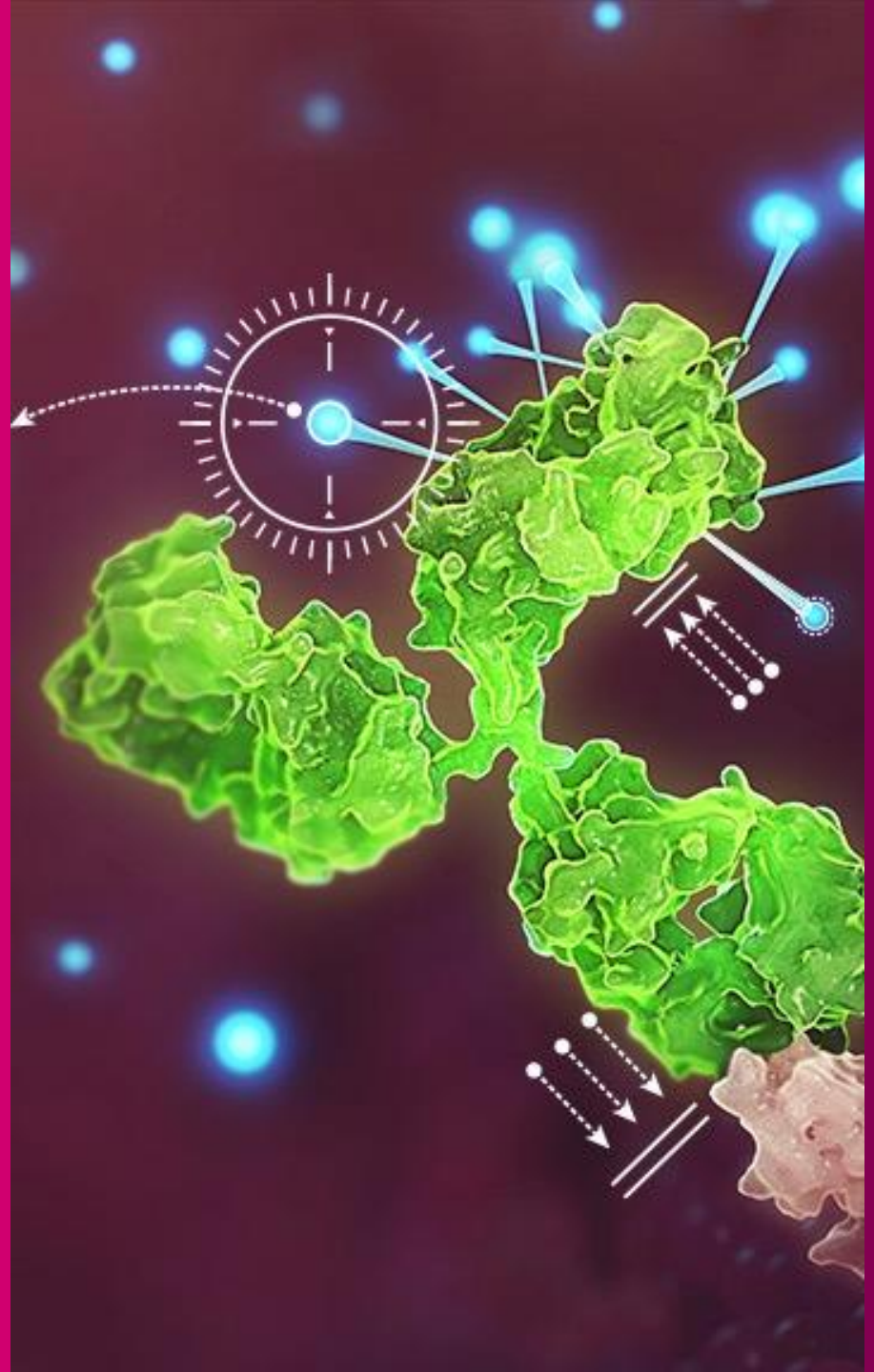
AstraZeneca

Nutty About Potency: Bolting Down a Robust Late-Stage Relative Potency Assay

Erika Farmer

28 Apr 2026

CASSS: Bioassays 2026



1

Introduction

Expectations for Lot Release Potency Method

Clinical Stage may not be fully MoA reflective, update required before commercial if so.

Non-Negotiable:

Method must be unbiased and consistent to allow confidence in results

Non-Negotiable:

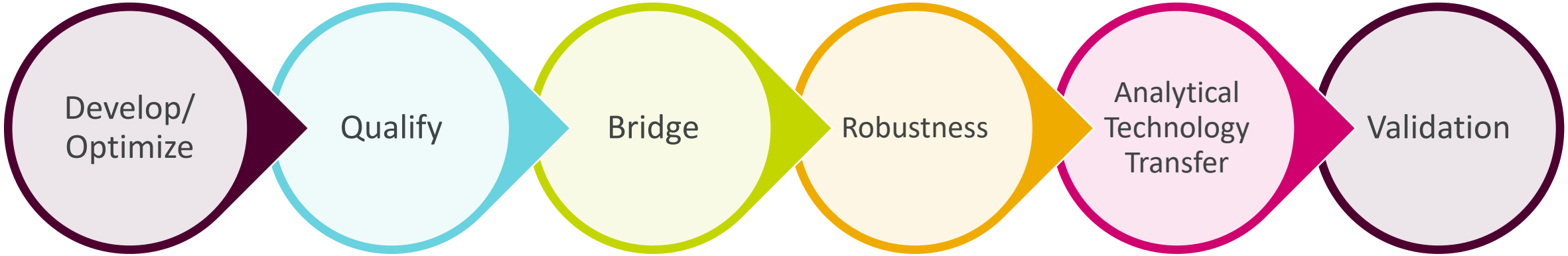
Data needs to be unique to product evaluated

Non-Negotiable:

Must be able to show a loss in potency

Optimizations may occur over time to increase robustness prior to commercial Setting

Process to Replace a Potency Method



- Decide new method format
- Utilize historical project knowledge to aid development
- Optimize method parameters for use in a QC space

- Perform standard method qualification
- Verify method can move forward

- Leverage existing data where possible
- Compares 2 methods to connect historical and future work
- Verify method can move forward

- Complete early enough to allow for method updates prior to ATT
- Verify method can move forward

- Transfer to new and/or existing Quality teams

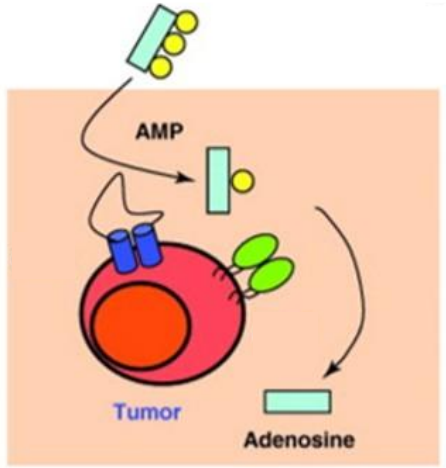
- Completed by Quality Organization

Project background

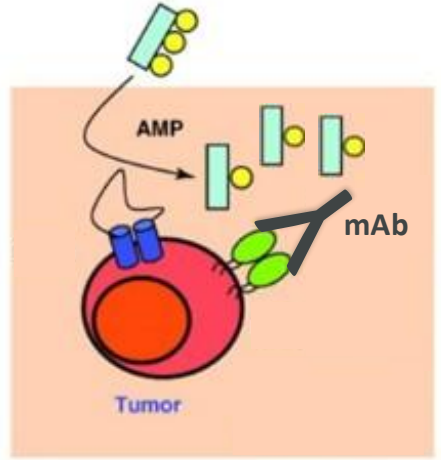
Information

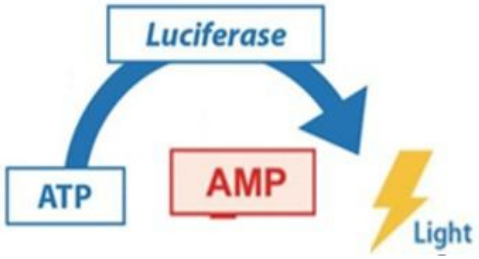
- mAb binding to receptor prevents AMP conversion to adenosine
- Project was progressing to pre-commercial stages
- Used clinical stage experience of method to inform next steps

Potency Method Design

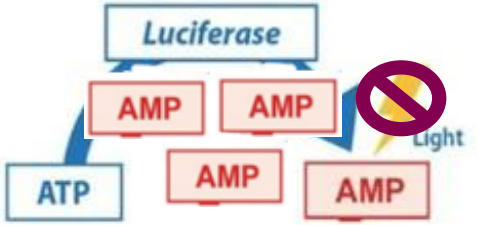


Part 1:
Cell-based MOA in assay plates





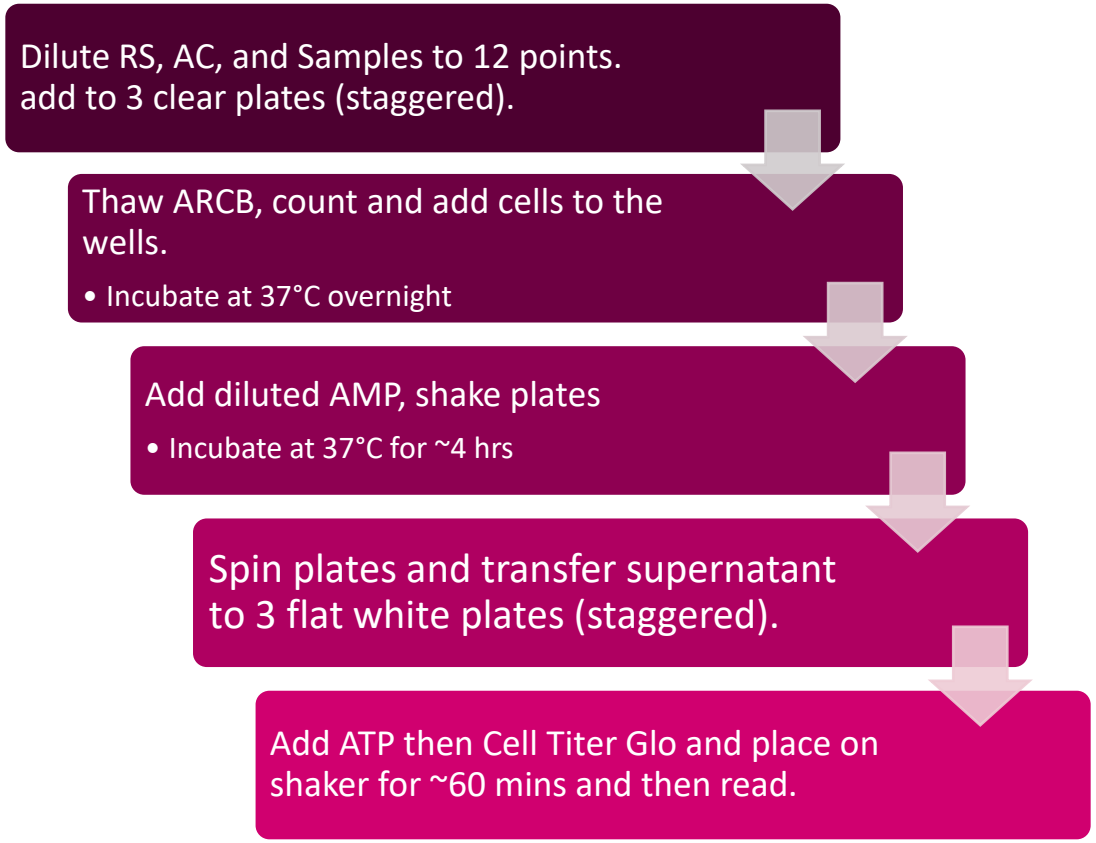
Part 2:
Indirect readout reaction in transferred supernatant



High mAb conc→high AMP→low signal

Why did we need to replace our method?

