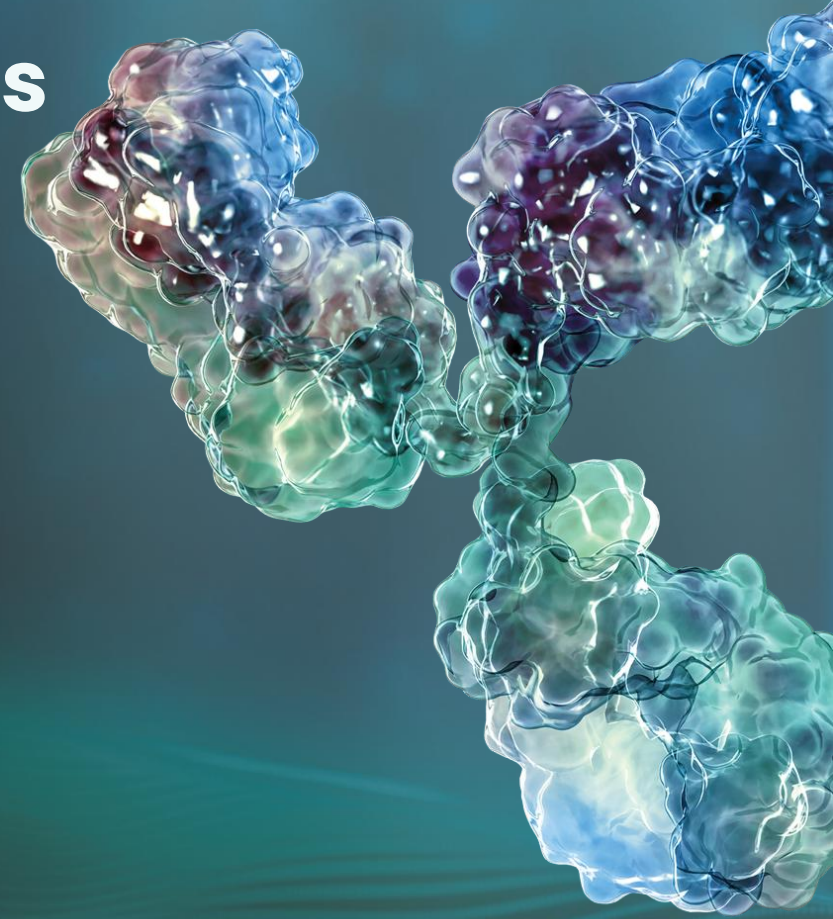


# Navigating Critical Reagent Challenges in Cell-Based Potency Assays: Case Studies on Variability and Solutions



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# Presentation Overview

**Challenges of Cell-Based Assays**

**Critical Reagents**

**Case Study 1: mRNA Flow Cytometry Assay**

**Case Study 2: Fusion Protein Assay**

**Concerns, Mitigations & Lessons Learned**

# Challenges in Cell-Based Bioassays

## BIOLOGY

- Cell Line Integrity
- Data Variability
- Reagent Inconsistency

## ASSAY & EXECUTION

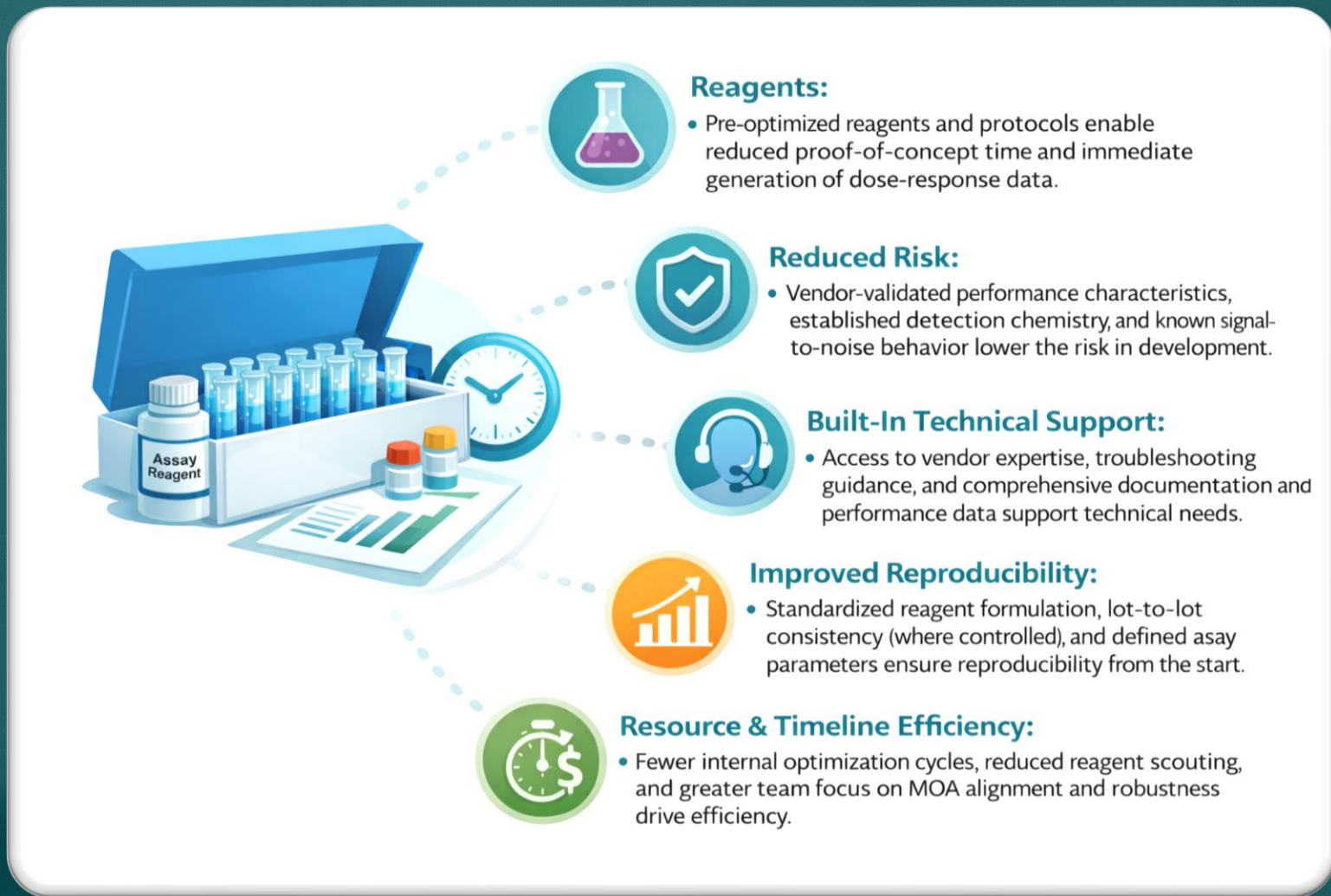
- Assay Design Gaps
- Tech Transfer Failures
- Infrastructure Limits

## STRATEGY & REALITY

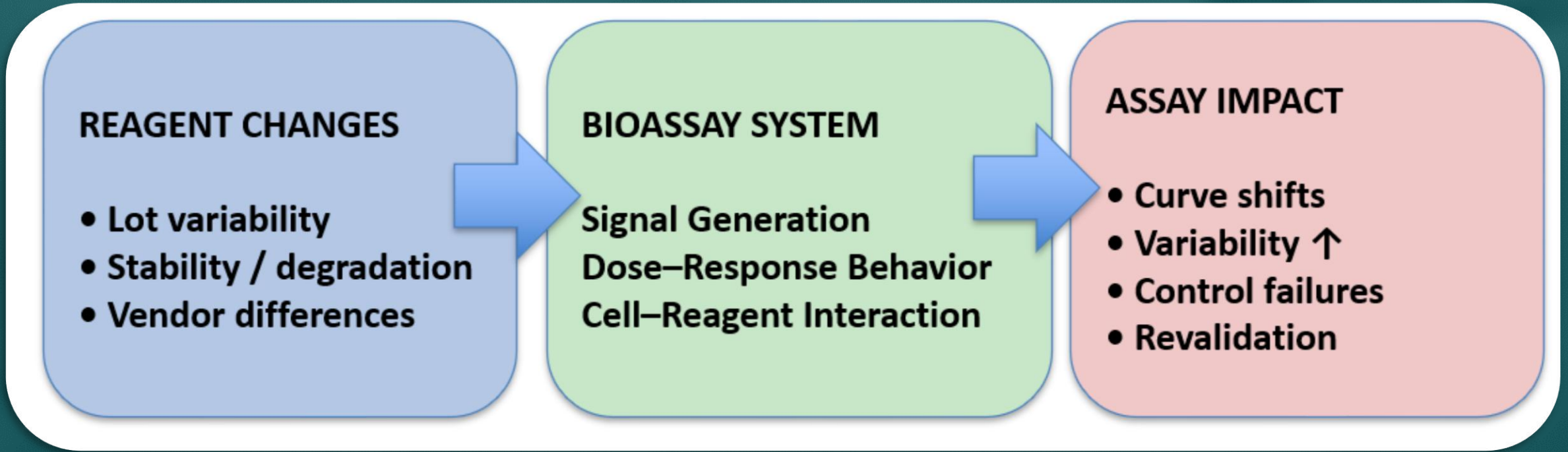
- Resource Constraints
- Incorrect assay format
- Regulatory Gaps

