
SWAr

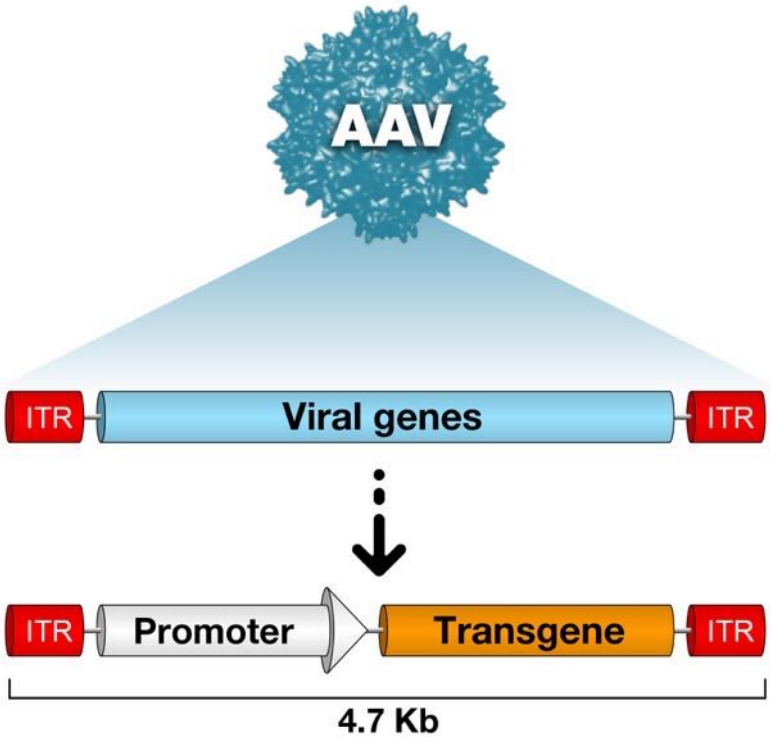
Answers in Life Science

Orthogonal Approach for AAV Immunogenicity Assessment: Evaluating Total and Neutralizing Antibodies

Dr. Michael G Tovey
Chief Scientific Advisor, Svar Life science

Adeno-Associated Viral Vectors (AAV)

- Today adeno-associated viral vectors (AAVs) are the predominate vector for performing *in vivo* genetic modifications.
- Non-pathogenic
- Transduction of dividing/quiescent cells
- Long-term expression
- Variety of serotypes with different tropisms
- High titer preps can be obtained



AAV-mediated Gene Therapy

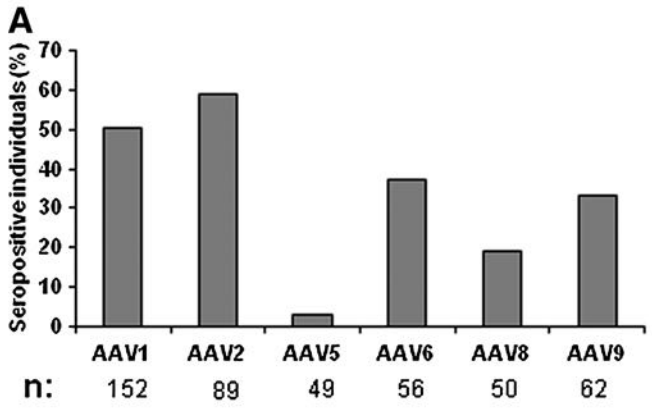
CHALLENGES AND HURDLES

- Low cloning capacity (4.7 Kb)
- Viral vectors carry the risks of toxicity, inflammatory responses, and targeting issues
- Long term therapeutical effect is still under evaluation
- Very high cost; Zolgensma $ 2.1 million per treatment
- Anti-AAV antibodies can reduce gene therapy effectiveness and trigger immune reactions.