Current method: Overnight (~24hr duration)

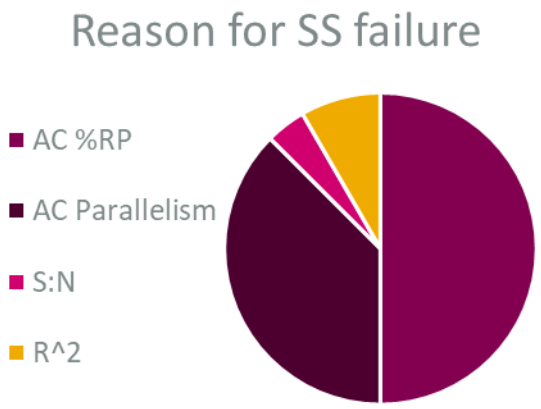


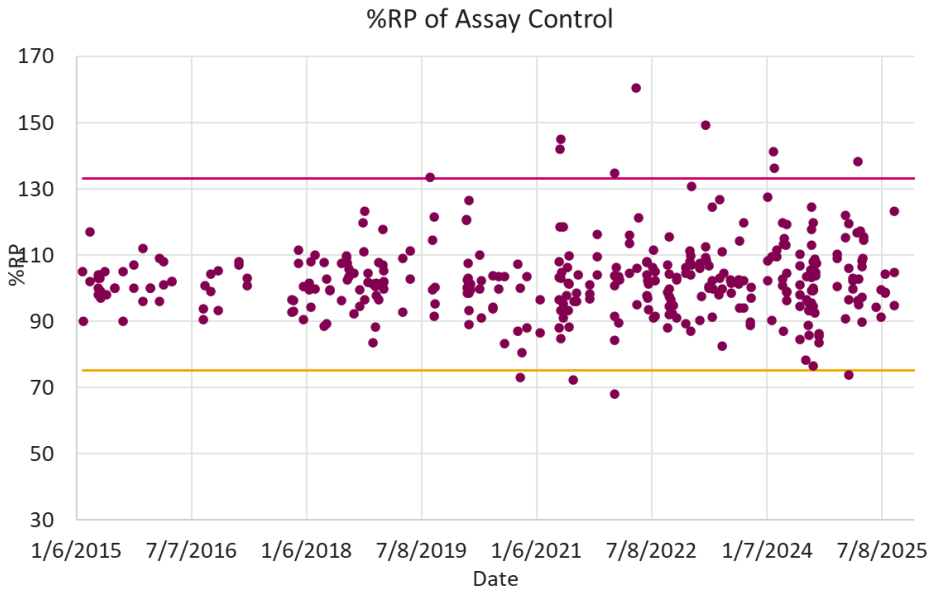
Not suitable for commercial stage

- Evaluation of historical data trend showed a high method failure rate; main reason being high Variability in Assay Control %RP
- TT to commercial site was difficult
- Timings were not analyst friendly

What could be causing the high variability?

- Enzymatic reaction may not need an overnight incubation. Are cells responding inconsistently during this step?
- Thaw and Use (ARCB) cell banks were variable lot to lot. Was the cell line consistently expressing the receptor?
- Were reagent concentrations at their optimal settings for the competitive part 2 of assay?





AC= Assay Control

2

Cell Line Analysis

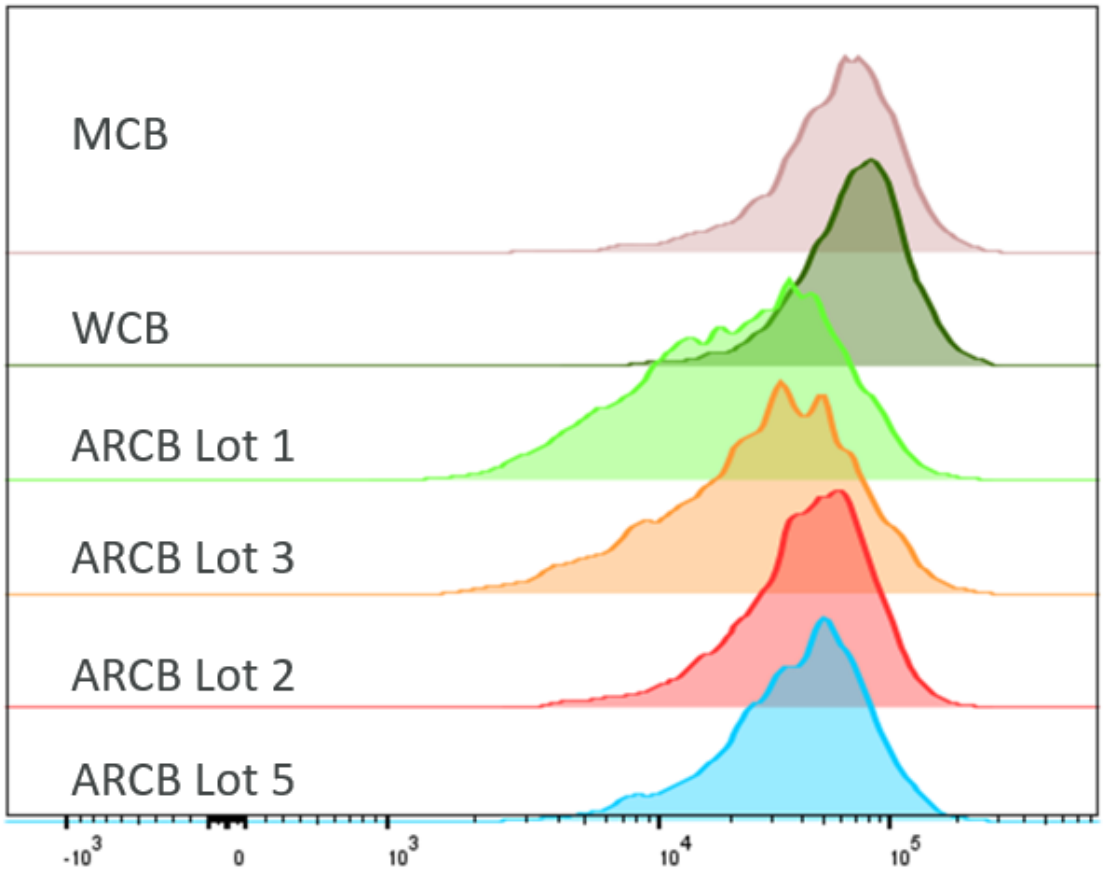
Evaluation of Current Cell Line for Receptor Expression

Not Ideal Cell Line

- Inconsistent expression across lots
- Loss of expression from parental banks
- Negotiation of commercial use license was difficult

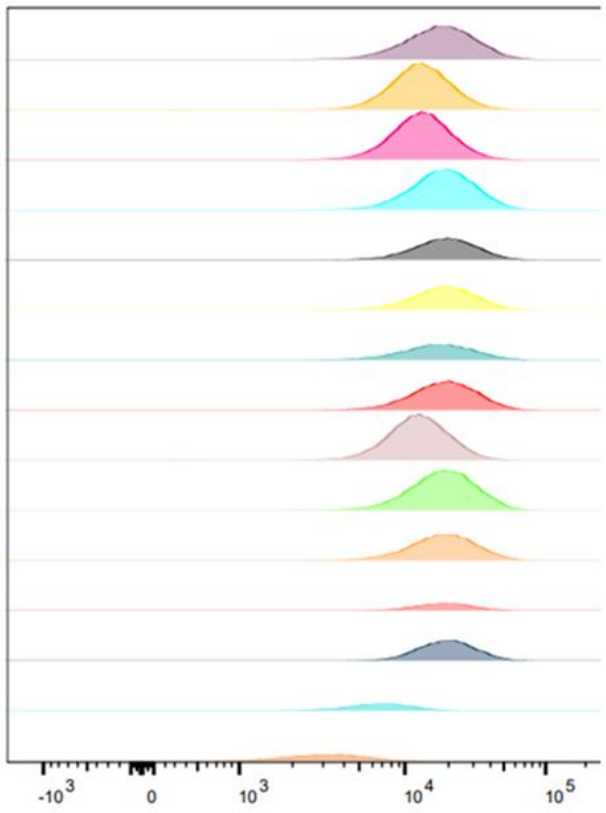
New cell line may improve method

Flow Results



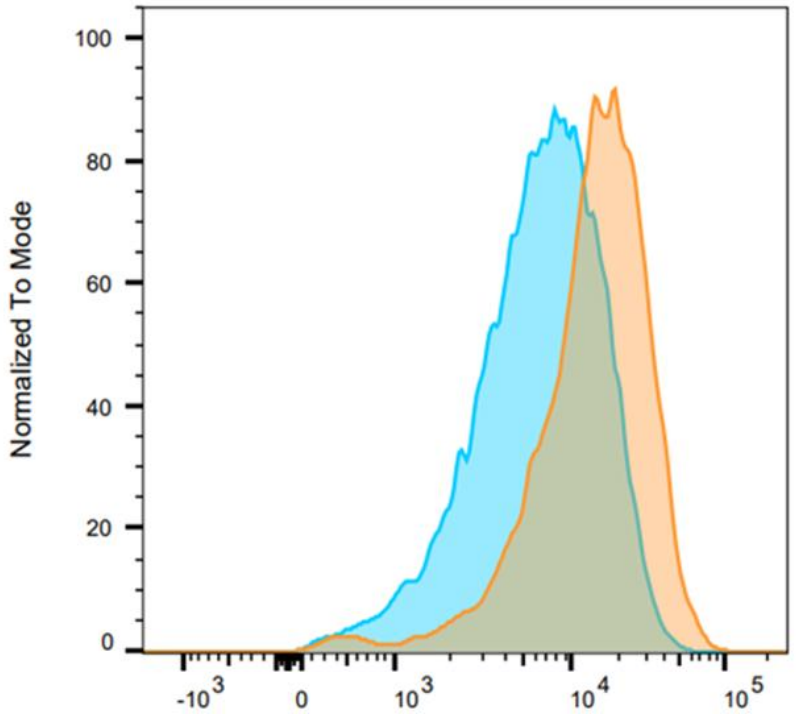
Potential New Cell Line Analysis

Receptor expression was maintained from passage 2 to passage 17.