**Timeline Expectations!**

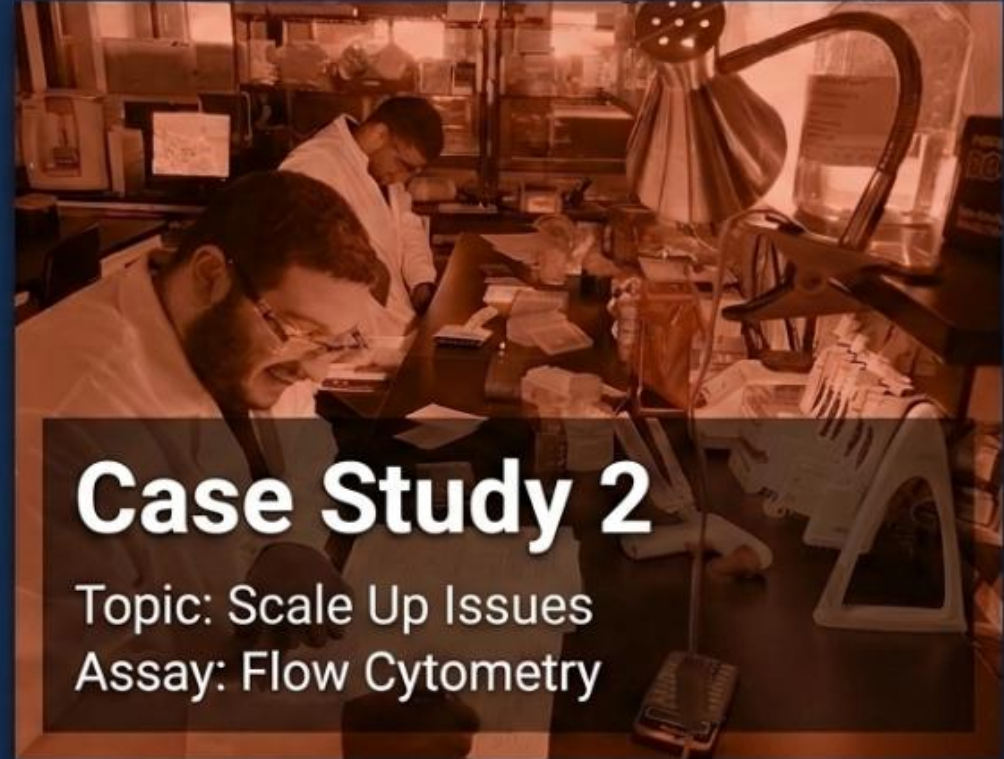
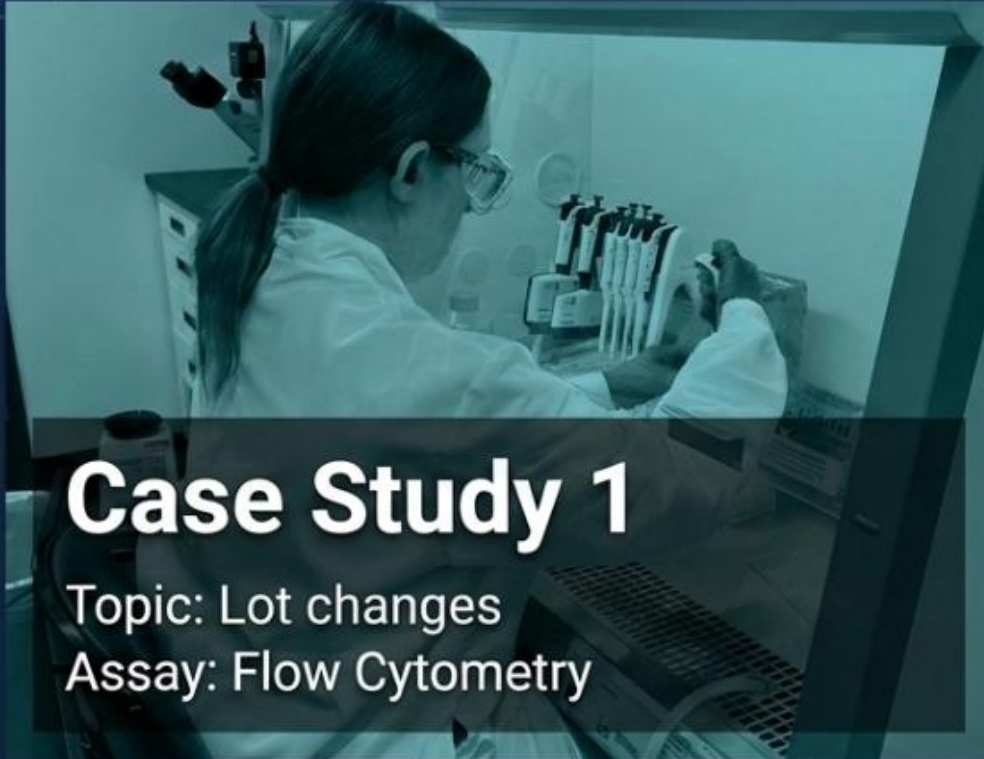
# Why This Topic Matters for Today's Biologics



