Development of Multiplex Sensitive Anti-Drug Antibody Assays for CRISPR/Cas9 Gene Therapies

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Overview of the presentation

- Immunogenicity Introduction
- Anti-Drug Antibody Assays for Cas9
- Anti-Drug Antibody Assays for AAV

Immunogenicity

- **Immunogenicity** is defined as the propensity of the therapeutic protein product to generate immune responses to itself and to related proteins or to induce immunologically related adverse clinical events.
- Patient immune responses to therapeutic protein products have the potential to affect product safety and efficacy.
- The immunogenic response generally includes both B cell-mediated humoral (antibody) and T cell-mediated cellular arms of the immune response.
- The development of sensitive, specific, and selective immunogenicity assays to assess ADAs and cellular responses is a key aspect of drug product development for biologics.

http://www.fda.gov/downloads/Drugs/.../Guidances/UCM192750.pdf

General principle

Risk-based approach for immunogenicity evaluation

The Assessment of Immunogenicity Risk

❑ **Patient- related**
- Pre-existing Abs

❑ **Product-related**
- In silico analysis
- *In vitro* non-cell assay
- *In vitro* cell-based assay
- Relevant animal models, mouse, rabbit, non-human primates

- High risk
- High probability
- Not meeting the requirement of TPP

Risk Mitigation

❑ **Avoidance**
- Patient stratification
- De-immunized trangene
- De-immunized vector

❑ **Reduction**
- Change regimen
- Actively induce tolerance

- Probably meeting the requirement of target product profile (TPP)

Immunogenicity Testing plan

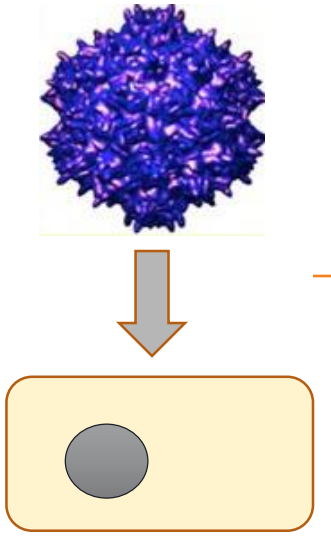
❑ **Correlation of Immunogenicity incidence and levels with safety, PK and PD (nonclinical and clinical)**

Monitoring Immunogenicity Throughout the Trial

AAV Gene Therapy and Immunity

Main risks to evaluate for an AAV gene product:

- Pre-existing immunity to viral capsid proteins
- Expected immune responses to AAV (humoral and cellular immune response)
- Potential immune response to transgene



