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

Bhoomi Jani Patel[1], *Sara Gholson*[1], *Shruti Patel*[1], *Lonzie Hedgepeth*[1], *Rizvan Uluisik*[1], *Manoj Baskaran*[1], *Andrew Taylor*[1], *Jeff Patrick*[1], *Jessica Weaver*[1]

[1]BioAgilytix Labs, 2300 Engert Drive Durham, NC 27713

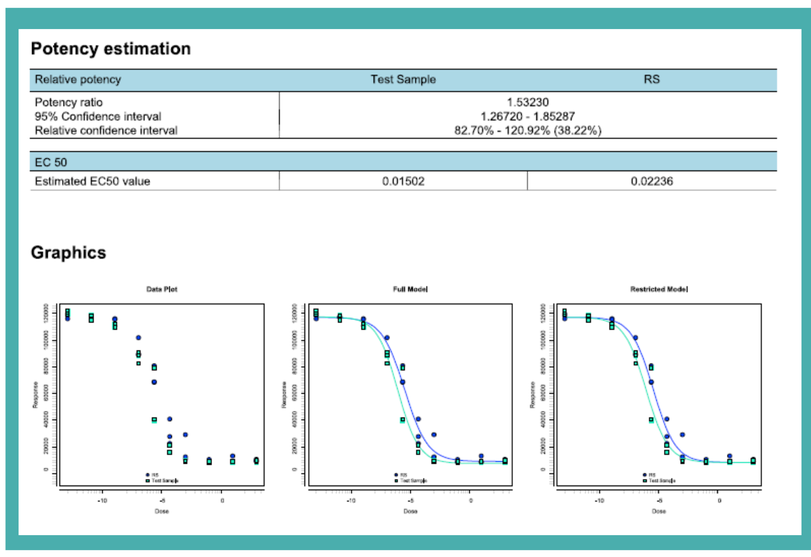
BACKGROUND

A critical reagent is a key component of an assay that directly impacts the assay's performance. Critical reagents are often biological molecules like antibodies, proteins, or cell lines. They are the backbone of cell-based potency assays (CBA), as such their variability can create major challenges in assay development, validation, and routine sample testing. Issues such as lot-to-lot inconsistencies, supply chain disruptions, and reagent discontinuation can lead to unexpected changes in assay performance, potentially impacting result consistency over time and regulatory compliance. This poster presents two real-world case studies that highlight these challenges and discuss practical mitigation strategies to ensure long-term assay sustainability and robustness.

CASE STUDY #1 – mRNA FLOW CYTOMETRY ASSAY

A flow cytometry-based potency assay faced multiple changes for its reference standard lots as well as the assay control lots throughout its lifecycle—from development to validation and through routine sample analysis. Each lot transition introduced variability which impacted the reported potency. Assay control recoveries were consistently trending high. This made assay qualification difficult and complicated regulatory submissions. The case underscores the importance of proactive lot-change management to maintain consistency.

Possible Solutions: A) Identify critical reagents early and assess their impact on assay performance. Assess and project the needs for the assay and manufacture a batch of critical reagents which lasts longer. B). Conduct parallel testing and stability monitoring of new and old lots to evaluate variability before transitioning. **C) Perform regular and routine evaluation of reference materials as part of method Lifecycle Management.**

