Development of a robust potency assay: the gateway to understanding the structure-function relationship in AAV gene therapy

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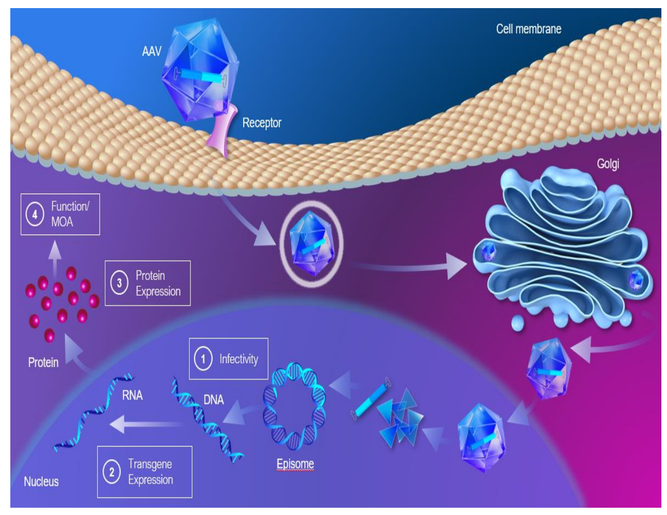
ABSTRACT

One of the essential components of successful drug development is product characterisation. Determining the critical quality attributes and the potential impact on efficacy helps to ensure consistency of product lots used in the clinic. The methods used to measure product potency should be robust, stability-indicating, and reflective of the mechanism of action. This presentation demonstrates an approach to develop a robust *in-vitro* method for evaluation of functional potency with minimal assay variability for AAV gene therapy. Additionally, it includes findings from a forced degradation study, using thermal stress to establish the structure and function relationship using a comprehensive suite of analytical assays. Thereby, it emphasizes the appropriate use of a functional potency assay to indicate changes in the stability of an AAV gene therapy candidate. This presentation will also highlight the AAV gene therapy candidate life cycle, by drawing a parallel between other potency assays that show the *in-vitro* activity of the AAV candidate at various stages of infection. Lastly, it attempts to provide an explanation for the observed loss in AAV drug stability and potency.

INTRODUCTION

FDA defines potency as “the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.” (21 CFR 600.3(s)). As per the regulations, a potency assay may consist of *in-vitro* or *in-vivo* tests, or both, is product-specific, and demonstrates the product’s potency as explained above. The following experiments were performed for development of a robust *in-vitro* potency assay that involves two steps for an end-point result measuring the enzymatic activity of the target enzyme.



RESULTS

1. ASSAY DEVELOPMENT

A step-by-step approach was used to develop the potency assay that involved several design of experiments to evaluate various parameters including cell line screening, substrate and second-step enzyme concentration, time of incubation, lysis conditions, cell seeding density, transduction time, as well as drug substance multiplicity of infection (MOI) concentration ranges. Plate variability was analyzed by performing plate uniformity and intraplate precision experiments. Method performance for accuracy, linearity and range was determined by prequalification experiments involving sample mimics spanning concentrations higher and lower than the Reference Standard.

Activity Optimization

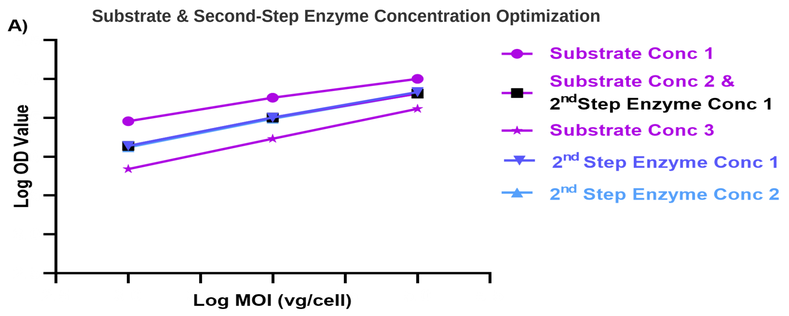


Figure 1A. Activity Optimization. Activity optimization included testing three different concentrations of both the substrate and the second-step enzyme to determine the optimal concentration.