# Critical Reagents:



# Unexpected Challenges: Case Studies



# Case Study 1:



## Background

- Flow cytometry-based potency assay for a biologic therapeutic
- Multiple reference standard lot changes across development, validation, and routine testing
- Phase 1

## Problem

- Each lot transition introduced  $\pm 15\text{--}25\%$  shifts in reported potency values
- Assay control recoveries consistently trended 110–130%
- Qualification runs showed assay drift

## Impact

- Regulatory submissions complicated by inconsistent trending data
- Re-validation required after 3 of 5 lot transitions

# Lot Changes : Assay Drift

## Development Data

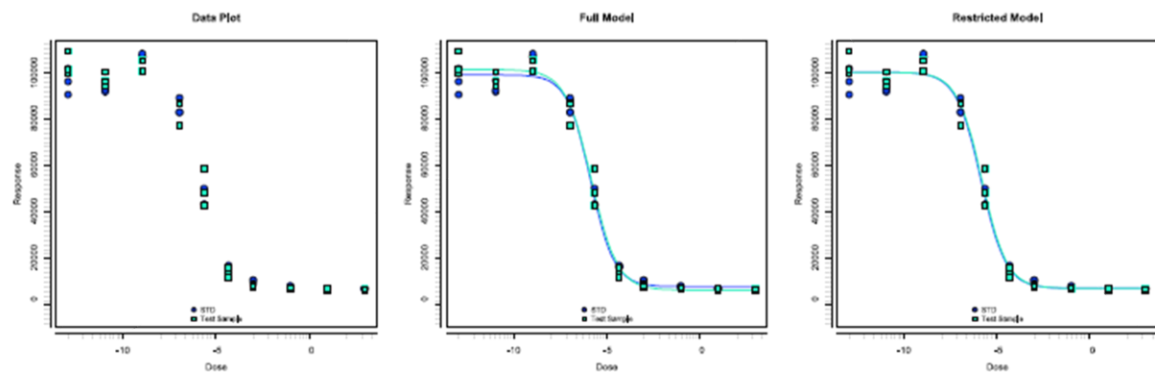
### Potency estimation

Relative potency	Test Sample	STD
Potency ratio	0.96409	
95% Confidence interval	0.87977 - 1.05650	
Relative confidence interval	91.25% - 109.58% (18.33%)	