AAV-mediated Gene Therapy

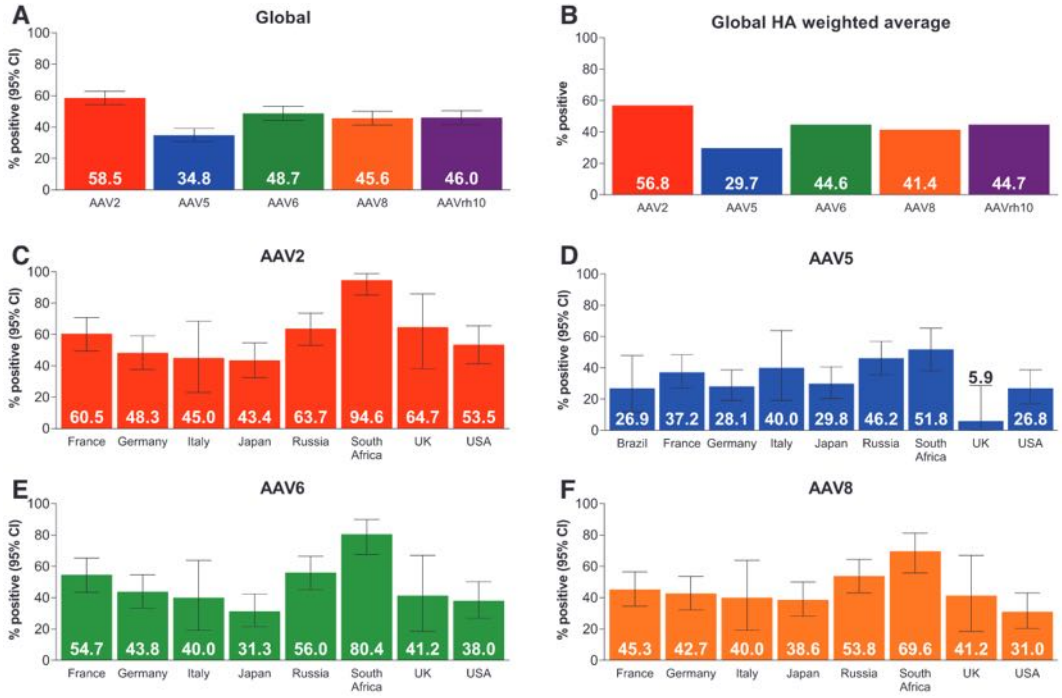
ANTI-AAV ANTIBODIES

Previous exposure to WT AAV virus in up to 90% of the human population, often during early childhood.



NAbs

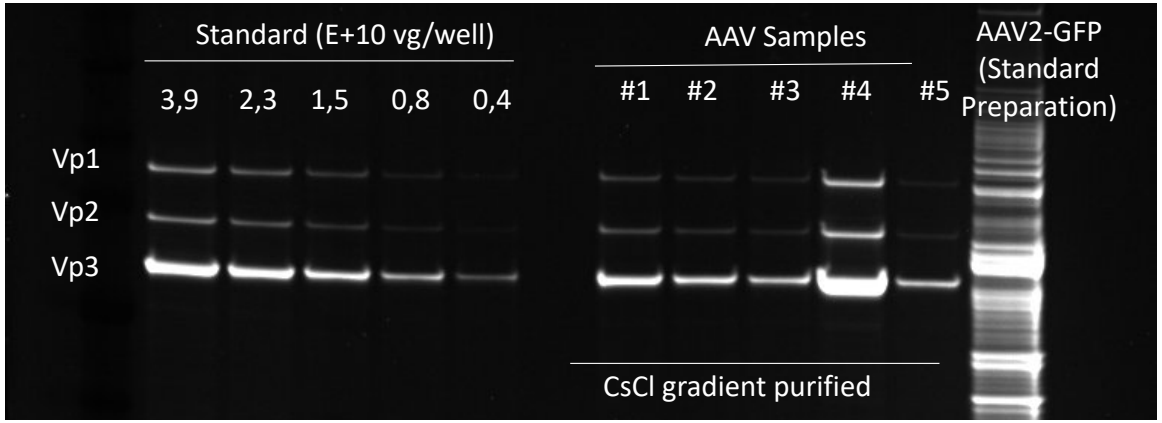
Cell-based assay
Boutin, S. *et al. Hum Gene Ther* 21, 704–712 (2010).



TAb

Bridging total antibody assay
Klamroth, R. *et al. Hum Gene Ther* 33, 432–441 (2022).