	Mode : FITC-A	Passage number
	16079	17
	12874	16
	12874	15
	19392	14
	19392	13
	18676	12
	14926	11
	20137	10
	12410	9
	17988	8
	20911	7
	17988	6
	21717	5
	7496	4
	3678	2

Consistent expression through bank lineage (similar peak width/height)



	Bank type	Mode : FITC-A
	newARCB	17988
	WCB	8953

3

Strategic Method Re-development

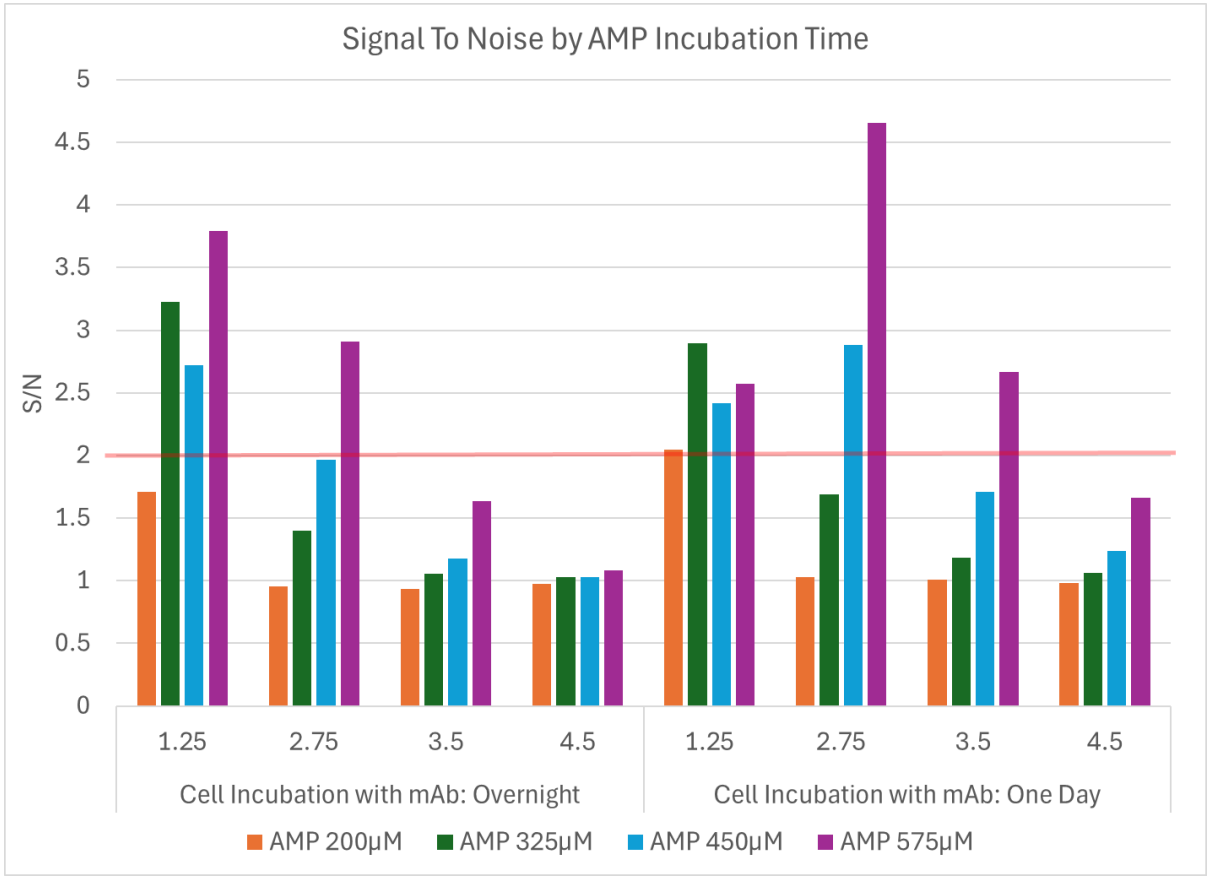
Key Driver of Signal:Noise = AMP Conc and Incubation Time

Assay Notes

- Used high mAb or no mAb to mimic max signal and lower asymptote
- Also tested cells+mAb dilutions overnight or 30mins

One day, AMP 450 µM for 2.75hrs selected for POC studies

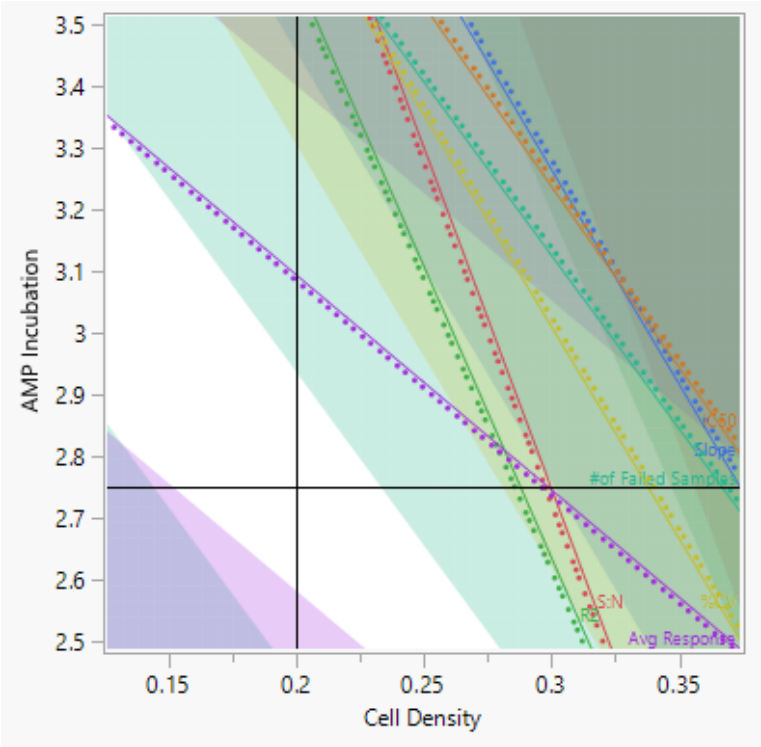
Minimum 2.0 is standard criteria

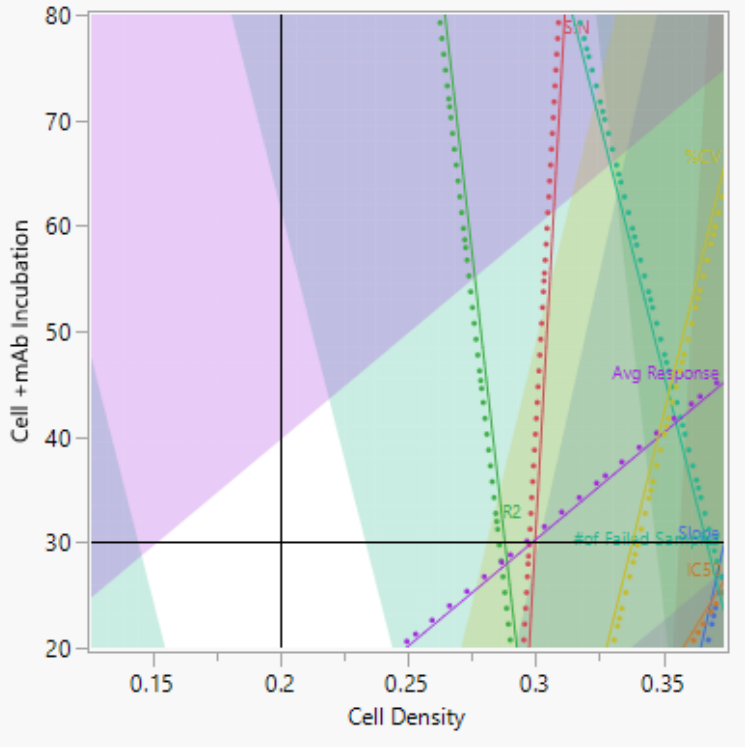


Defining Design Space Limits

- Screen possible #cells/well; AMP incubation times and cell&mAb incubation times
- Data demonstrates that cell density was the most critical aspect of method design
- Optimal operational ranges had to be determined

Source	Logworth	PValue
Cell Density	2.449	0.00356
AMP Incubation	1.042	0.09076
Cell +mAb Incubation	0.728	0.18701