Humoral Immune Response

Cellular Immune Response

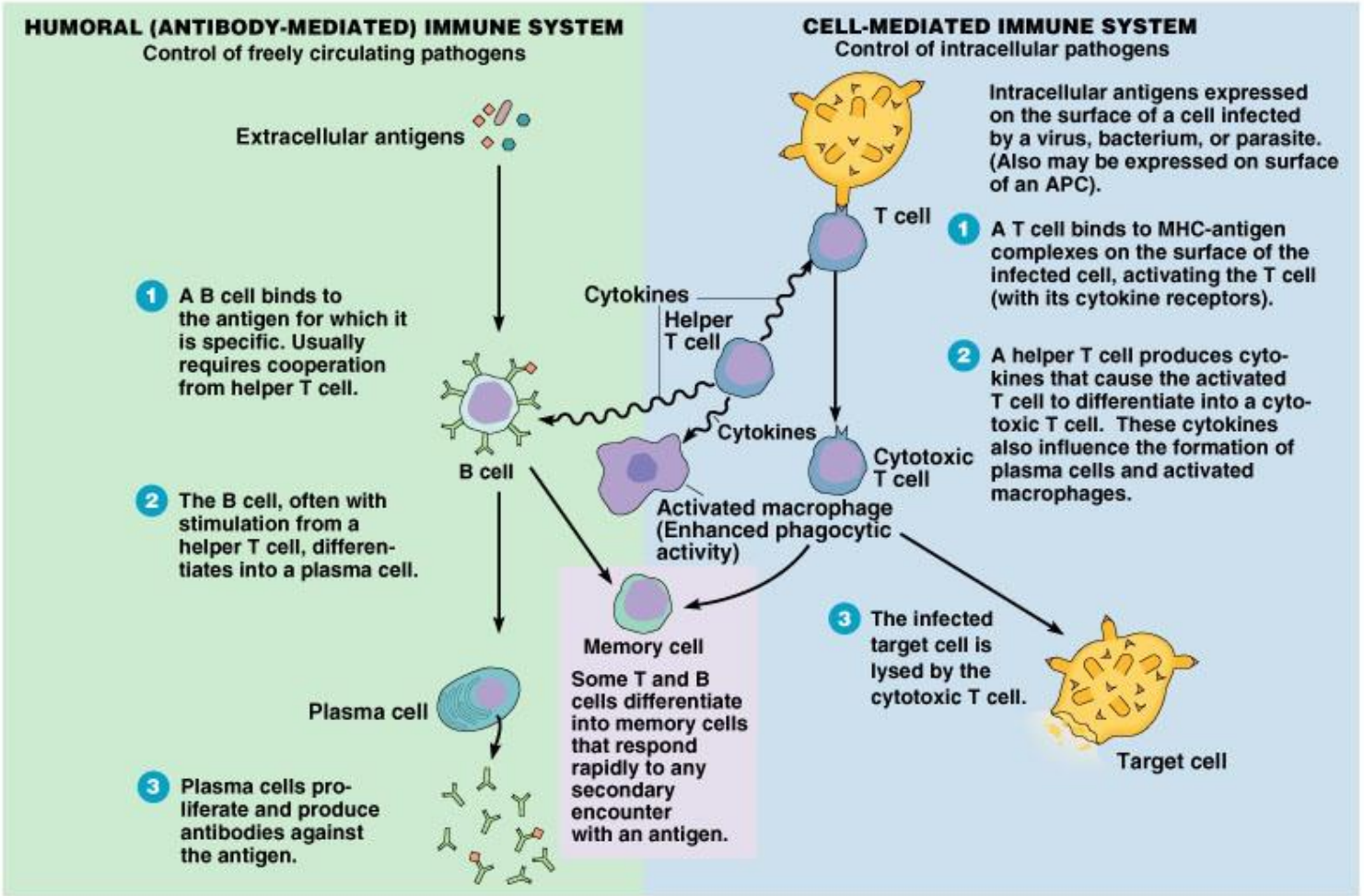
ADA assays

- Antibody response to Cas9 and AAV

PBMC T cell assays

- T cell responses to Cas9 and AAV

Humoral and cell-mediated immune responses



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Anti-Drug Antibody Assays for Cas9

ADA screening and confirmatory assays

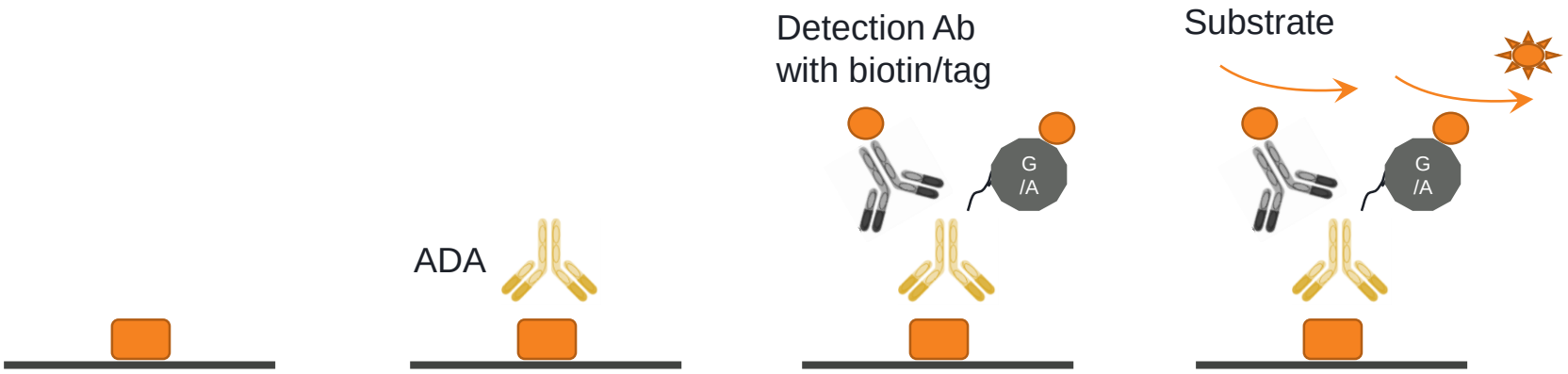
- Anti-drug antibody (ADA): An immunoglobulin (Ig) with specific binding properties to a drug.
- ADAs are typically evaluated using both screening and confirmatory assay methods using a tiered approach.
- In the first tier, ADA screening assays are used to detect all antibodies that bind to drug and transgene.
- Assays for detection of ADA facilitate understanding of the immunogenicity, safety, and efficacy. The detection of ADA is dependent on key operating parameters of the assays, such as sensitivity.
- The second tier comprises a confirmatory method based on a competitive inhibition with excess exogenously added drug that determines the specificity of binding antibodies to drug.

