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Potency Assay Development for Gene Therapy Products – Approaches and Points to Consider in Data Processing

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PURPOSE

Gene therapies – including AAVs, engineered viruses and polymer formulated oligonucleotides/plasmids – are among the highest growth area in biopharmaceuticals. They provide great promise as therapeutics but present many development challenges owing to their complex mechanisms of action (MOA). Potency assays play a pivotal role in the development of nearly every biologic and are an expectation of global regulatory agencies. These assays take many forms depending on the MOA of the biologic and include analysis of cell activity using ELISAs, Flow Cytometry, and ddPCR/qPCR. The cells and the readout are critical but so is the appropriate processing of the data to derive a relative potency. Here case studies for representative gene therapies have been provided as examples of the challenges encountered in acquiring and processing data. Different data modelling is applied to achieve robust outcomes that meet the requirements of a GMP Relative Potency assay. We include discussion of phase appropriateness and the risks and benefits of differing approaches. The modalities include a plasmid gene therapeutic as well as AAVs. Dilution series, ranges and other aspects of the assay execution are optimized to better fit the best processing paradigm.

OBJECTIVES

- Provide examples of potency and related assays for gene therapies and other ATMPs
- Demonstrate how alternative data processing, extraction and manipulation can affect or be beneficial in deriving results.

METHODS

In all cases, cells were cultured and maintained per vendor recommendation or established conditions. Flow cytometry was performed using a Cytoflex LX and plates were read using a BioTek SynergyNeo2. Data was processed using Gen5, PLA or Excel. Dosing of the gene therapy was performed using the nominal established concentrations (vector copy and MOI). Transfection or transduction was facilitated using various agents including Lipofectamine, PEI, Ad5, or other related compounds. Exploratory runs were performed across large concentration and dilution ranges to identify the optimal dosing window. Acquired data was processed for potency determination and comparison made to reference materials and/or controls. Acquisitions were in triplicate throughout.

Analytical platforms used:
Cytoflex LX (Beckman Coulter, Indianapolis, IN)
BioTek Synergy NEO2 (Agilent Technologies, Santa Clara, CA)
Data Analysis: Gen 5 Secure version 3.02

Figure 1: (A) Dose range screening for identification of dilution schema and (B) resultant assay-friendly dilution schemes for a DNA-polymer formulated drug. Cells were transfected using the plasmid DNA and lipofectamine with HEK293 cells. Detection was achieved using a Sandwich ELISA.

Rigorous exploration of dilutional schemes allow for the development of more robust assays with critical density in the most important parts of the curve. Simple 4PL will work consistently.

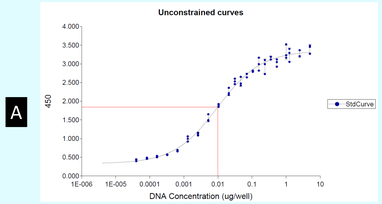


Figure 2. Transfection can cause issues with cell health. As seen in (A) transduction indicates an observed roll-over (hook effect) in the assay. This is also visible in the images shown below in (B) for a healthy (left) and stressed (high dose transduction) cell. In these instances if cell health cannot be recovered across the range then use of the linear portion of the dilutional curve can provide appropriate capacity for the needed dose-response relationship. This is demonstrated in (c) for the 130% Nominal Dose (NDC) where it is compared with the 100% reference. The offset parallel lines indicate appropriate comparability and can then be used to calculate relative potency. Note that this employs 5 dilutions (concentrations) to permit robust determination of comparability. Less than 5 could compromise the integrity of the evaluation and determination.

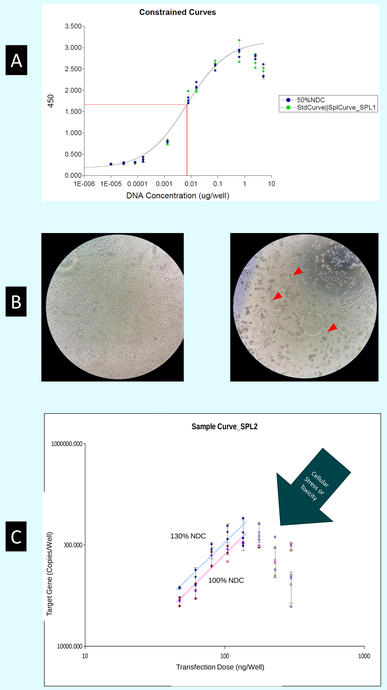


Figure 3(A): Traditional 4PL analysis of AAV2-like vector. Transduction was affected using lipofectamine (other agents also tested). Shown are three fits for data (raw), full 4PL model and restricted 4PL model. In each case the analysis is limited by the absence of an upper asymptote. This is (likely) due to the limit of cell number: AAV particle limit for effective response. Wobble in the projected upper asymptote causes uncertainty in the curve fit which impacts both determined potency and curve comparability assessment.

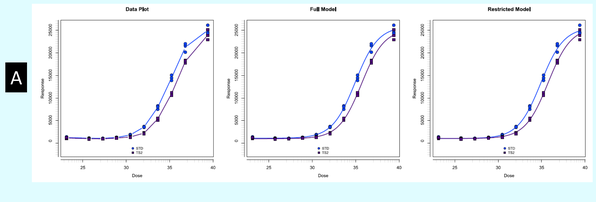
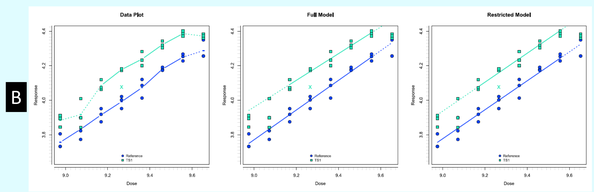


Figure 3(B): By changing the numeric representation and employing a logarithmic-logarithmic plot with a linear interpretation. The double transformation provides for a robust dilutional range (8 points) and ample opportunity for a linear analysis of the data (log v log). Of the included dilutions, 6 of the 8 provide a robust linear response and strong comparability. A single outlier (x) is show in the plots.



By evaluating and selecting the best dilution schemes and data transformations, more effective and robust data generation can be achieved. This can contribute significantly to enhancing the performance and consistency of potency and related assays.

CONCLUSIONS

1. Comprehensive scouting of dilutional schema can lead to a more robust assay format. This enhances curve character for 4PL determinations of relative potency and makes evaluation of comparability more robust and meaningful.
2. Characteristics observed in dilutional curves may be attributed to physical and biological phenomena which may provide constraints to data analysis. Confirmation of the source (here visual assessment of cells) can confirm effect and support alternative data evaluation processes.
3. Even with robust dilutional series data transformations may provide benefit. Often – as shown here – changes to dilutions may not be necessary and log-log or other transformations may be useful to overcome limitations of traditional 4PL analysis of relative potency determinations. This can also provide better data for curve comparability assessments.

REFERENCES

1. US Pharmacopeia <1030>, <1032>, <1033> and <1034>
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3. Guidance for the Industry – Potency Tests for Cellular and Gene Therapy Products (US FDA, Jan. 2011)
4. C.J. Robinson, et al, BioProcess International, 12 (1), 30, 2014