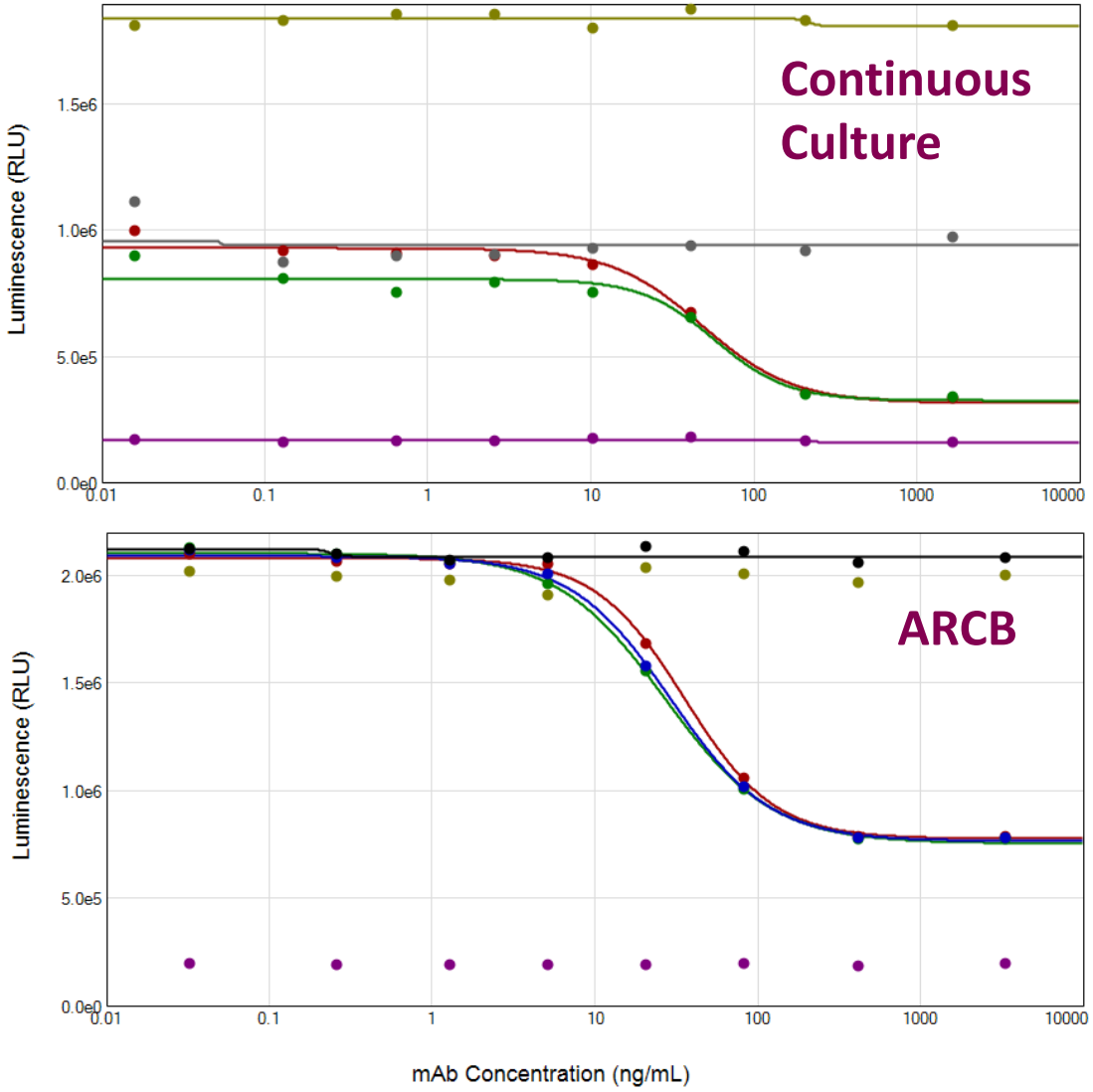


Determination of Design Space

Application to Cell Preparation

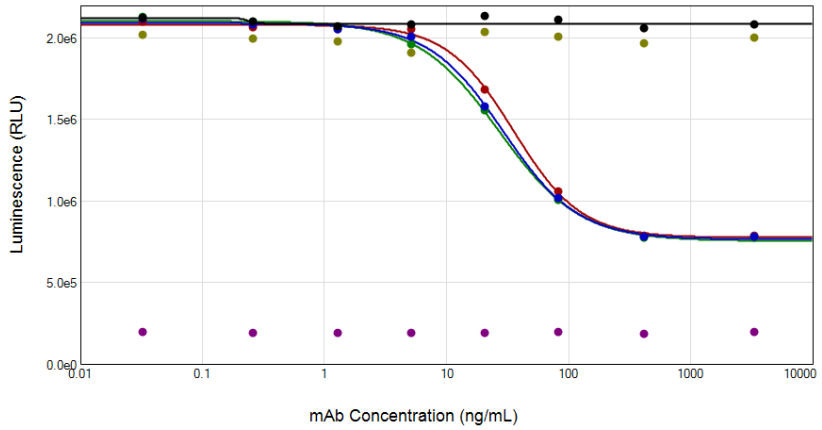
- **Olive**: ATP and CellTiter-Glo Only (max possible)
- **Purple**: AMP/ATP and CellTiter-Glo (background)
- **Gray**: Cells, AMP/ATP and CellTiter-Glo (upper asymptote possible)

Result: ARCB gives upper asymptote near max possible

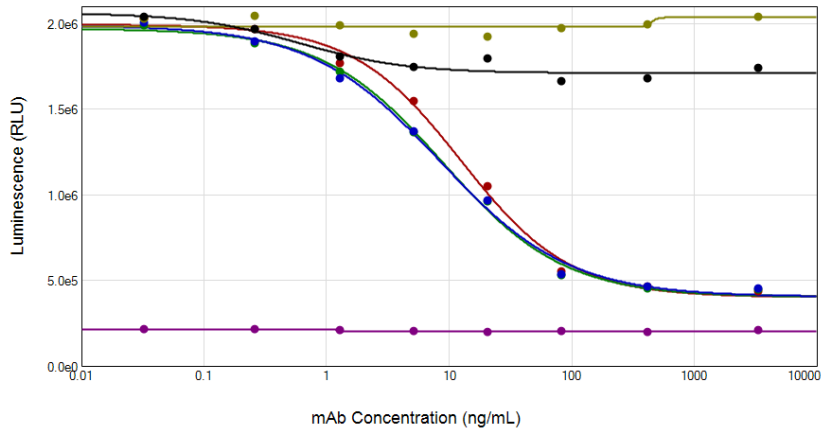


Design Space to Determine #cells/well

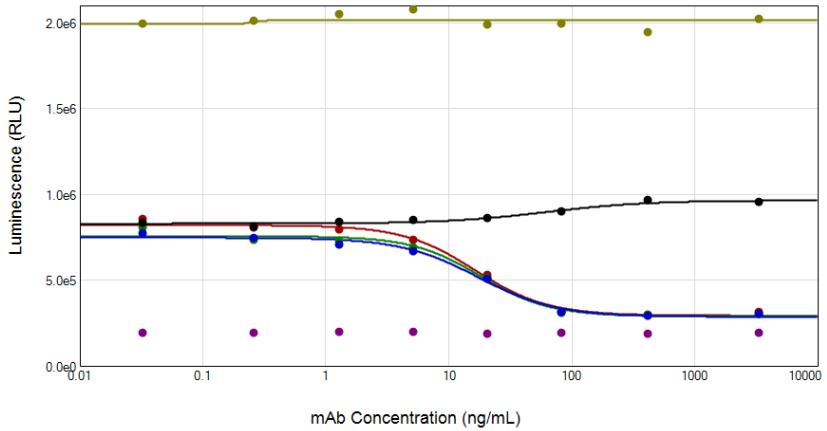
15k/well



10k/well



5k/well



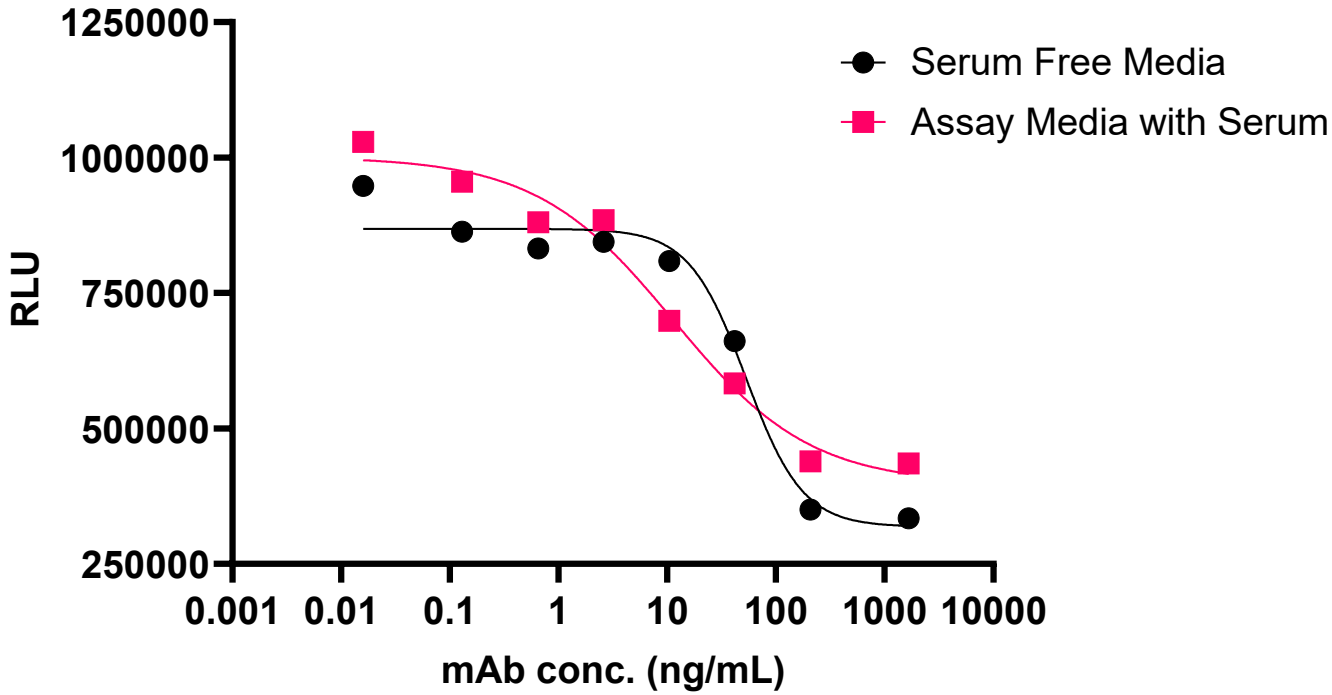
Cells/well	Signal:Noise	Note
15k	2.7	High Background
10k	5.0	Good Span!
5k	2.8	Not enough response

Cells are the most important factor.