Analysis of AAV Preparations



NB: CsCl density gradient ultra-centrifugation can not be used to purify clinical material since CsCl is a neuro-toxin

AAV Titration: Sypro Ruby Staining

Immune Response to AAV

All preparations of recombinant AAV vectors, including those administered to patients contained in addition to whole virus expressing a transgene:

- Empty capsids often in a large excess
- Individual capsid proteins
- Non-encapsulated viral DNA
- Cellular components from the packaging call

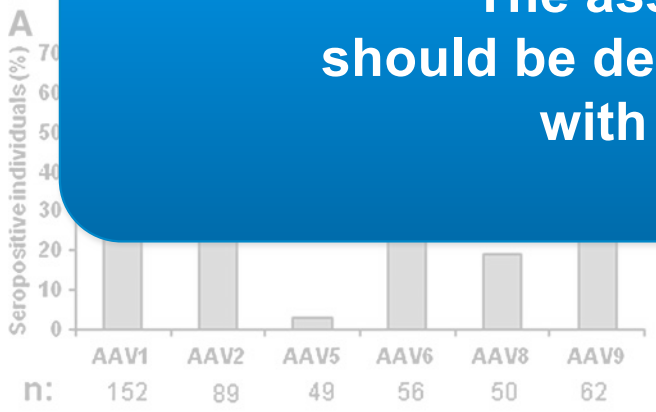
All these components are potentially immunogenic

AAV-mediated Gene Therapy

ANTI-AAV ANTIBODIES

Previous exposure to WT AAV virus in
up to [illegible]
ofte[illegible]

The assessment of anti-AAV antibodies should be determined prior to treatment of patients with AAV-mediated gene therapies



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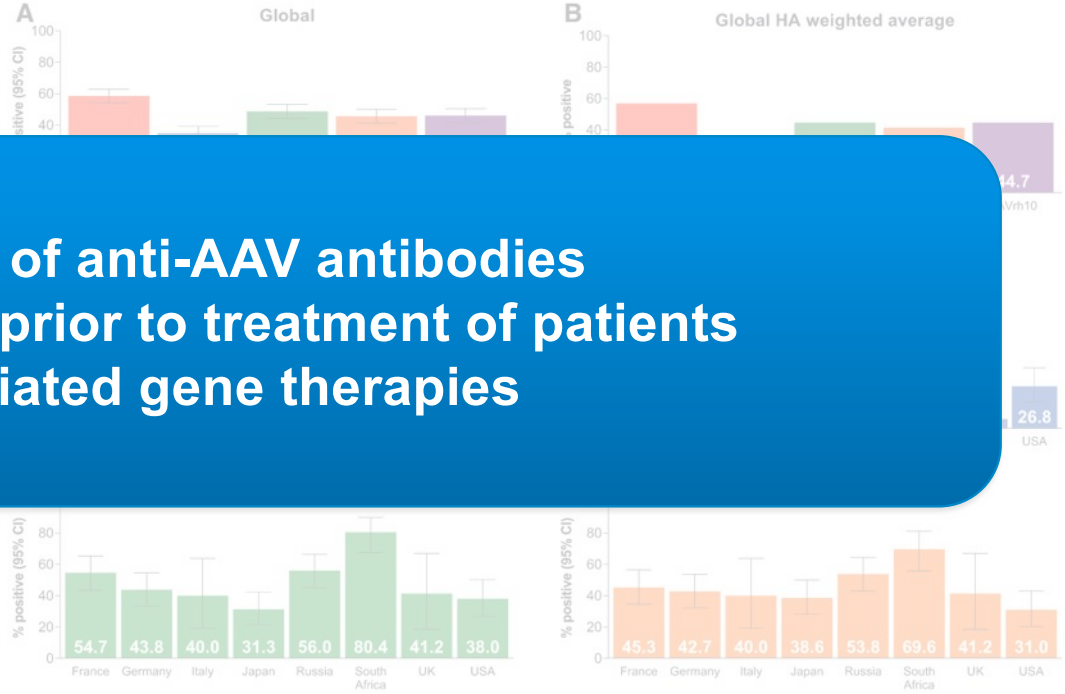

