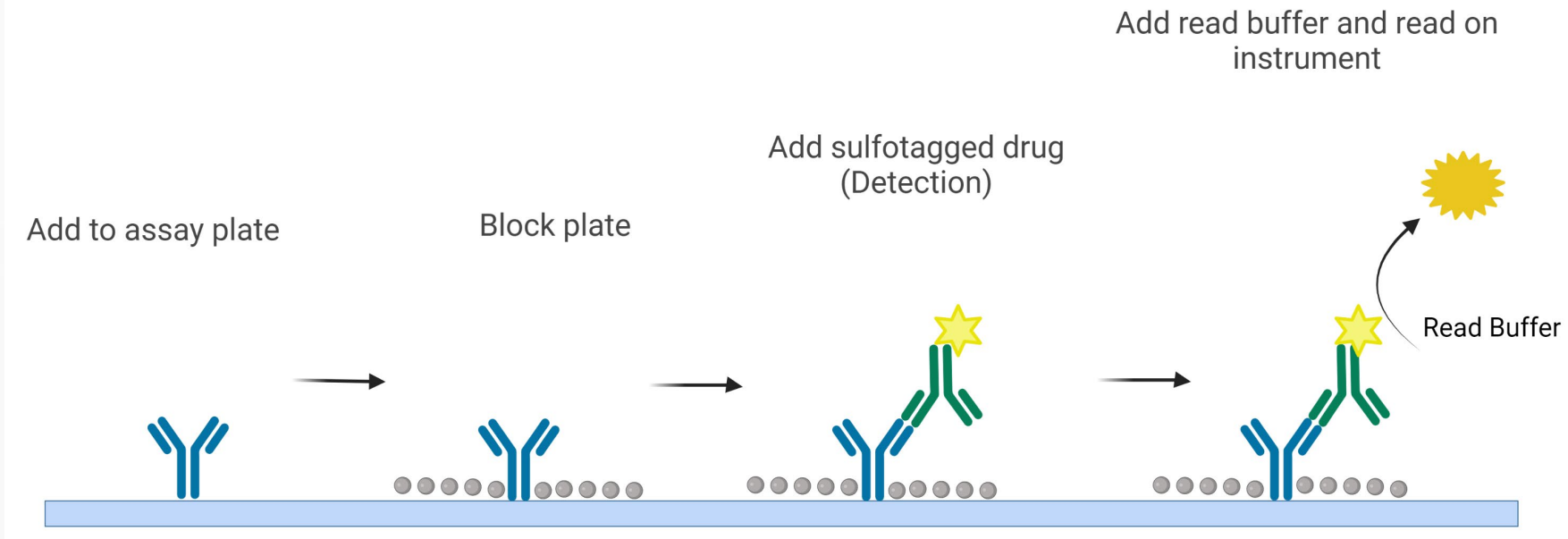
Case Study 3: Improving precision in BEAD assays (ADA)







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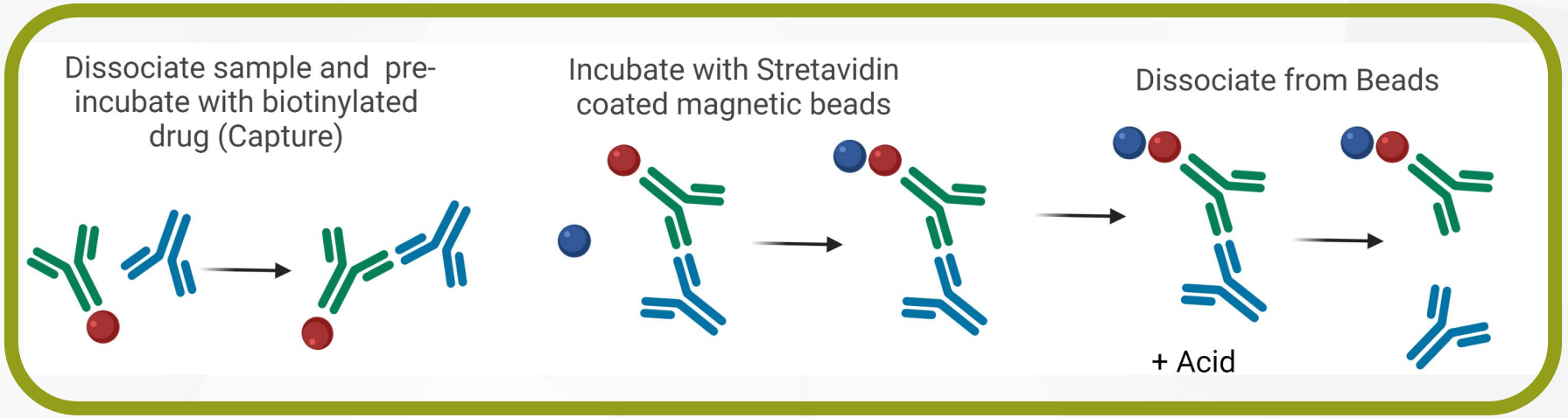
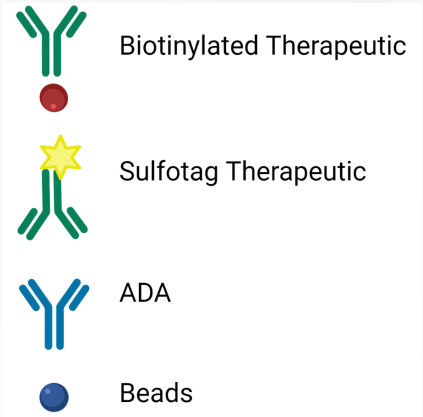
Case Study 3: Improving precision in BEAD assays (ADA)