Serum could interfere with Enzymatic Reaction

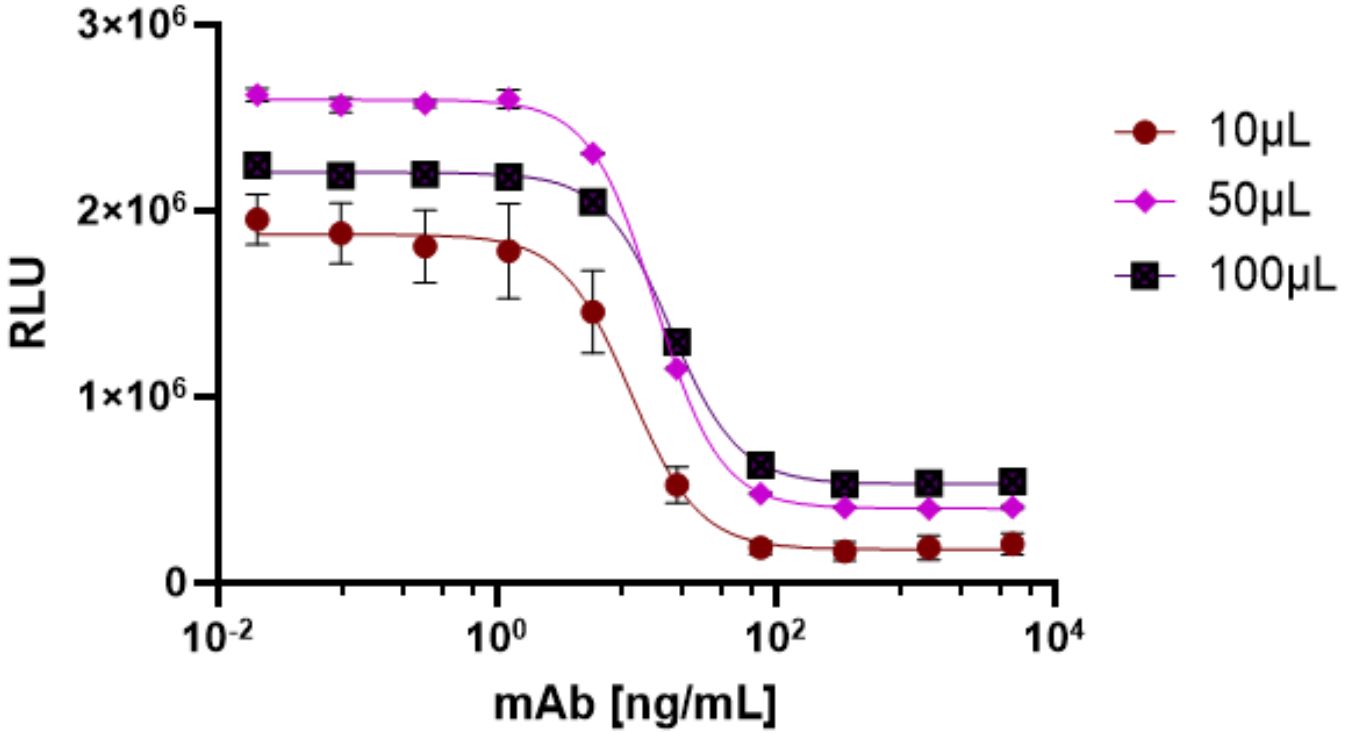
Removal of Serum results in:

- Higher slope
- Increased asymptote stability
- Impacted IC_{50}
- Easier transfers to other sites!



Altering CellTiter-Glo volumes affects Signal:Noise

- CellTiter-Glo reaction is driven by ATP interaction with Luciferin (which is inhibited by AMP)
- Altering ratio of AMP&ATP mixture to luciferin impacts outcome
- 50µl (half of previous volume) provided a steady upper asymptote with increased Signal:Noise



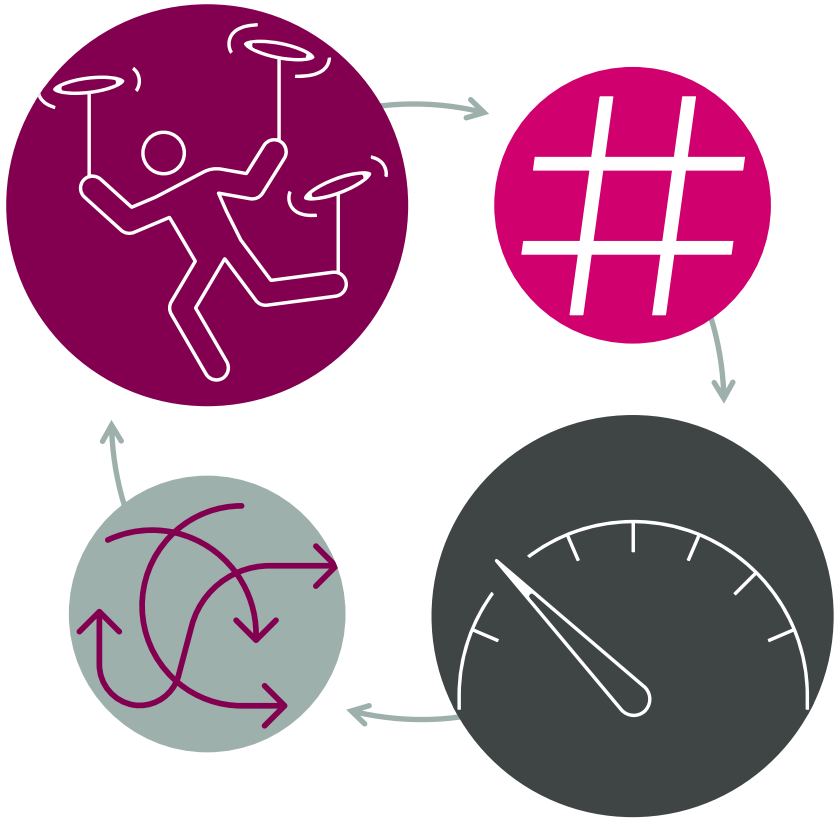
Unique Consideration for Enzymatic Assay: Plating Timings

# of plates per assay

Assay designs with multiple plates will take longer per addition step

Orientation of each addition

Will addition by mAb dilution point or by unique sample impact results?



# of additions

Each reagent addition will have numerous pipetting steps

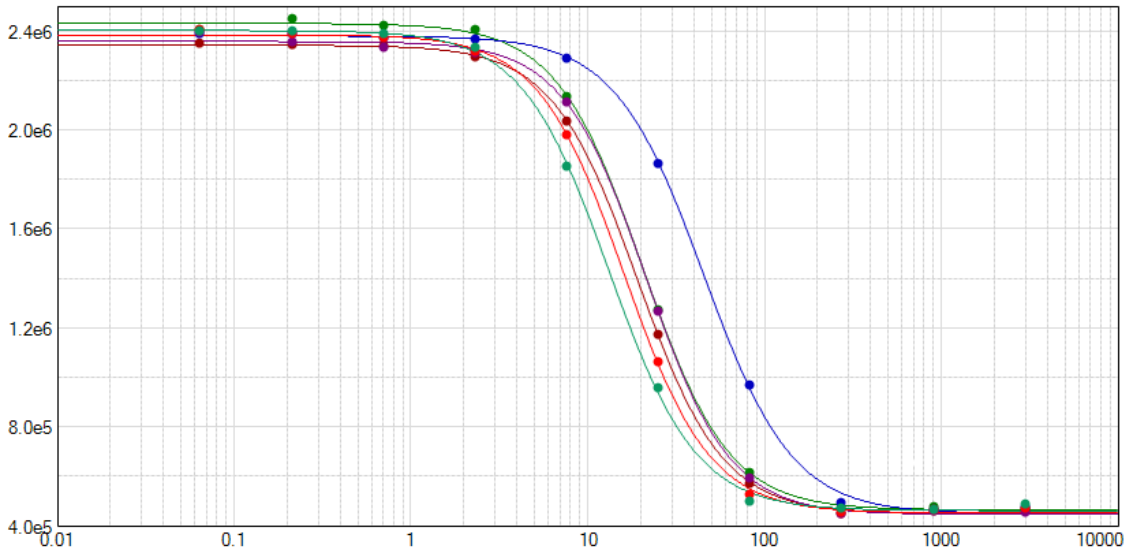
Reloading of manual or electronic pipette can be a factor

Speed of analysts

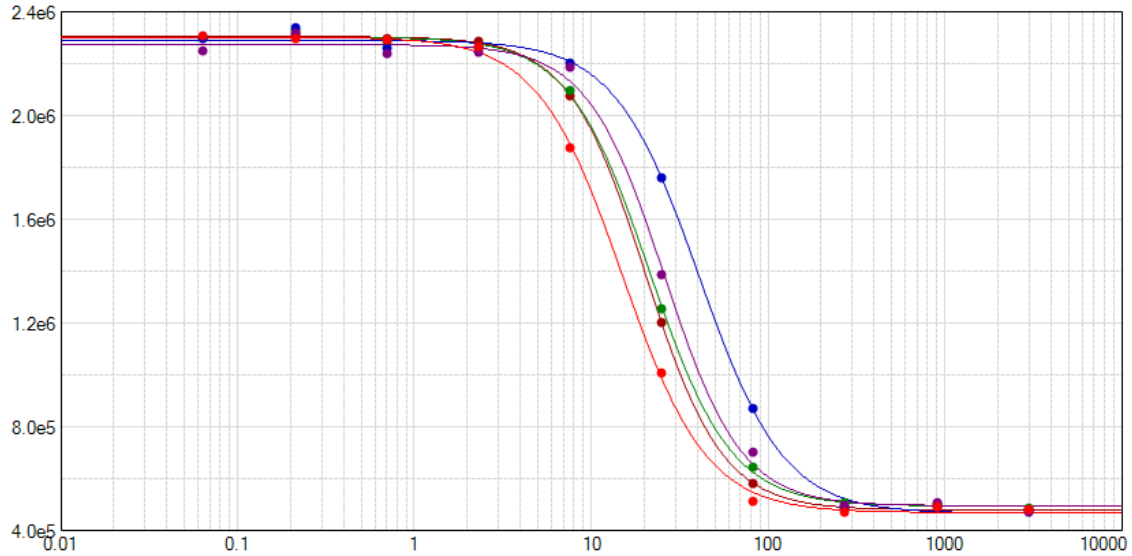
Variations in timings will exists between different analysts