EC 50		
Estimated EC50 value	0.01771	0.01725

### Graphics



## Pre-Validation Data

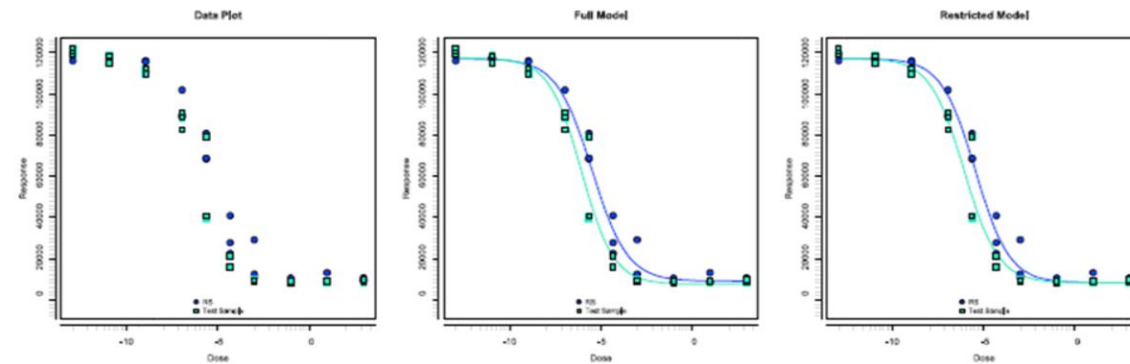
### Potency estimation

Relative potency	Test Sample	RS
Potency ratio	1.53230	
95% Confidence interval	1.26720 - 1.85287	
Relative confidence interval	82.70% - 120.92% (38.22%)	

EC 50		
Estimated EC50 value	0.01502	0.02236

### Graphics



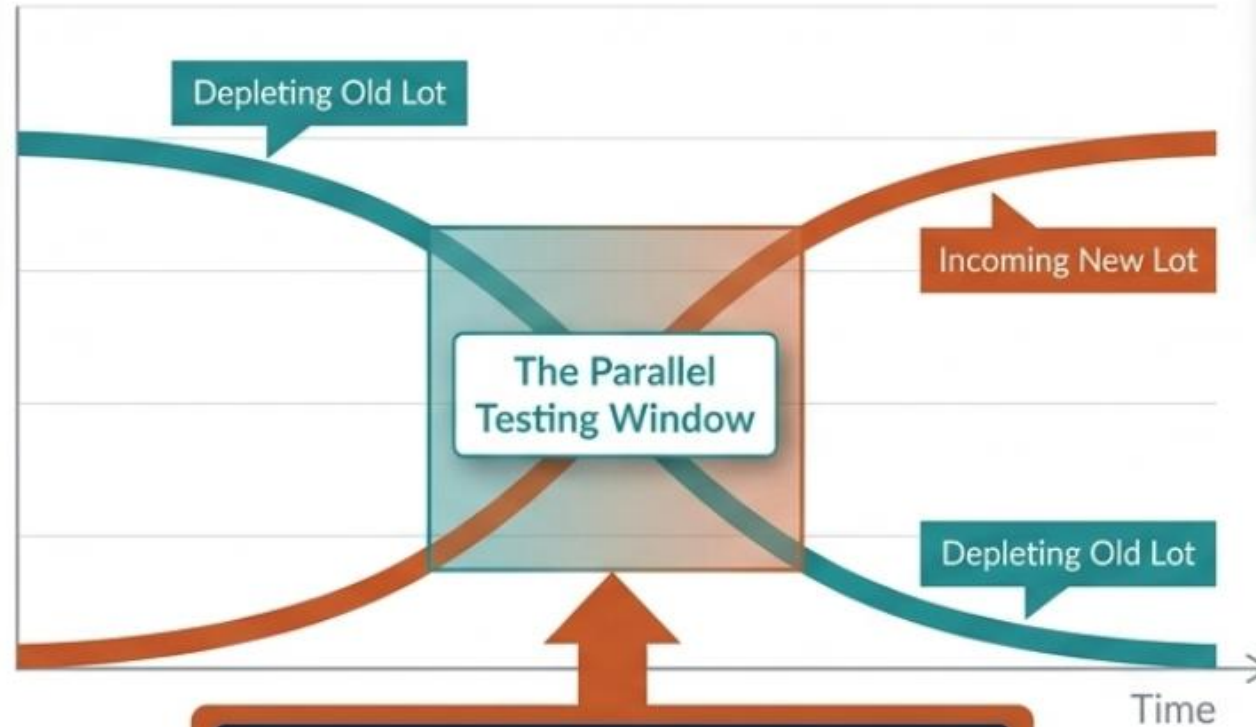
# The Mitigation: Proactive Reagent Bridging

## Identify Early

Assess needs upfront and manufacture a large batch of critical reagents to extend the timeline between transitions.