http://www.fda.gov/downloads/Drugs/.../Guidances/UCM192750.pdf

Universal ADA Immunoassays for nonclinical studies

Direct/Indirect ADA assay format

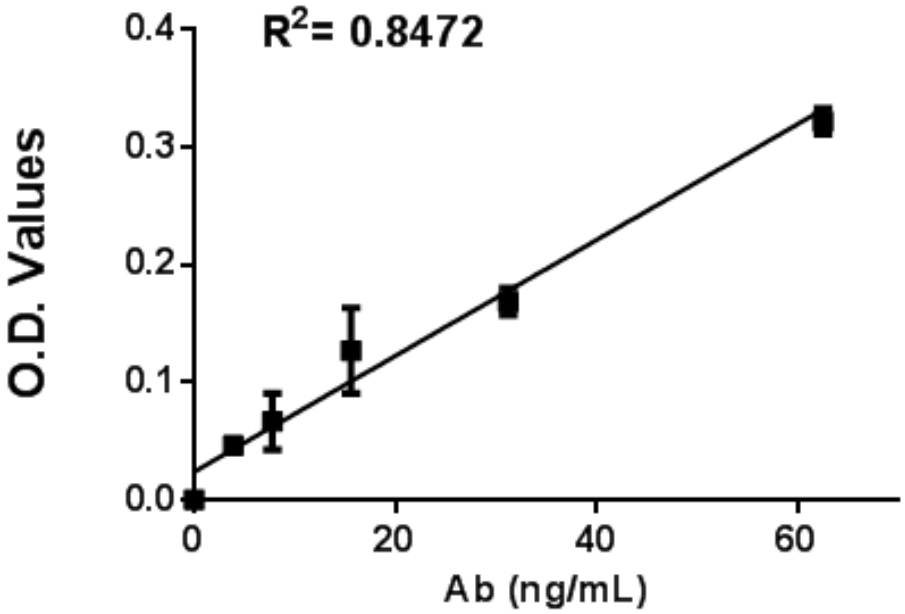
Capture drug on surface → Sample containing ADA added to well drug is captured → Add detection antibody or protein G/A with tag → Detection of captured ADA



- Do not require labeling of drug and is ready to use as it is.
- Using one assay condition across different species saves the assay development time.
- The streamlined immunogenicity assessment strategy speeds up the assay validation.