Reagent Addition by Dilution Point Improves Assay

Additions by Sample



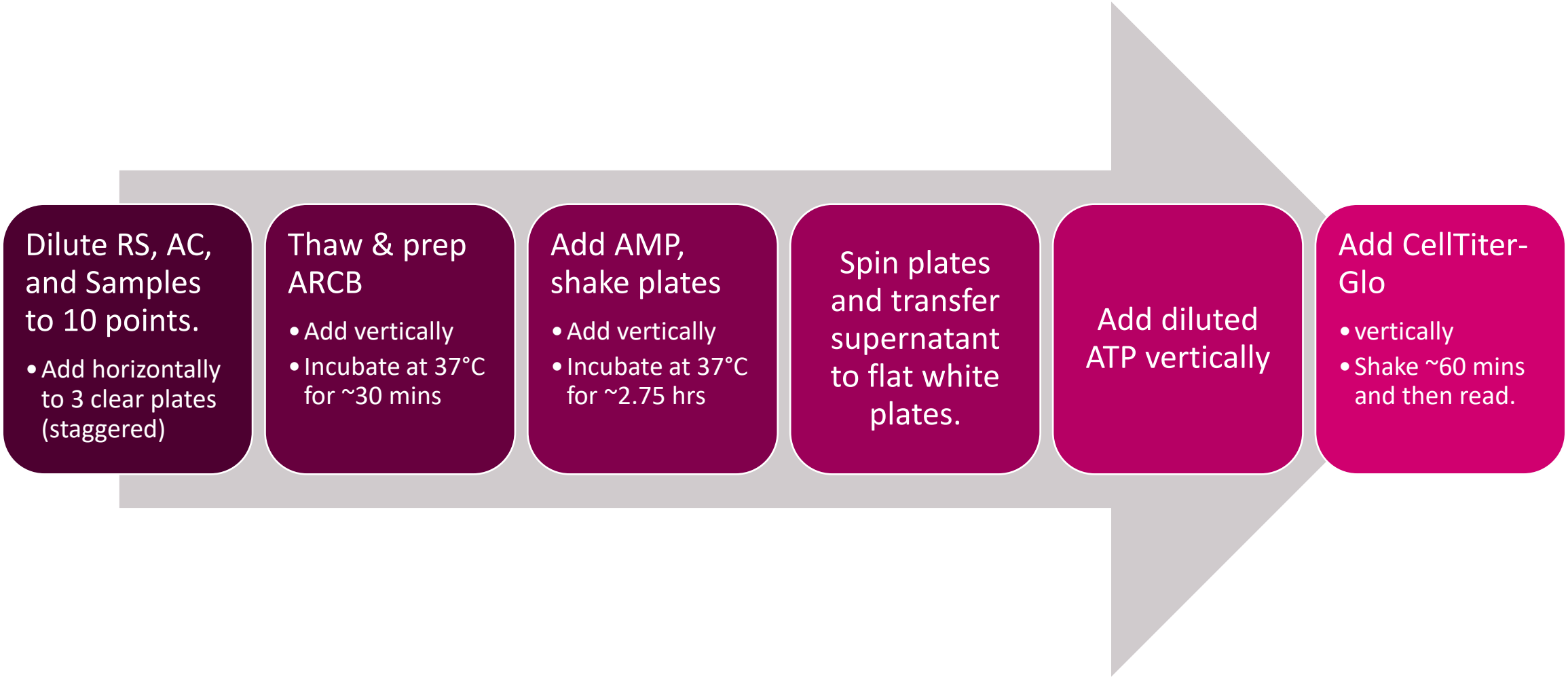
Additions by Dilution Point

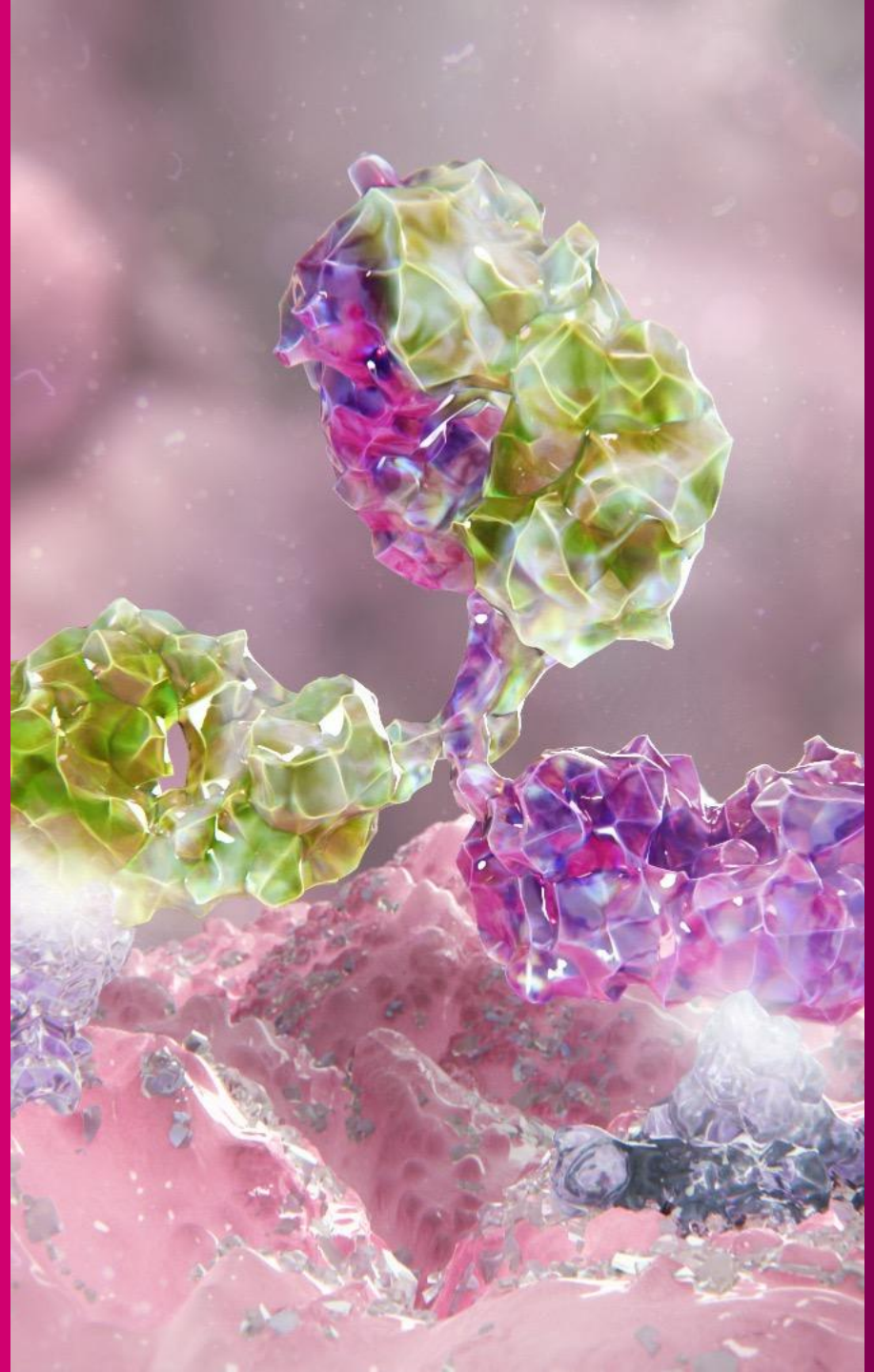


Upper Asymptote consistency achieved by change to reagent addition orientation

Final New Method (~6hr total assay time)

Went from 2 day→1 day





4

Outcome

Completed New Method Development

Existing method not suitable for commercial testing. New method needed with improved accuracy, robustness and ease of use.

New method development completed, converting ~24hr assay to ~6hr assay with fewer runs per reportable result required due to new method having improved accuracy, robustness and lower system suitability failure rate.

Problem	Solution
Assay Length was unnecessarily long for enzymatic MoA	Shortened assay procedure to ~6hr total
Enzyme expression in cell line 1 was inconsistent	Switched to cell line 2 with consistent target expression
Reagent concentrations/incubation times were not optimal	Used DoE results to ensure optimal conc. and incubation pairing
Serum in assay media may interfere with reaction	Successfully switched to serum free assay media
Signal to Noise Ratio was consistently low	Altered ratio of CellTiter-Glo to Supernatant/ATP on the plate
Variability in results between runs in upper asymptote consistency and EC50 values	Switched to plating all reagents perpendicular to the mAb dilutions

Our new method introduction

Method Development

Utilized history for strategic/streamlined development

3 months for full new method development/optimization

Method Qualification

Plan, Assays and Report required.

Assays completed in 6 business days by 2 analysts!

Robustness

Plan, Assays and Report required.

Assays completed in 2.5 weeks using our DoE approach.

Validation occurred during DS PPQ runs

Formally asked to re-develop method

New Cell Line Cell Banking

Banked MCB through ARCB. MCB→WCB→ARCB

Full lineage in 8 weeks!

Bridging

Plan, Assays and Report required.

Utilized hybrid approach of multi-purpose linearity samples (qualification/bridging)