## Lifecycle Management

Perform regular, routine evaluation of reference materials.



## Bridge Proactively

Conduct parallel testing and stability monitoring of new and old lots simultaneously to evaluate variability before the hard transition.

# Case Study 2:

- **Therapeutic Type:** Fusion Protein
- **Assay Mode:** Flow Cytometry Potency Assay

**The Catalyst:** The assay relied on a highly specific in-house critical reagent (engineered cells).

**The Pivot:** As the project advanced, the required volume of reagent exceeded in-house capacity, mandating an immediate shift to external outsourcing.



# Overcoming Reagent Capacity Limits in Potency Assays

## Problems

- The assay relied on a highly specific in-house critical reagent (engineered cells).
- As the project advanced, the reagent had to be “commercially available”, threatening assay continuity.

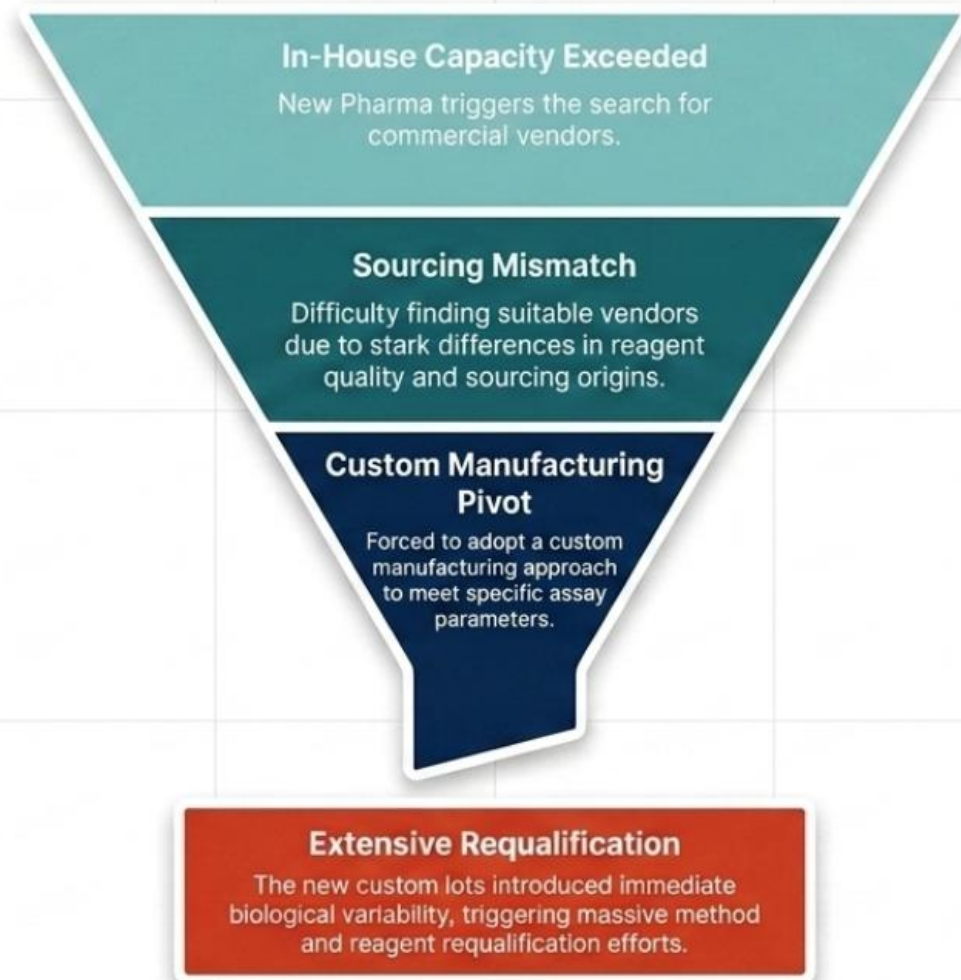
## Solutions

- To address the shortfall, the team executed an immediate shift to external outsourcing for reagent production, ensuring uninterrupted assay operations and project timelines.