Bautista A. C., Salimi-Moosavi H., Jawa V. Universal immunoassay applied during early development of large molecules to understand impact of immunogenicity on biotherapeutic exposure. The AAPS Journal. 2012

Application of the ADA assay in preclinical screening of Cas9 antibodies in human



ELISA to Luminex Technology

ELISA

- Basic principle behind all immunoassays
- Plate-bound assay format with color absorbance detection
- A single analyte approach
- Requires large sample volume – 50 μL
- More drug needed to coat plates
- Sensitivity: ng/mL

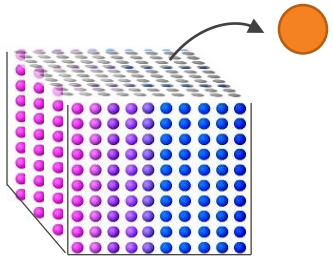
Luminex bead assays

- Same principle as ELISA using beads with specific detection
- Large dynamic range: Typical ≥4.5 logs
- Multiplex capability up to 500 analytes per run
- Small sample volume – 10 μL or less
- Customizable and automated
- High sensitivity: pg/mL
- 21 CFR Part 11 compliant

ADA Luminex assays for AAV/Cas9 gene therapy

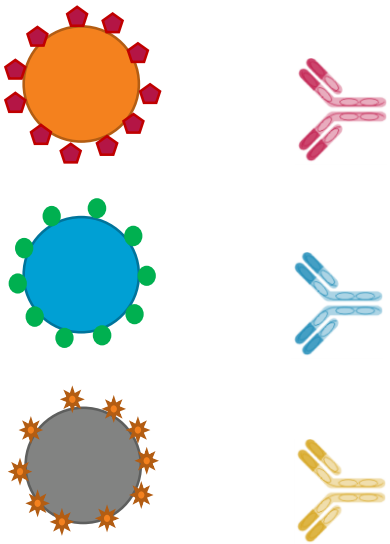
Luminex bead system

Each of 500 beads can be identified by its unique proportion of component dyes.



- SpCas9
- SaCas9
- AAV5 (custom made for Editas)

Incubate drug coated beads with sample containing ADAs



ADAs

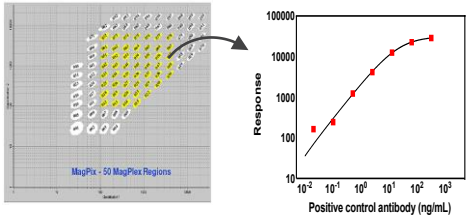
- Anti-SpCas9 (Commercial)
- Anti-SaCas9 (Made in house)
- Anti-AAV

Detection

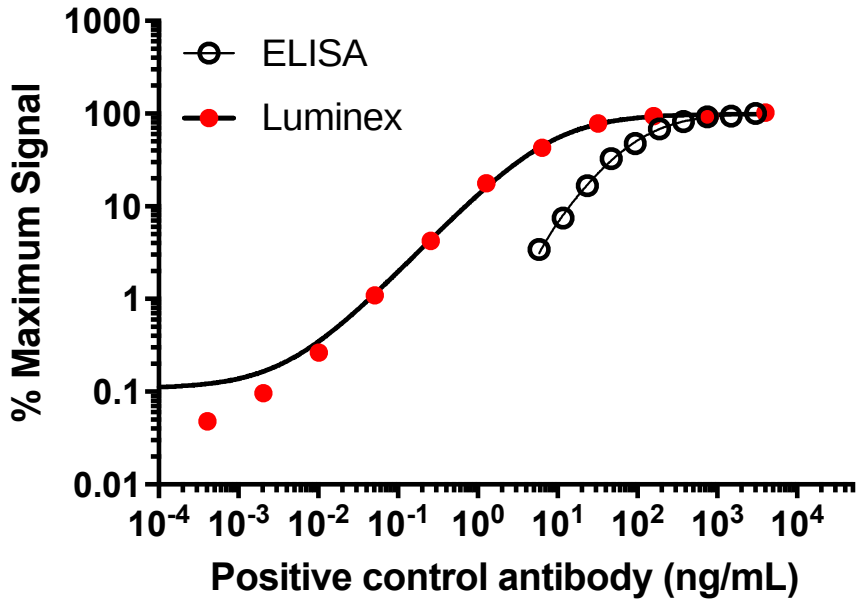
Secondary antibody conjugate or protein G/A with biotin