Assays completed in 2.5 weeks

ATT

4 Operations Analysts trained over 4 weeks and trained Commercial site Analyst in their building.

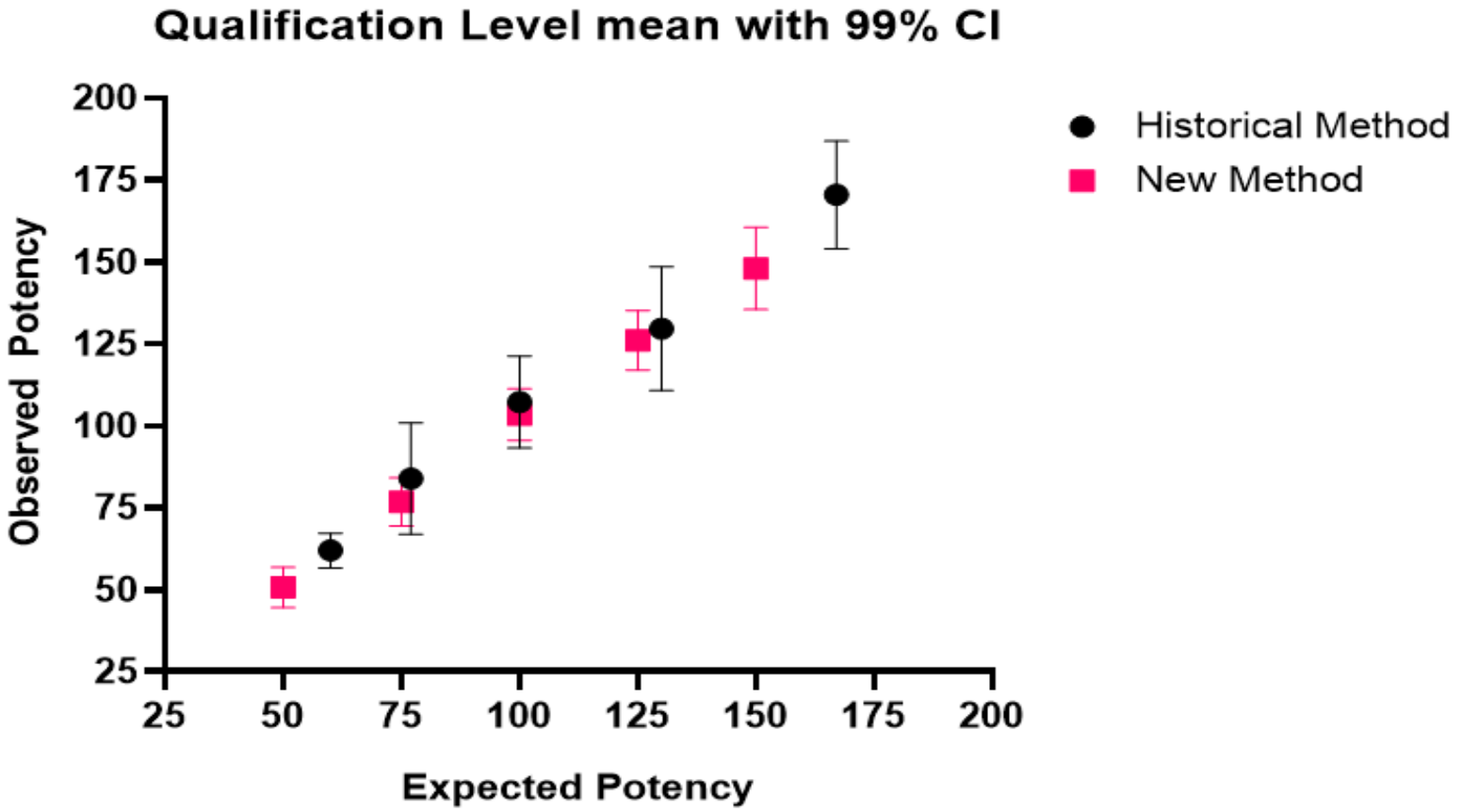
ATT successfully completed, <6 months from method re-development start

~6 months start to finish!!

Very Accurate

Overall Accuracy
Historical: 104.3% ± 8.7%
New: 101.4% ± 5.4%

Reduced #of runs per result



Historical method qualified with a geometric scale, new method with a linear scale

Method is more Robust

DoE analysis done in JMP for both methods

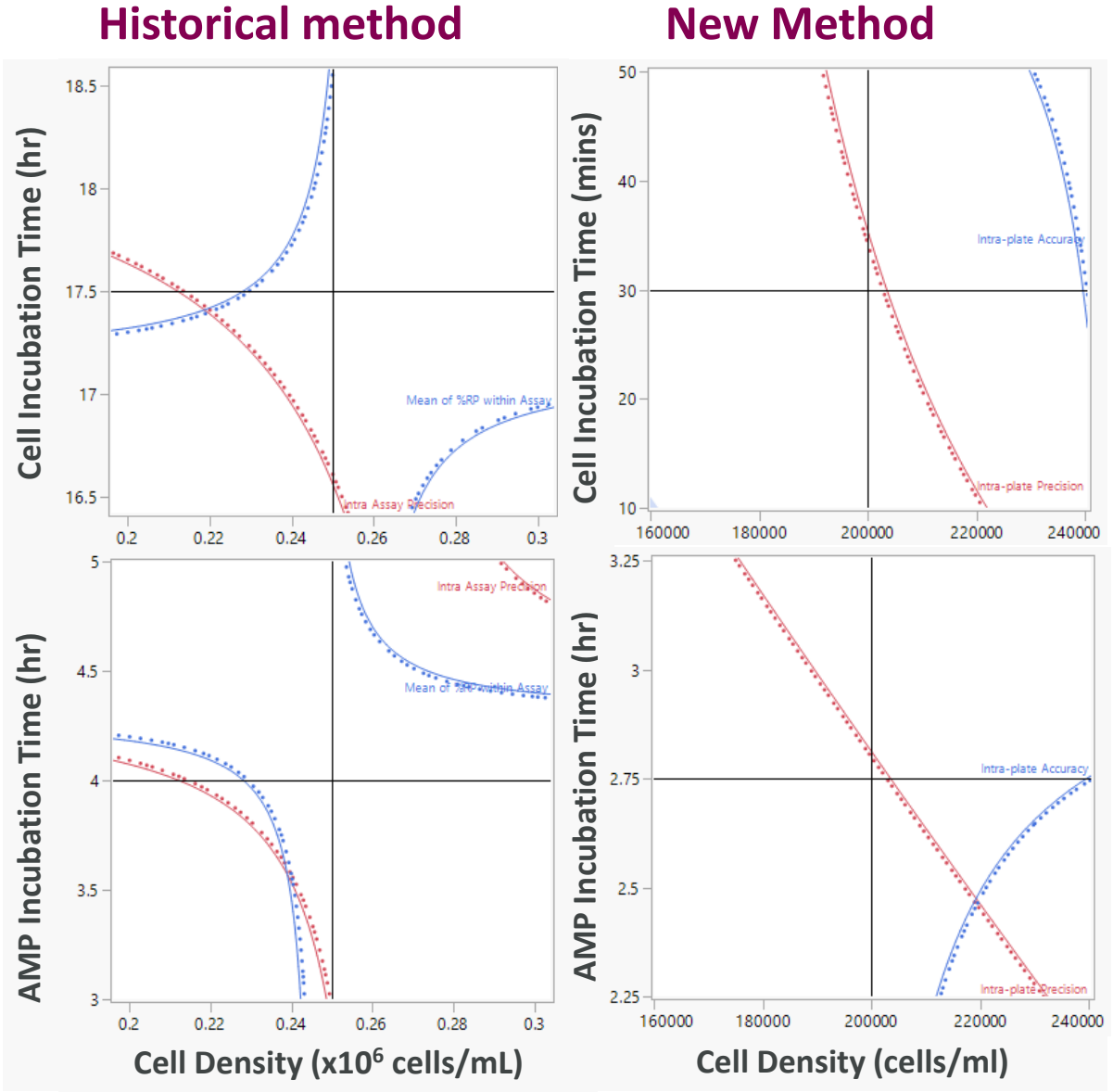
Parameter	Prob. For Intra-assay Precision (%CV)		Prob. For Accuracy (%RP)	
	Historical Method	New Method	Historical Method	New Method
Cell Density	0.4064	0.3325	0.5536	0.3908
Cell/mAb Incubation Time	0.5001	0.7877	0.8403	0.6696
AMP Incubation Time	0.5808	0.4804	0.8878	0.7800
AMP Conc.	0.8030	NT	0.5891	NT
Cell Density*Cell Incub. time	0.4019	0.8384	0.1192	0.7408
Cell Density*AMP Incub time	0.0451^	0.9828	0.0694	0.2817
Cell Density*AMP Conc.	0.2703	NT	0.3207	NT
Cell Incub*AMP Incub	0.0226^	0.3129	0.5623	0.6996
Cell Incub*AMP Conc	0.1205	NT	0.7832	NT
AMP Incub*AMP Conc	0.2398	NT	0.7175	NT

Went from 2 significant interaction effects to no significant factors/interactions!

^ statistically significant, NT: Not tested

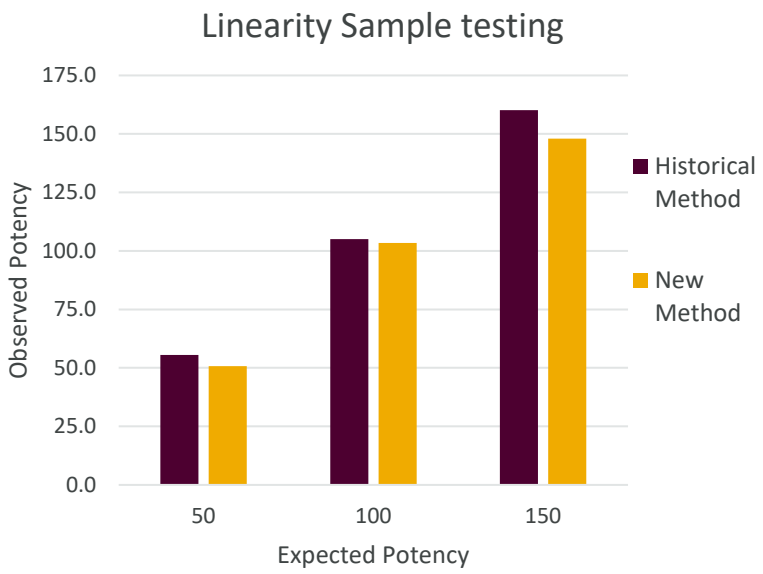
Method is Robust

- Both methods had plenty of white “robust” space.
- Improved robustness with no significant interaction effects.