Transduction Optimization

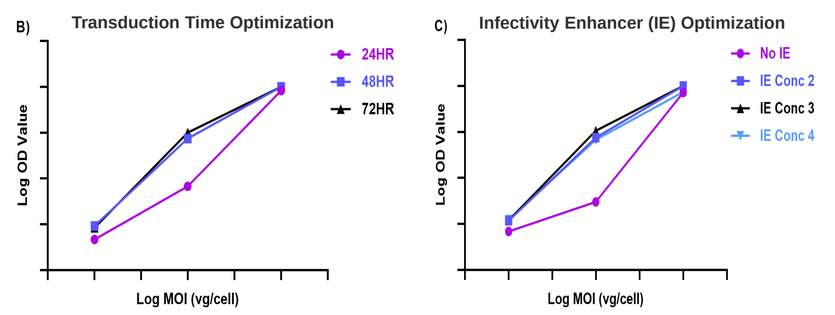


Figure 1B-C. Transduction Optimization. As a part of optimizing transduction conditions, three time points for transduction time (1B) and three concentrations of the infectivity enhancer (1C) were analyzed.

MOI Curve Optimization

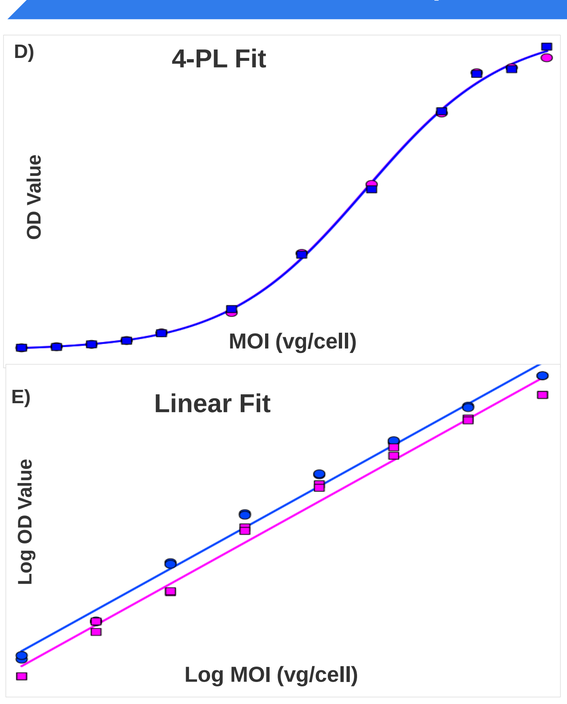
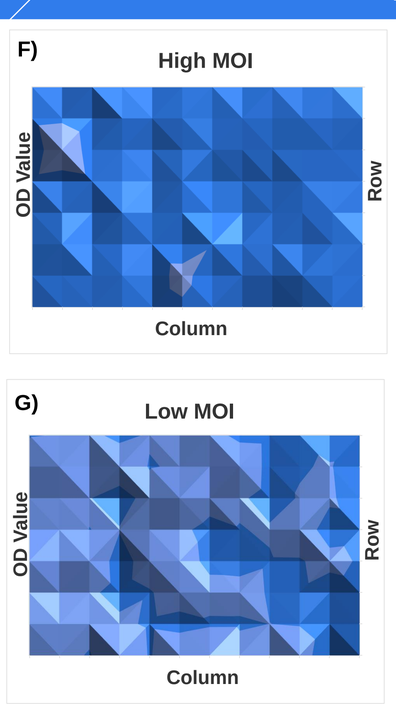


Figure 1D-E. MOI Curve Optimization.

To represent the data with an appropriate statistical model, both, 4PL curve fit (1D) and linear regression (1E) were evaluated.