- **Biotin**

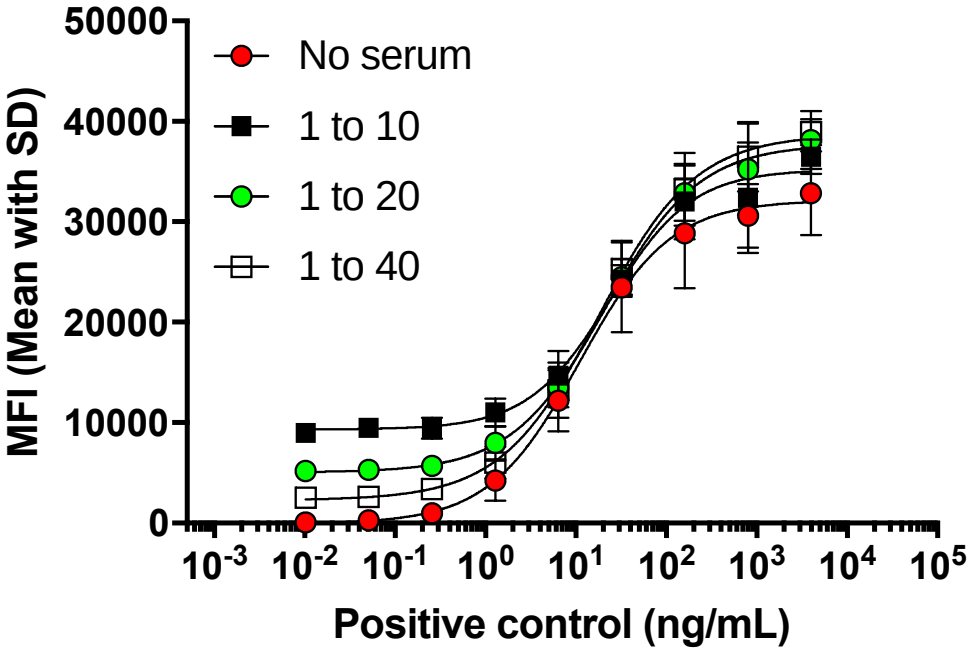
Streptavidin - PE



Platform comparison (Sensitivity)



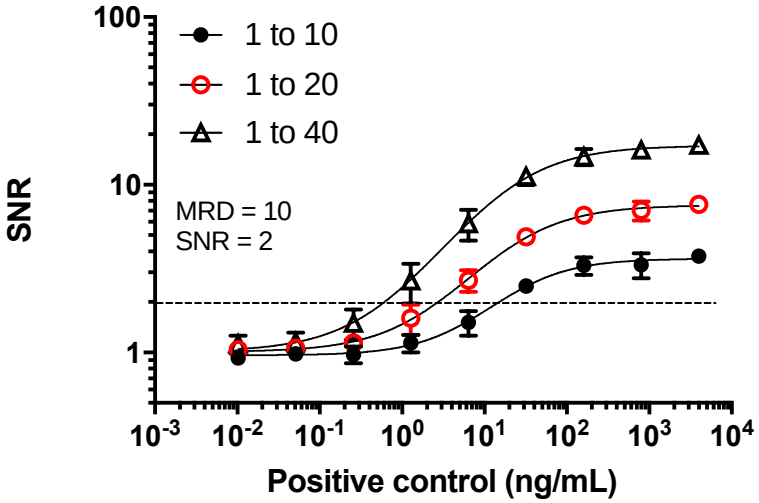
Evaluation of serum matrix interference in Cas9 ADA assay (non-human primates)



Assay summary (5% Cyno serum):
Sensitivity: 2 ng/mL
Minimum Required Dilution (MRD): 20
Analytical range: 2 ng/mL to 1000 ng/mL

