Development of a high-throughput, miniaturized, semiautomated rapid transduction inhibition assay (TIA) for the characterization of anti-AAV antibodies in gene therapy

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Abstract 772

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Introduction

- Adeno-associated virus (AAV) is a commonly used vector for gene therapy because it is relatively simple to engineer and is non-pathogenic.[1]
- However, most people will be exposed to at least one strain of AAV during their lives, resulting in development of anti-AAV antibodies that can reduce or eliminate the efficacy of AAV-based gene therapy.
- Transduction inhibition assays (TIAs) are functional cell-based assays that estimate the extent of AAV neutralization in patient plasma to determine patient eligibility for AAV gene therapy.[2]
- We previously described the development of a 6-hour (rapid) and 24-hour TIA method, which was subsequently validated.[3]
- This assay is suitable for determining titers of neutralizing antibodies (NAbs) in prospective gene therapy patients, but throughput limitations hinder use in population-based seroprevalence studies.

Objective

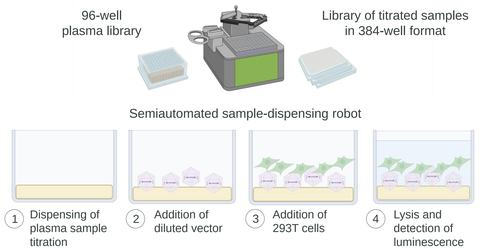
- To develop a high-throughput TIA via miniaturization and automation that will enable more widespread seroprevalence assessments and novel capsid screening.

Methods

Development of a semiautomated, additive assay workflow

- 96 plasma samples (76 male and 20 female) were purchased from a commercial supplier of normal donor plasma and aliquoted into a screening-ready library in a 96-well format (**Figure 1**).
- A precision liquid handler (Mantis®, Formulatrix) was used to develop a protocol for serial titration and dispensing of samples and controls into a 384-well plate, allowing simultaneous assay of 8 samples per plate.
- Previously validated 96-well TIA parameters, such as plasma dilution, multiplicity of infection (MOI), and cell number, were retained and developed into an additive format with automated dispensing of samples, viral vector, cells, and detection reagent.

Figure 1: Outline of assay workflow for HTS-TIA



384-well additive TIA workflow for high-throughput screening

Optimization of MOI and assay workflow

- To enable testing of combinations of AAV serotypes and cell lines with reduced transduction efficiency, MOIs of 5 and 50 were investigated.
- To facilitate screening, a continuous and an 'interrupted' workflow, in which plates with pre-dispensed plasma sample dilutions were stored at −20° C for a prolonged period prior to running the TIA, were tested.

Screening of the plasma library

- The 96-sample plasma library was screened using the assay workflow for AAVS3 vector in human embryonic kidney 293T (HEK293T) cells.
- Half-maximal inhibitory concentration (IC50) and goodness-of-fit data were extracted from four-parameter fitting software, and signal to background (S/B) ratios and Z′ values were calculated.

Results

Effect of MOI on assay performance

- Five plasma samples of increasing neutralizing capacity with an MOI of 5 (validated) and 50 (10-fold increase) were tested.
- No significant differences in IC50s were observed (**Figure 2**).

Effect of 'interrupted' workflow on assay performance

- Eight plasma samples of increasing neutralizing capacity with the continuous and 'interrupted' workflows were tested.
- No significant differences in IC50s were observed (**Figure 3**).

Figure 2: Effect of MOI on assay performance

Sample ID	IC50, MOI 5	IC50, MOI 50	% Difference
A3	2428	1874	77.2
B3	2209	2701	122.3
D3	1310	973	74.3
E3	2036	1374	67.5
H3	42	47	111.5
IVIG	327	357.2	109.2
		Average (SD)	104.2 (28.6)
		%CV	27.5

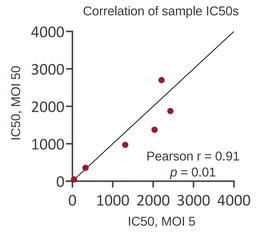
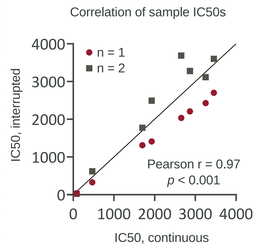


Figure 3: Effect of 'interrupted' workflow on assay performance

Sample ID	IC50, continuous (n = 1)	IC50, interrupted (n = 2)	% Difference
C3	3454	3152	91.2
A3	3254	2771	85.2
B3	2867	2743	95.7
E3	2655	2863	107.8
F3	1926	1953	101.4
D3	1699	1543	90.8
H3	86	34	39.4
IVIG	469	475	101.2
		Average (SD)	89.1 (21.3)
		%CV	23.9



Screening of the plasma library and assay performance characteristics

- The 96-sample plasma library was screened using 15 assay plates over three occasions, the last of which retested out-of-range samples.
- The mean S/B ratio was 474, and the mean Z′ value was >0.5 (**Figure 4**).
- Intravenous immunoglobulin (IVIG), the positive control included on each plate, had an IC50 of 0.63 µg/mL and R^2 of 0.97 (**Figure 4**).
- Median (range) IC50 across samples was 3040 (50, 32000).
  - Ten samples exceeded 32000, and 11 samples were under the detection limit of 50 (**Figure 5**).
- Median IC50 values were 2825 and 4166 across 76 male and 20 female donors, respectively.

Figure 4: Summary of assay performance

Occasion	S/B ratio	Z′	IVIG IC50 (µg/mL)	R^2
1	495	0.59	0.57	0.97
2	271	0.63	0.70	0.97
3	656	0.72	0.61	0.96
Average (SD)	474 (193)	0.64 (0.07)	0.63 (0.06)	0.97 (0.01)
%CV	40.8	10.3	10.3	1.0

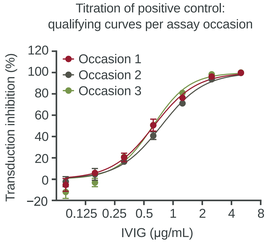
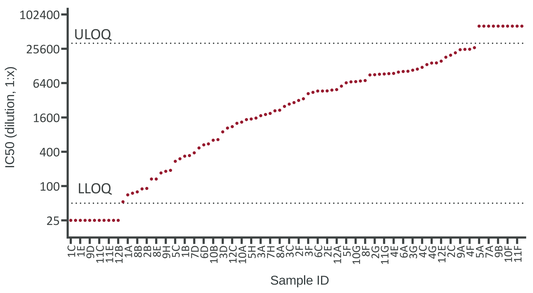


Figure 5: IC50 of 96 plasma samples by AAVS3 HTS-TIA



Conclusions

- A high-throughput 384-well TIA was developed, which showed a 6-fold increase in productivity compared to a prior validated TIA.
- There were no significant differences in IC50s at MOIs of 5 and 50, which enables testing of different serotype and cell line combinations.
- This work shows that once cumbersome functional cell-based assays, such as TIA, can be scaled up and standardized, thereby facilitating pre-clinical and clinical development of gene therapies.

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Abbreviations: AAV, adeno-associated virus; CV, coefficient of variation; HEK293T, human embryonic kidney 293T; HTS-TIA, high-throughput screening transduction inhibition assay; IC50, half-maximal inhibitory concentration; IVIG, intravenous immunoglobulin LLOQ, lower limit of quantification; MOI, multiplicity of infection; S/B, signal to background; SD, standard deviation; TIA, transduction inhibition assay; ULOQ, upper limit of quantification

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