Plate Uniformity & Intraplate Precision



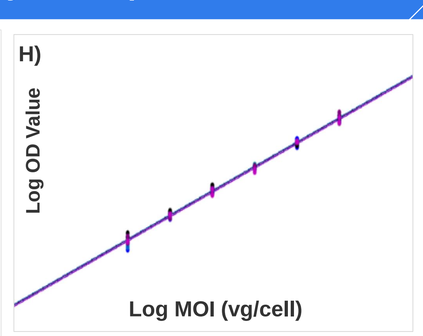
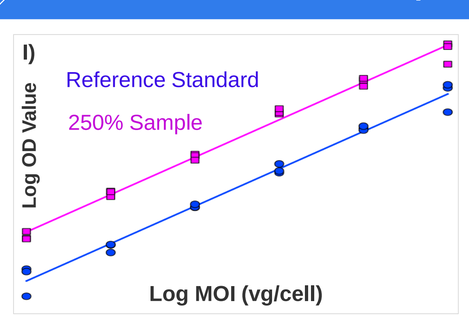
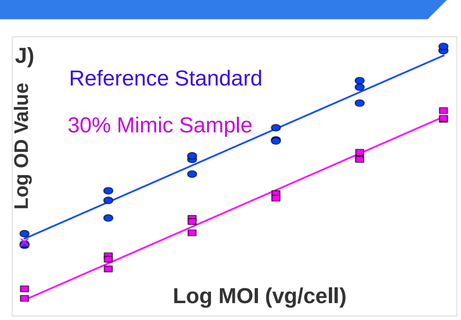


Figure 1F-H. Plate Uniformity & Intraplate Precision. Plate uniformity experiment consisted of one plate with maximum signal using high MOI (1F) and the other plate with minimum signal using low MOI (1G), to assess the edge effect. Intraplate precision (1H) was evaluated by running six replicates of the MOI curve on one plate to determine any plate-related variabilities.

Pre-qualification





K) PRE-QUALIFICATION DATA		
MIMIC LEVEL	%RP	%RECOVERY
250	248	99
200	198	99
150	141	94
100	96	96
75	71	95
30	31	103

Figure 1I-K. Prequalification. The purpose of prequalification is to get an early estimate of method performance in Quality Control (QC). Prequalification consisted of running sample mimics with concentrations higher and lower than the Reference Standard to determine the range of the assay. Fig 1I demonstrates results for 250% mimic level, Fig 1J demonstrates results for 30% mimic Table K demonstrates prequalification results expressed as %relative potency (%RP).

2. STABILITY CHARACTERISATION

In addition to specificity, the potency assay must also be stability-indicating to ensure consistency of all the lots released. Following attributes were analyzed for stability-assessment by subjecting the drug candidate to thermal stress at 25°C and 40°C over multiple time points.