- High 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

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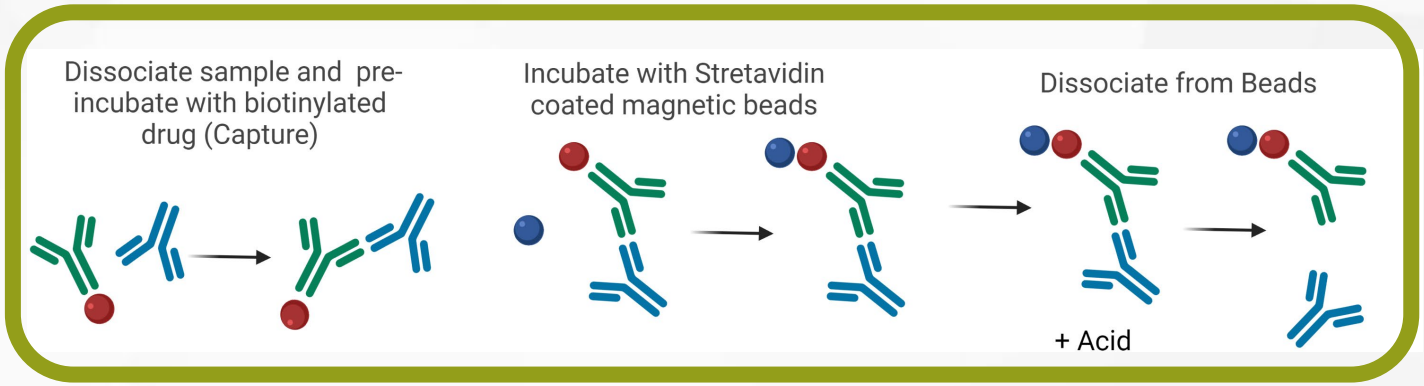
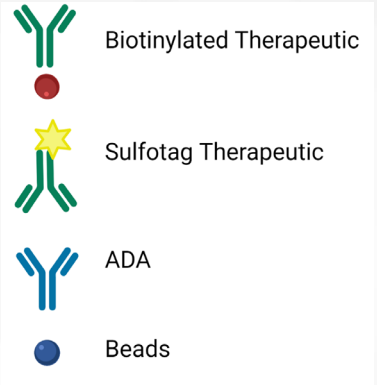
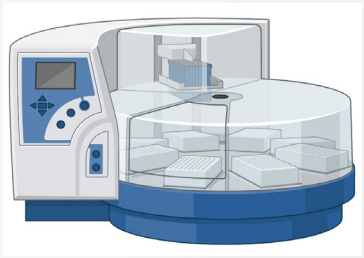
Case Study 3: Improving precision in BEAD assays (nAbs)





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



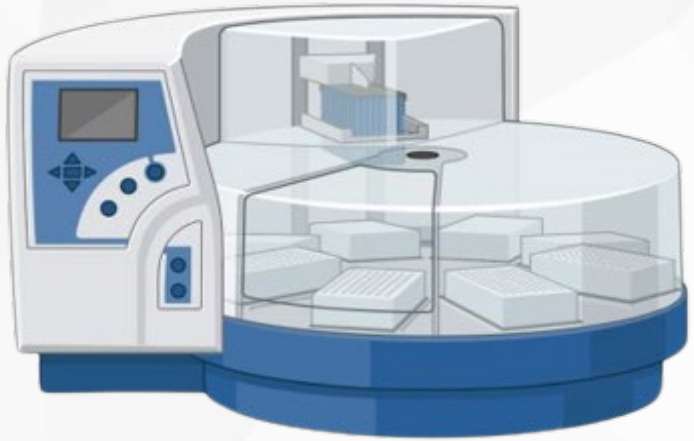
Intra-assay precision using automated bead processing

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



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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
- 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?

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