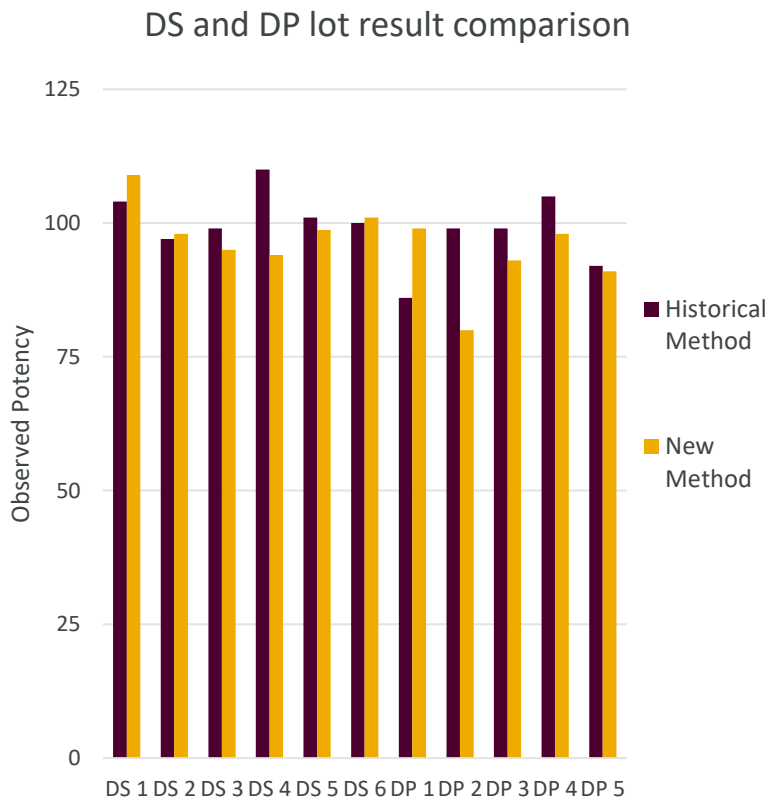


Successful Bridging

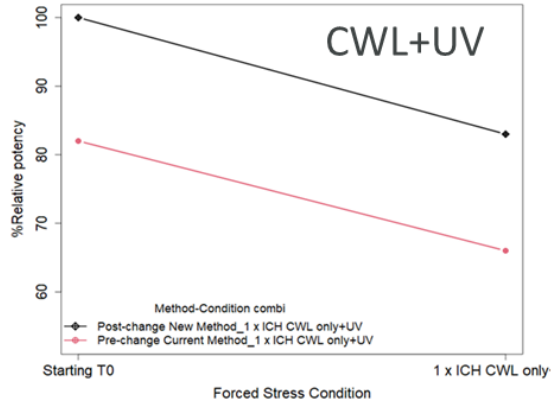
Same samples tested

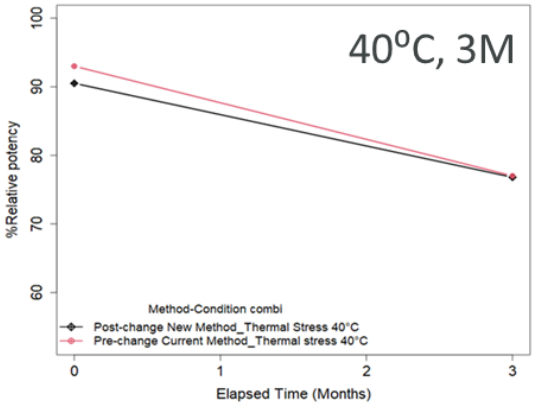


Historical data compared to retains tested in new method



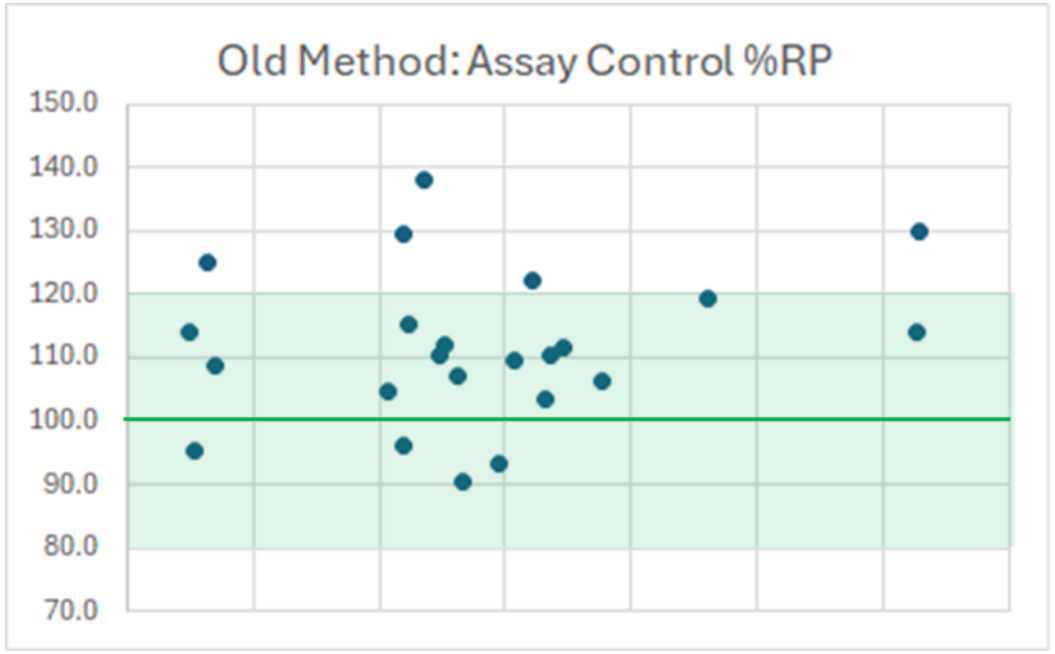
Similar Stability Profiles



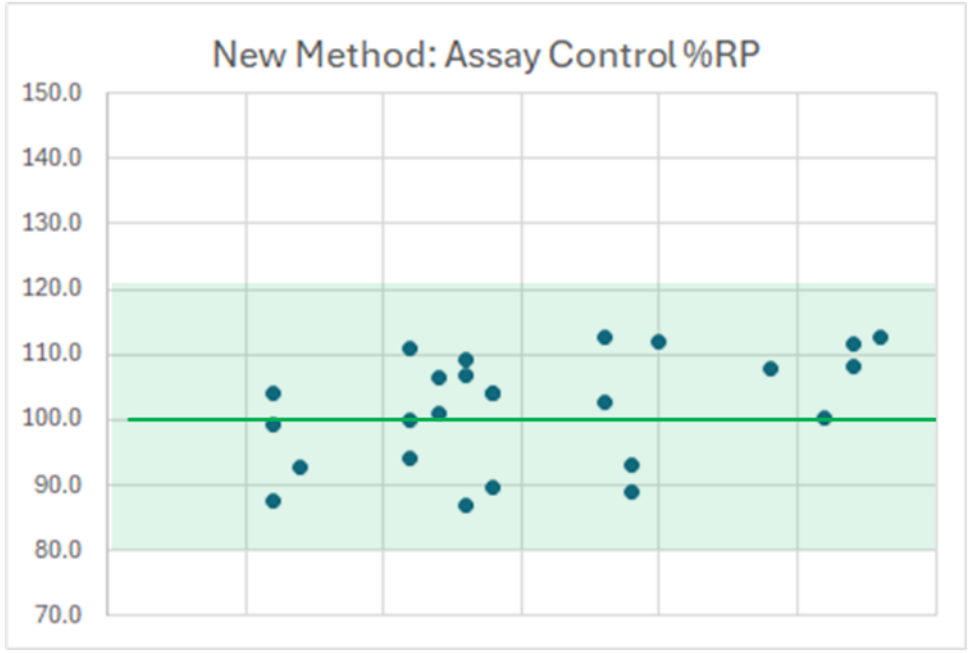


Comparison of 25 training runs at the commercial site of old vs new method

- New method has less failures and better consistency



112 ± 12% RP



102 ± 8% RP

Lessons to be Learned

Method Design

- Carefully evaluate how method design reflects project MoA
- Are there design elements that make it inherently more variable? Less QC friendly?

Trend Data

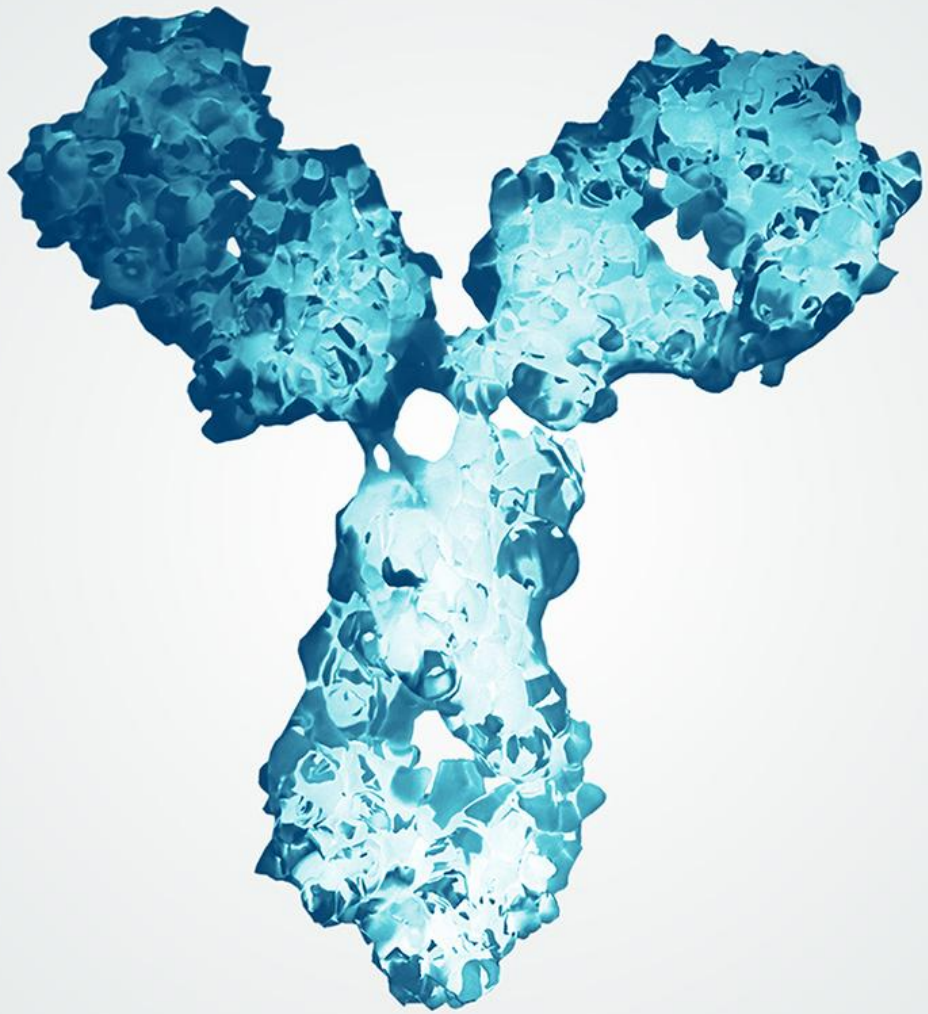
- Review trend data regularly and update methods as necessary

Robustness Study

- Complete early enough to allow time to act upon factors found to be significant

Acknowledgements

- Weimin Chen
- Felix Feng
- Sreedevi Danturi
- LeeAnn Machiesky
- Scott Umlauf
- Varna Kishore
- Liz Christian



Thank you.

AstraZeneca PLC, 1 Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge, CB2 0AA, UK
+44(0)203 749 5000
www.astrazeneca.com