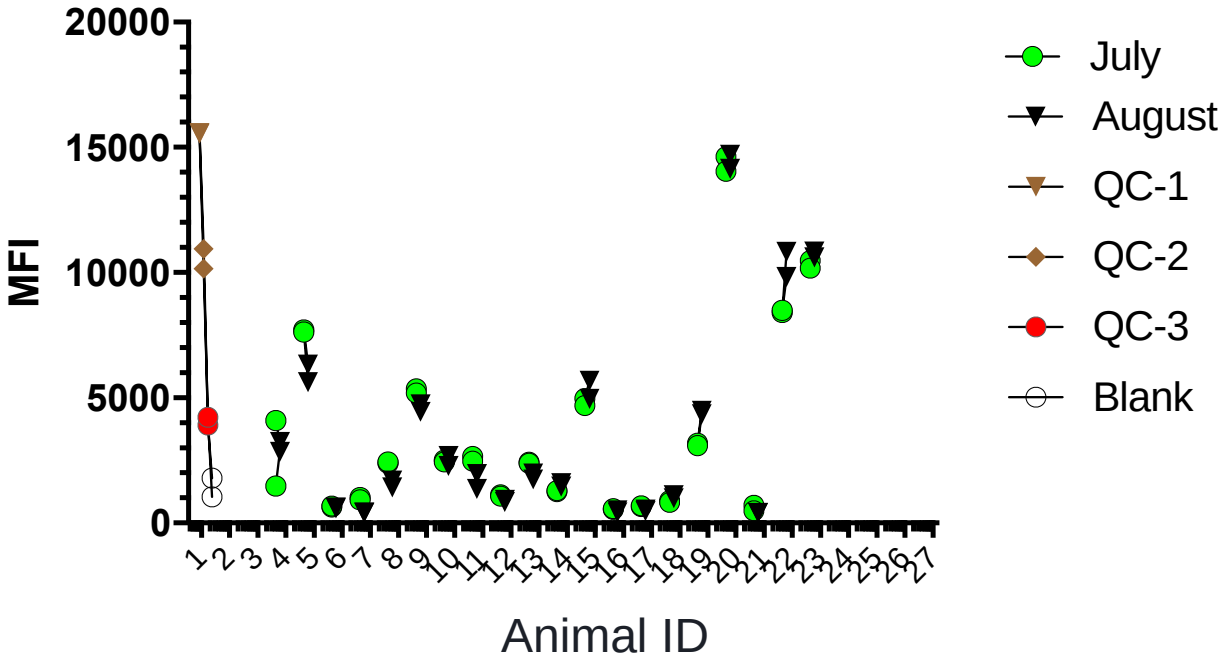
Minimum Required Dilution (MRD)

MRD is the sample dilution that yields a signal close to that of the assay diluent and allows for the highest signal-to-noise ratio.



Implementation of Cas9 ADA screening assay in preclinical non-human primate studies