TAb

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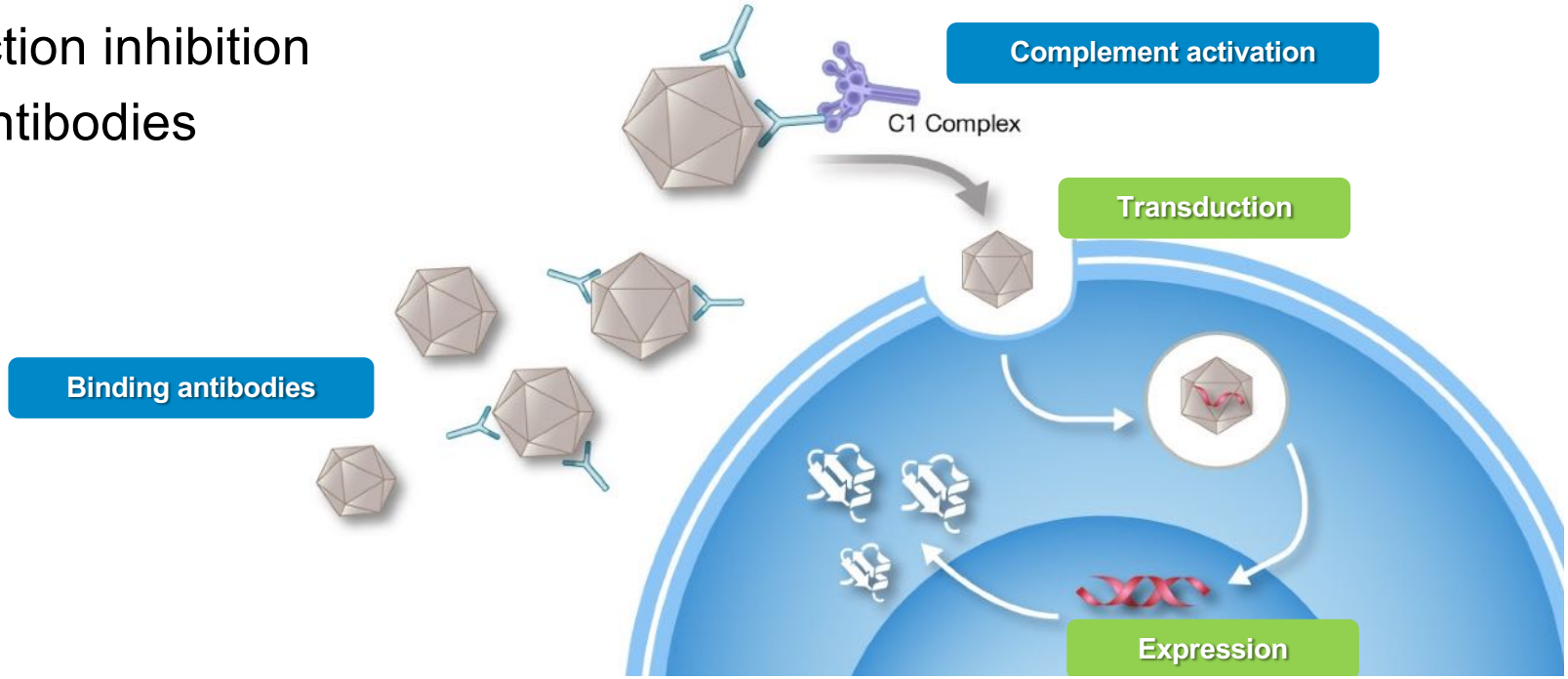
Assays for AAV Gene Therapy
ANALYTICAL PLATFORMS for HUMORAL IMMUNITY

Assays for AAV Gene Therapy

ANALYTICAL PLATFORMS

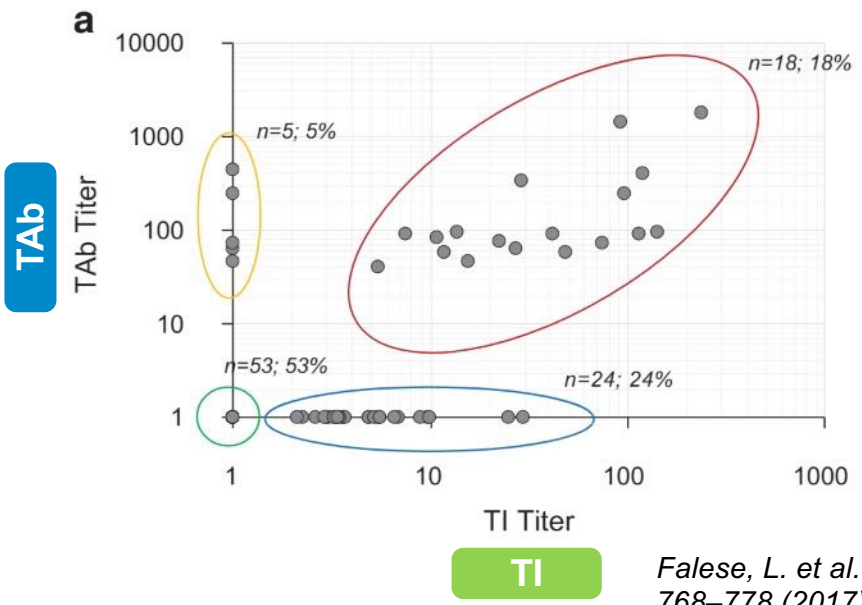
- Immunogenicity – Humoral response
  - TI - Transduction inhibition
  - TAb - Total antibodies