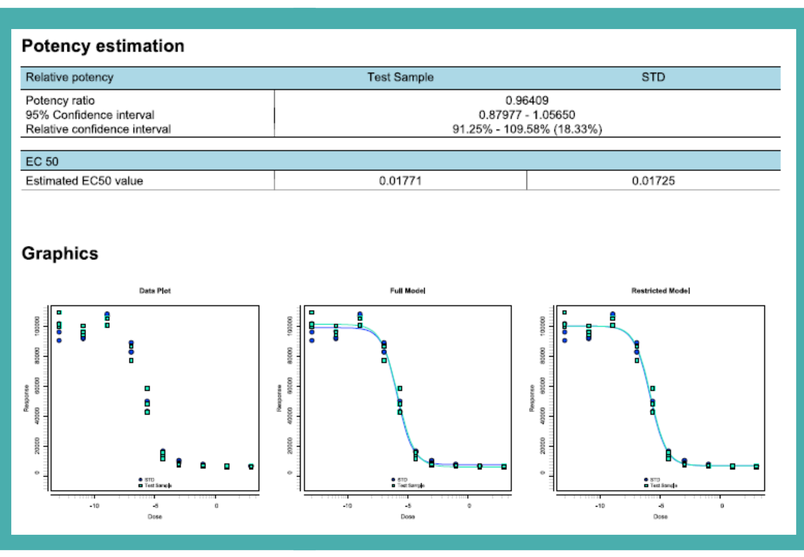


Potency estimation

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

Figure 1. Assay Performance Trends During Early Development

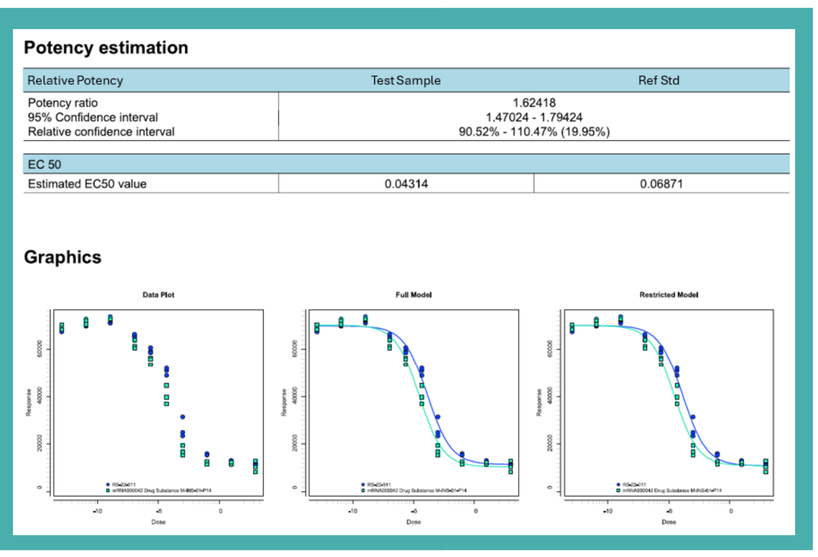


Potency estimation

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

Figure 2. Impact of Lot Changes on Assay Qualification



Potency estimation

Relative Potency	Test Sample	Ref Std
Potency ratio	1.62418	
95% Confidence interval	1.47024 - 1.79424	
Relative confidence interval	90.52% - 110.47% (19.95%)	
EC 50		
Estimated EC50 value	0.04314	0.06871

Graphics

Figure 3. Sample Analysis Variability Due to Reagent Differences

CASE STUDY #2 – FUSION PROTEIN FOR PHAGOCYTOSIS

A flow cytometry potency assay relied on an in-house critical reagent (engineered cells), but as the project scaled up, outsourcing became necessary to meet requirements for volume of reagent. Finding a suitable vendor proved difficult due to differences in reagent quality and sourcing. A custom manufacturing approach was ultimately chosen, but the new lots introduced additional variability, requiring extensive method and reagent requalification efforts.

Possible Solutions: A) Conduct early risk assessments to identify potential sources. B) Develop backup strategies, including alternate suppliers. C) Establish long-term agreements with suppliers to ensure batch-to-batch consistency. D) Consider custom manufacturing with strict quality control measures.

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

Figure 4. Performance Comparison: In-House Engineered Cells

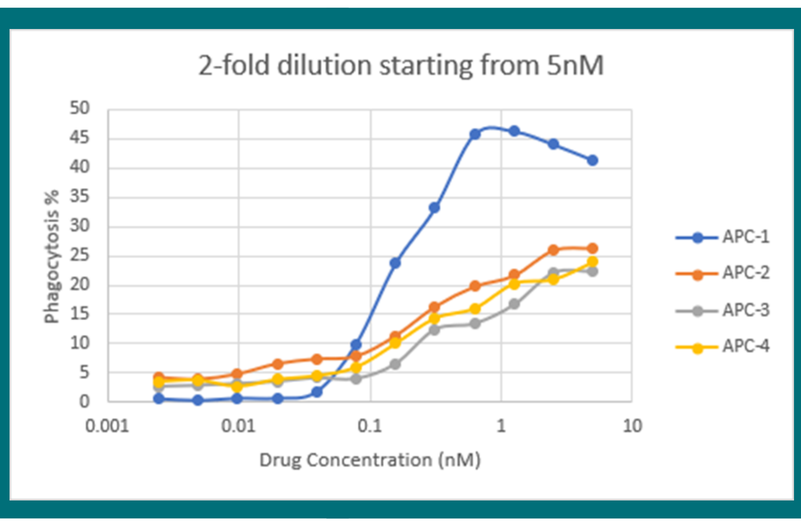


Figure 5. Performance Impact of Vendor A's Cells on Assay Results

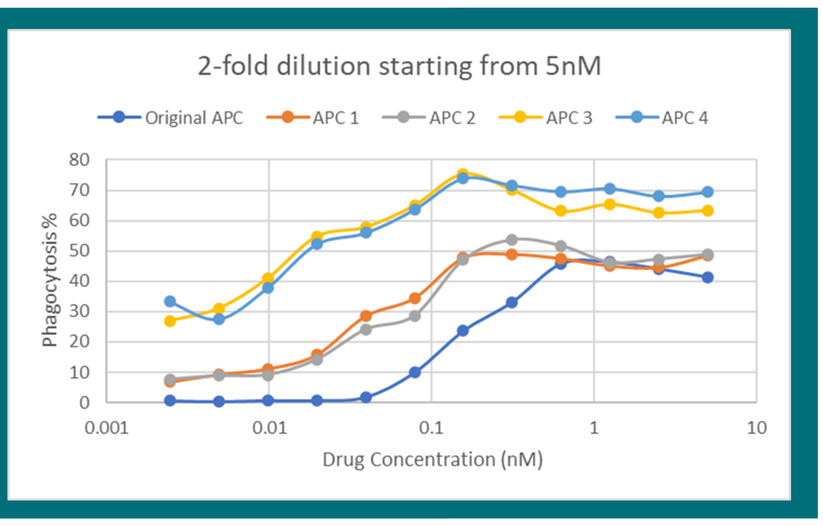


Figure 6. Performance Impact of Vendor B's Cells on Assay Results.

CONCLUSIONS

By taking a proactive approach to critical reagent management, companies can: A). Improve assay reliability & maintain consistency during drug development. B). Reduce the burden of frequent requalification and regulatory delays. C). Strengthen supplier agreements to mitigate risks associated with reagent variability. Early risk assessment, robust supplier partnerships, and long-term reagent planning are essential to ensuring sustainable potency assay performance.