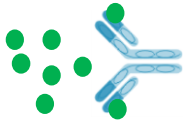
Reproducibility



Assay principle to dissect ADA specificity

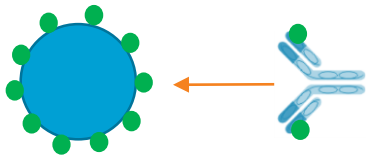
SaCas9 ADA confirmatory assay

ADA preincubation with excess Drug



Incubate drug coated beads with sample containing ADAs

Screening positive ADA



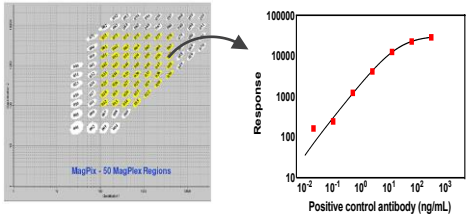
No binding

Detection

Secondary antibody conjugate or protein G/A with biotin

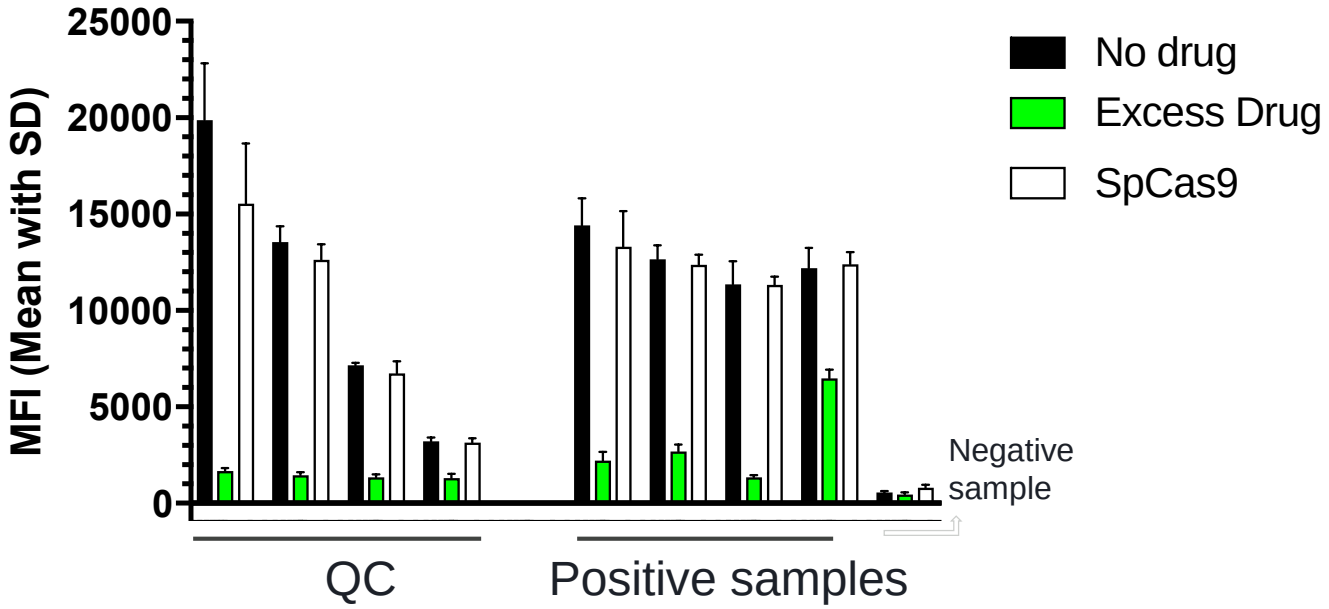
- Biotin

Streptavidin - PE



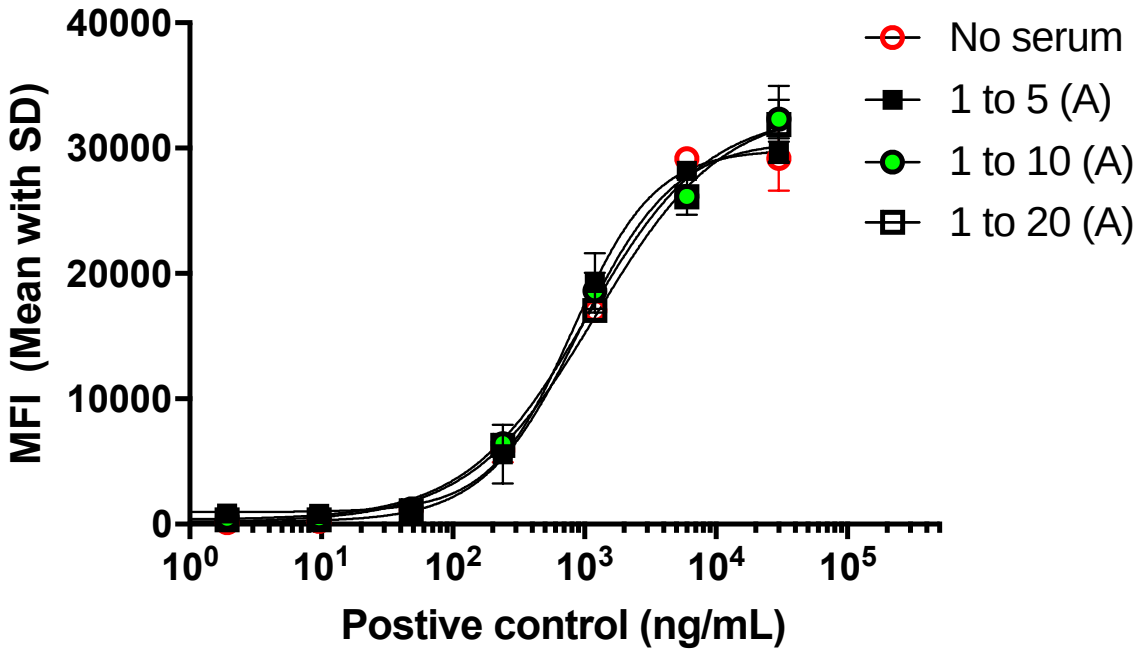
Determination of the specificity of binding antibodies to drug by competition

