Challenges surrounding 100% pre-exiting antibody incidence in matrices; tricks to develop/validate an anti-Ad5 vector TAb assay

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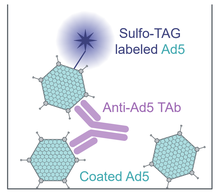
Novelty

This poster presents novel approaches to develop/validate an anti-Ad5 vector total antibody (TAb) assay when a very high incidence of samples have pre-existing antibodies against Adenovirus serotype 5 (Ad5), often at high levels. Traditional tactics such as a multi-tiered immunogenicity testing cascade, use of matrix to dilute samples and cut-points which are statistically determined were modified: testing only included the titer tier, assay buffer was used to dilute samples and the titer cut-point was determined empirically.

Assay setup

The assay is a bridging assay which uses Ad5 coated on 96-well assay plates to capture TAbs in serially diluted samples. Detection is then performed using sulfo-TAG-labeled Ad5 (Figure 1). The dilution factor producing the signal equivalent to the titer cut-point multiplied by the minimum required dilution (MRD) gives the titer result.

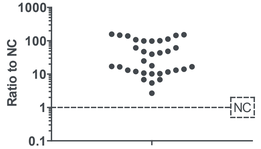
Figure 1 Assay setup



Immunogenicity cascade

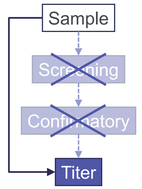
During assay development, it quickly became apparent that most/all available matrices from the healthy population had relatively high levels of pre-existing anti-Ad5 TAbs (Figure 2). Use of the traditional screening and confirmatory immunogenicity cascade tiers where positivity of sample is based on differences with the signal of negative samples was not considered applicable; only the titer tier was kept for relative measurements (Figure 3).

Figure 2 Presence of TAbs in all tested serum lots



- Every symbol represents the result for one lot
- A negative sample should have a ratio to NC of ~1

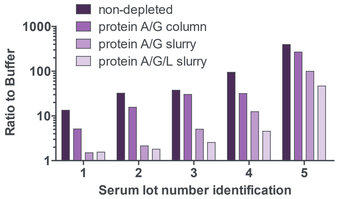
Figure 3 Simplified ADA immunogenicity cascade



TAb-free matrix

Although TAb-free matrix is not readily available, it remains essential to have matrix devoid of TAbs to prepare negative (NC) and positive control (PC) samples. Different antibody-depletion strategies were tested (Figure 4).

Figure 4 Reducing TAbs in matrix



Protein A/G/L slurry was the best tested option. Although multiple iterations of protein A/G/L treatment were shown to remove most TAbs even when initial concentrations were high, it was considered preferable to pre-select matrix lots with relatively low pre-existing TAbs quantities to generate “negative” matrix using only one depletion treatment.

Since that all samples in this study require dilutions for the titer assay, the need for rare TAb-free matrix normally used for dilutions would be significant. In an attempt the reduce consumption of TAb-free matrix and based on the elevated MRD, results from dilutions in diluted matrix versus buffer were compared for PC samples and serum samples (Figure 5 and Table 1)

Figure 5 Dilutions of a PC sample in matrix versus buffer

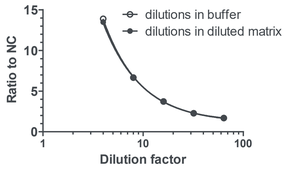


Table 1 Dilutions of matrix samples in matrix versus buffer

Matrix lot number	Dilution factor at ratio to NC of 2 Dilutions in:	
	Buffer	Diluted matrix
1	2	2
2	239	238
3	69	69
4	8	8
5	18	16
6	71	70

Titration curves for PC samples and dilution factors at the titer cut-point when testing matrices were quasi-identical, confirming that samples could be diluted in buffer instead of matrix without any impact on the reported titer values.

Titer cut-point

Titer cut-points are traditionally statistically established using screening data from multiple testing of multiple matrix lots and based on a 0.1% false-positive rate. An alternative empirical approach to establish the titer cut-point was used:

- Due to the elimination of the screening assay tier, there was no screening assay data available to determine a titer cut-point
- Since all study samples are expected to have TAbs:
  - The “false-positive” approach predicated on results from negative sample is not possible
  - High sensitivity generated using the statistical approach is not critical
- Reproducibility was favored over sensitivity

The empirical titer-cut point was determined during assay development: multiple PC sample titrations were performed, and reproducibility of the interpolated dilution factor at arbitrary cut-points of 1.5, 2.0 and 3.0 were compared (Figure 6 and Figure 7)

Figure 6 PC sample titrations to establish titer cut-point

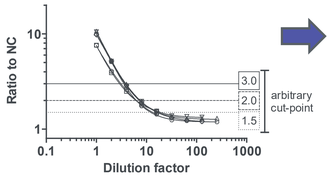
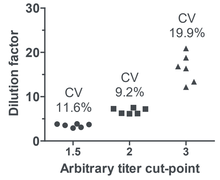


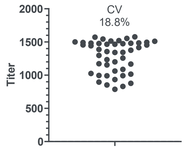
Figure 7 Precision of dilution factors at different titer cut-points



The tested arbitrary cut-points of 2.0 was considered optimal because it was further away from the generally variable portion of the titration curves (near the bottom plateau), generating reproducible interpolated dilution factors; it was selected moving forward.

Precision of the mean titer of the titer PC sample using the titer cut-point of 2.0 was evaluated with data produced throughout assay validation. As presented in Figure 8, the CV was higher than during assay development; this is due to the generation of data over a much longer period of time. A CV of 18.8% remains acceptable for a titer assay where changes in titer values are considered significant when fold-changes reach 4 (± 2 dilution factors).

Figure 8 Titer PC sample precision (validation)

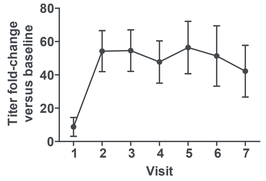


Every symbol represents the result for one titer PC sample

Sample analysis

Using the validated method, sample from 52 patients in a clinical study were analyzed to evaluate changes in TAbs through time. Patients received multiple doses of the gene therapy vector drug treatment between visits. Average fold changes in TAb titers versus baseline are presented in Figure 9.

Figure 9 Sample analysis results summary



- Points represent means of available data from 52 subjects
- Error bars represent standard errors on the mean

Results showed an increase in TAbs up to the 2nd visit (6 weeks after first treatment) where they stabilized.

Sample analysis confirms that the validated method along with the selected titer cut-point were adequate for the purpose of semi-quantification of TAb against the vector and measurement of drug-boosted antibodies.

Conclusions

The adapted tactics for immunogenicity testing of an immunotherapeutic viral vector were successfully used to validate the method for its intended use:

- Non-informative screening and confirmatory assay tiers were eliminated
- Use of precious reagents such a TAb-free matrix were reduced
- The titer cut-point determination was simplified

Purposeful TAb titer results interpretation from a clinical study evaluating drug-boosted antibody production was performed with this method.

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