## Impact

- Maintained uninterrupted flow cytometry potency assay operations
- Enabled timely data delivery for the fusion protein project
- Mitigated risks of project delays due to reagent shortages
- Established a scalable model for future reagent needs

# The Hidden Bottlenecks of Reagent Outsourcing

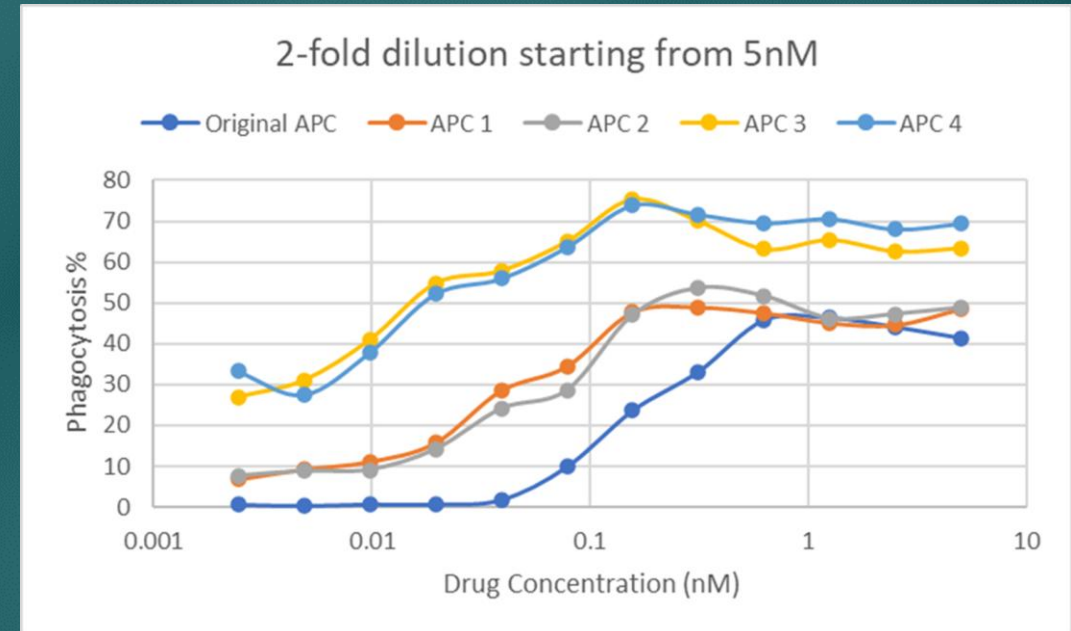
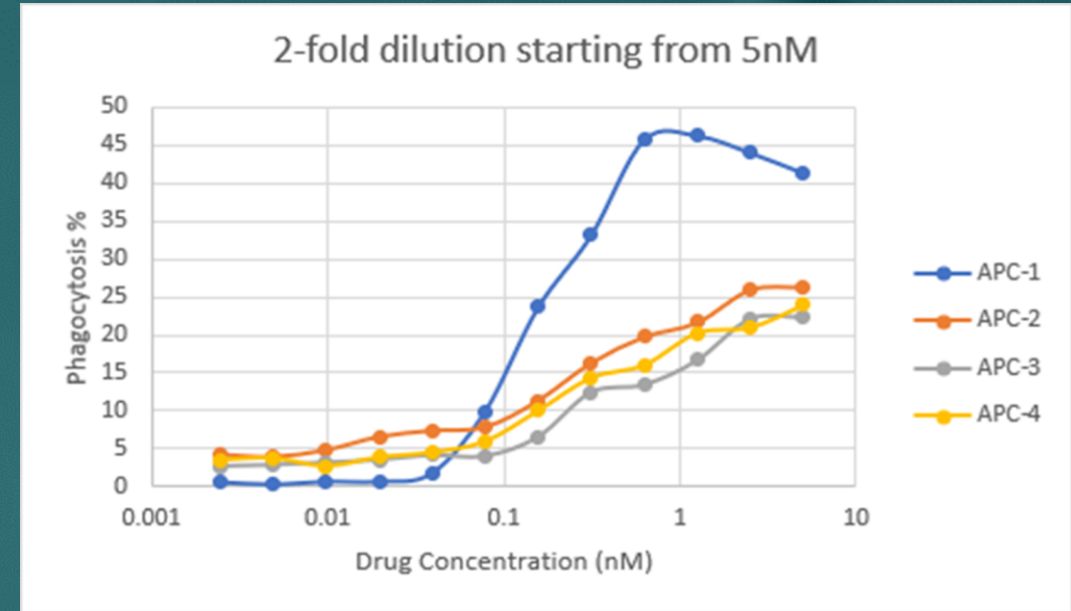
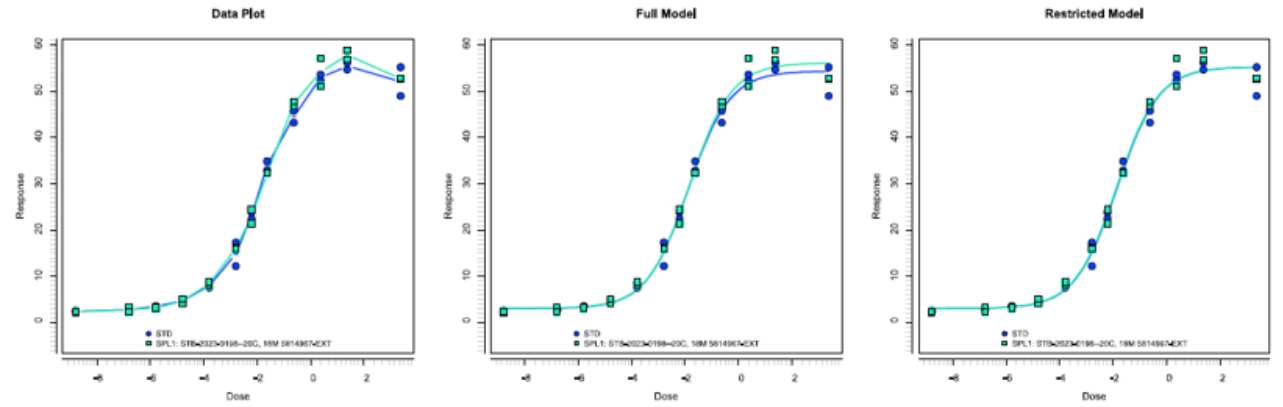


