Evaluating Singlicate Analysis for Cell-Based Neutralizing Antibody Assays

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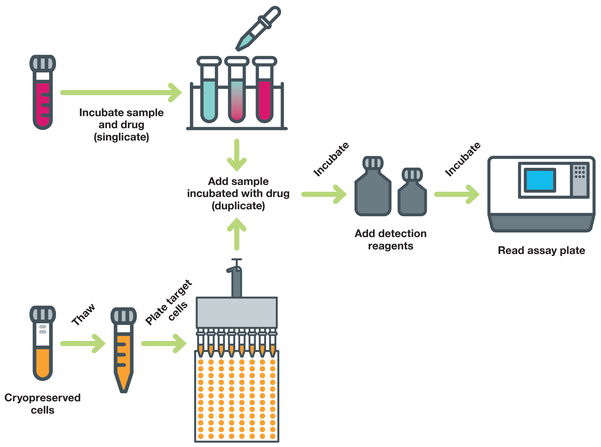
BACKGROUND

Cell-based neutralizing antibody (nAb) assays are typically performed in duplicate or triplicate, a practice driven by convention rather than scientific rationale. This study evaluates whether replicate testing meaningfully improves assay reliability and explores the impact of singlicate analysis as a more efficient alternative without compromising data quality.

METHODS

*PathHunter® eXpress cell lines in AssayComplete Cell Culture with HitHunter® cAMP Assay detection.

**HEK-Blue™ Cells with Quanti-blue Solution Kit



RESULTS

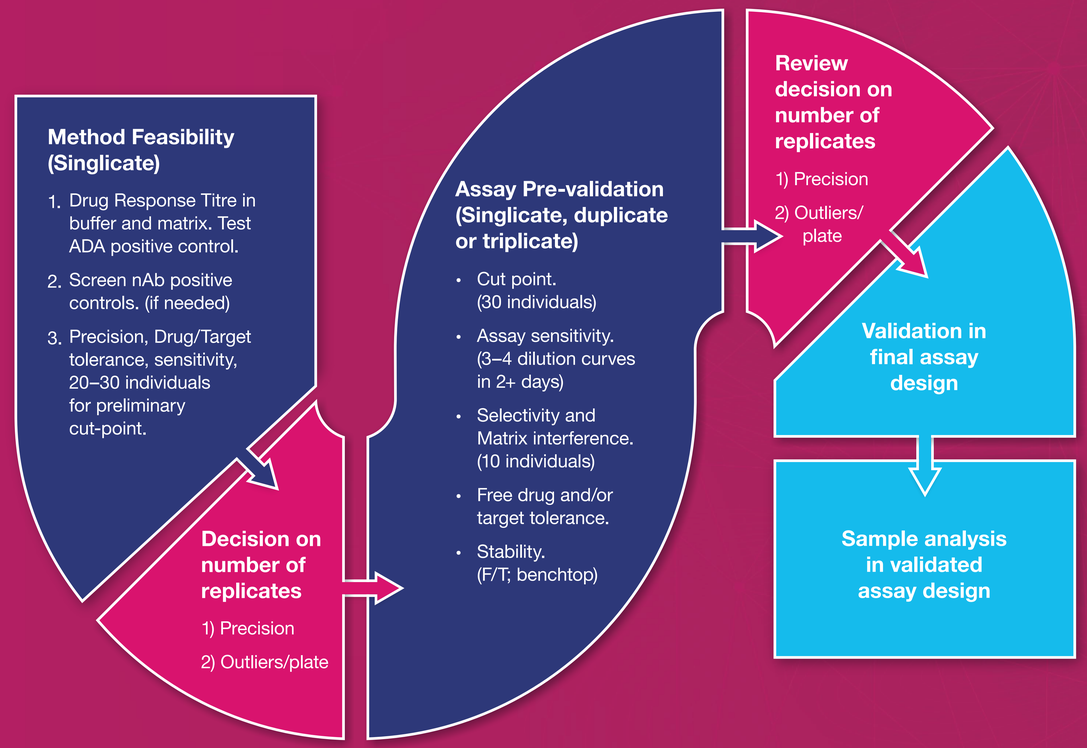
- Cell based assays can be as precise as ligand binding assays
- Mean %CV <7%. Very few %CV exclusions
- No impact on interpretation (positive/negative)

CONCLUSION

- Singlicate analysis can work well for cell-based assays
- Precision for cell based nAb inhibition assays is often best around the cut-point, making positive/negative interpretation accurate
- Enormous savings on expensive cell line reagents and laboratory time
- Additional information through more control-wells per plate
- Streamlined Method Development and Validation—saving many days of laboratory work through additional throughput on plates

Cell-based nAb assays can deliver excellent precision. Singlicate analysis offers significant cost savings without compromising data quality or interpretation.

Workflow for establishing a method in singlicate based on experimental assessment:



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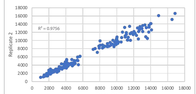
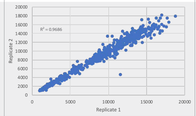
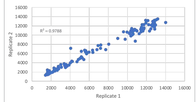
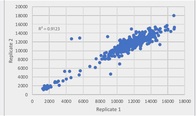
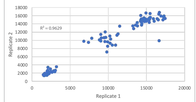
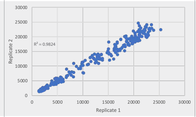
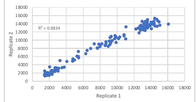
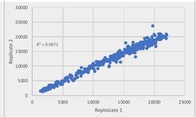
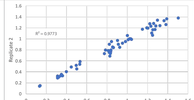
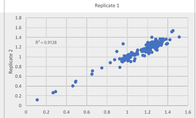
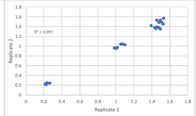
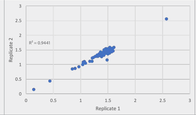
Assessment of 6 Cell-Based Neutralizing Assay Studies:

	Replicate 1 vs 2	%CV Statistics
Study 1* (Positive and Negative controls)		Mean: 5.7% N: 180
Study 1* (Samples)		Mean: 5.0% N: 581 CP (1%) Mean = 0.733 CP (1%) Rep 1 = 0.741
Study 2* (Positive and Negative controls)		Mean: 5.2% N: 120
Study 2* (Samples)		Mean: 5.1% N: 387 CP (1%) Mean = 9079 CP (1%) Rep 1 = 8860
Study 3* (Positive and Negative controls)		Mean: 6.8% N: 110
Study 3* (Samples)		Mean: 6.0% N: 305
Study 4* (Positive and Negative controls)		Mean: 5.8% N: 150
Study 4* (Samples)		Mean: 4.7% N: 471
Study 5** (Positive and Negative controls)		Mean: 3.8% N: 56
Study 5** (Samples)		Mean: 3.1% N: 167
Study 6** (Positive and Negative controls)		Mean: 3.1% N: 24
Study 6** (Samples)		Mean: 2.3% N: 105

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