SaCas9 ADA confirmatory assay



Anti-Drug Antibody Assays for AAV

Evaluation of Cyno serum matrix interference in ADA screening assay for AAV



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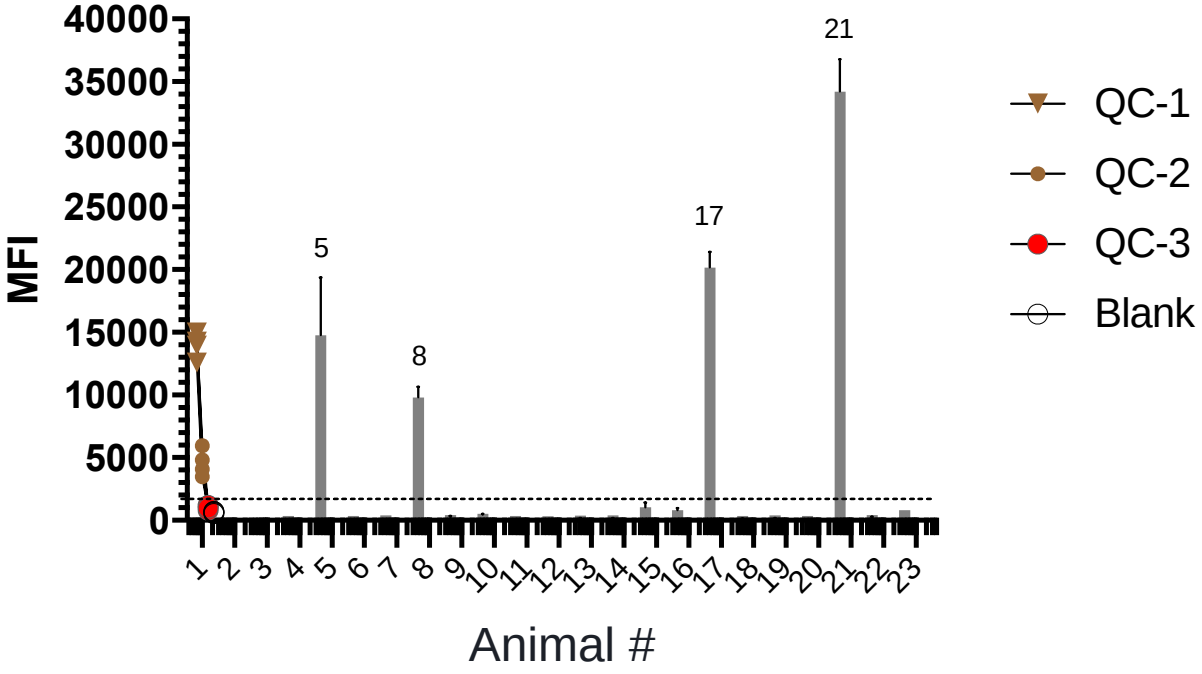

Summary

- AAV vectors can elicit efficient humoral and cellular responses against the vector and transgene product. Therefore it is important to develop immunogenicity assays in the early stage of drug development to understand the factors which influence PK, efficacy of the drug or induce unwanted side effects.
- A sensitive Luminex-based method has been developed and implemented in preclinical non-human primates and human studies to screen for antibodies against AAV and transgene Cas9. The greater sensitivity allows for the detection of low levels of ADAs.
- The flexibility of the luminex xMAP technology allows multiplex ADA or other protein analysis which could be important for small samples, such as tear, mouse tail blood, eye vitreous and aqueous humor. It could also save on time and resources.