# Raw Data Comparison

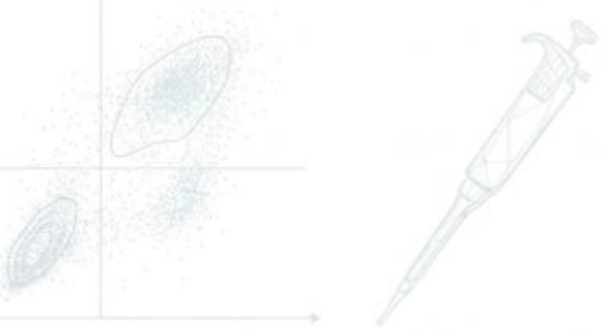
## Potency estimation

Relative Potency	Test Sample	Ref Std
Potency ratio	1.01849	
95% Confidence interval	0.93437 - 1.11018	
Relative confidence interval	91.74% - 109.00% (17.26%)	
EC 50		
Estimated EC50 value	0.27857	0.26975

## Graphics



# The Critical Reagent Lifecycle Management Playbook




**Monitor Continuously.**  
Treat reference materials as living components. Implement routine evaluation as a strict pillar of continuous method lifecycle management.

Monitor Continuously

Rigorous  
Governance  
=  
Timeline  
Immunity

Assess Early



Identify critical reagents during initial design. Project lifecycle volume needs immediately and manufacture/source the largest possible foundational batches.

Bridge Proactively

**Never execute hard transitions.**  
Mandate parallel testing and stability monitoring of new and old lots simultaneously to capture biological variability.

# Lessons learned & Key Takeaways

## Reagents Drive Variability

Biological materials are a primary source of assay drift

## Lot Changes Need Strategy

Plan for comparability—don't react to failures

## Robustness Is a Lifecycle Requirement

Development decisions echo into validation and routine testing

## Scalability Matters Early

What works in development may fail at scale

## Flexibility Protects Timelines

Strategic sourcing enables continuity

## Standardization Improves Confidence

Control, automation, and validation reduce noise

# Acknowledgements

BioAgilytix US CMC Analytical Operations Teams

Sponsors for the projects to demonstrate case studies today

Thank you for your time today.

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