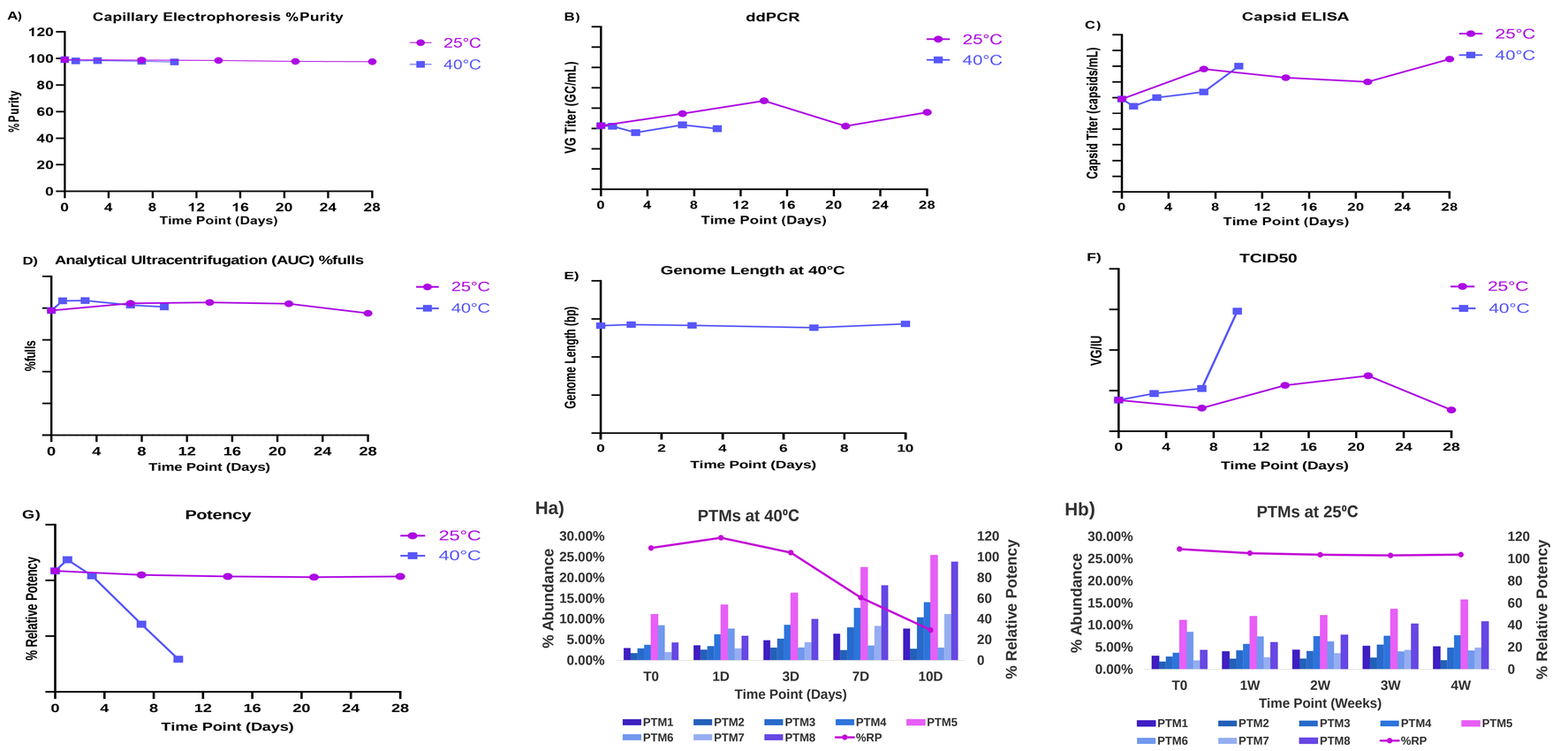


Figure 2A-H. Stability Indicating Assessment. A representative sample was heat-treated either at 25°C for up to 28 days or 40°C for up to 10 days, then tested in a range of analytical methods. A) CE-SDS for % purity, B) VG titer by ddPCR, C) Capsid titer by ELISA, D) AUC for %fulls E) TapeStation Analysis for genome length, F) Infectivity (TCID50) by ddPCR and G) biological activity (*in vitro* potency)., H) LCMS for post-translational modifications on AAV capsid.

Figure 2 Summary:

- No change in VG titer, capsid titer or VP purity was detected over time at 25°C and 40°C.
- TCID50 and potency methods detected a loss in product efficacy over time at 40°C.
- Increase in deamidation and isomerization was observed over time at 40°C which can be co-related with potency loss.
- The potency assay may be more sensitive to changes in stability than TCID50.

CONCLUSIONS

- OXB Solutions uses a systematic approach for potency assay development that involves optimization of various parameters. This approach ensures development of a robust assay with minimal variability that is QC-ready.
- A well-developed potency assay is a true stability-indicating assay that reflects changes in potency of the drug candidate on subject to thermal stress.

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