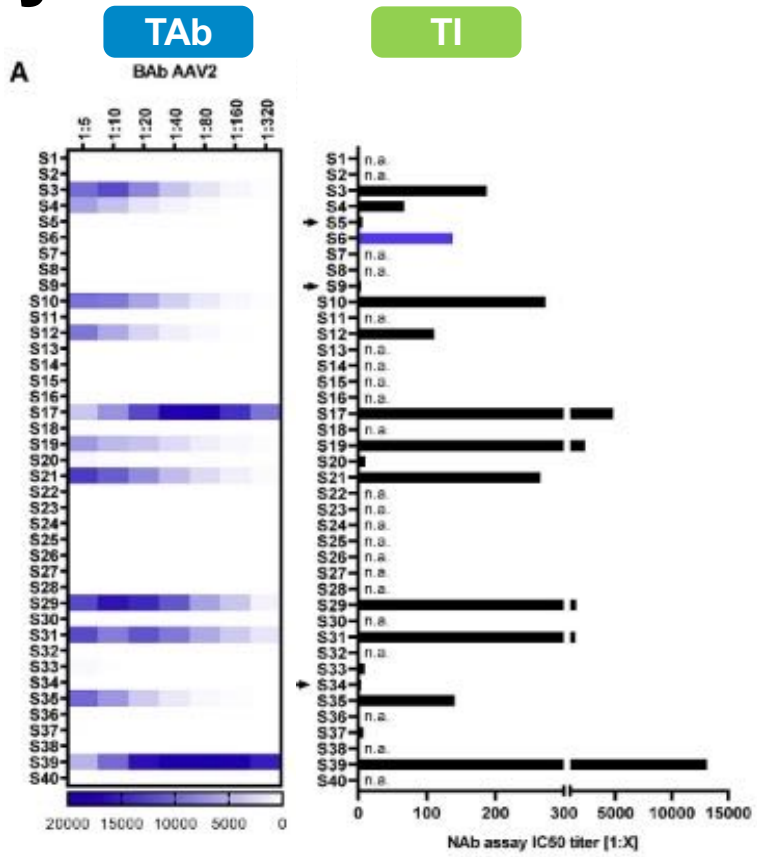
Assays for AAV Gene Therapy

ANALYTICAL PLATFORMS

Discordances observed between TI & TAb assays.



Falese, L. et al. Gene Ther 24, 768–778 (2017).



Haar, J., et al. Mol Ther - Methods Clin Dev 25, 360–369 (2022).

Assays for Gene Therapy

ANALYTICAL PLATFORMS

- Dual assay screening testing proposed for CT
  - Falese, L. *et al. Gene Ther* 24, 768–778 (2017).
  - Stanford, S. *et al. Res Pract Thrombosis Haemostasis* 3, e12177 (2019).
  - Long, B. R. *et al. Mol Ther* 29, 597–610 (2021).

Assays for Gene Therapy

ANALYTICAL PLATFORMS

> *However,* [the assessment of both TI and Tab...] *is likely impractical given the difficulties with standardizing assays*

Schulz, M. *et al.* **Binding and neutralizing anti-AAV antibodies: Detection and implications for rAAV-mediated gene therapy.** *Mol Ther* 31, 616–630 (2023).

"*Another approach could include the generation of pre-clinical data establishing the relationship between these two platforms [*on eligibility criteria*] using well-characterized and robust TI and TAb assays*"


Determination of TAbs
Against AAV2 & AAV6

Assays for Gene Therapy

HUMORAL RESPONSES

Total binding assay measure the total amount of binding antibodies.

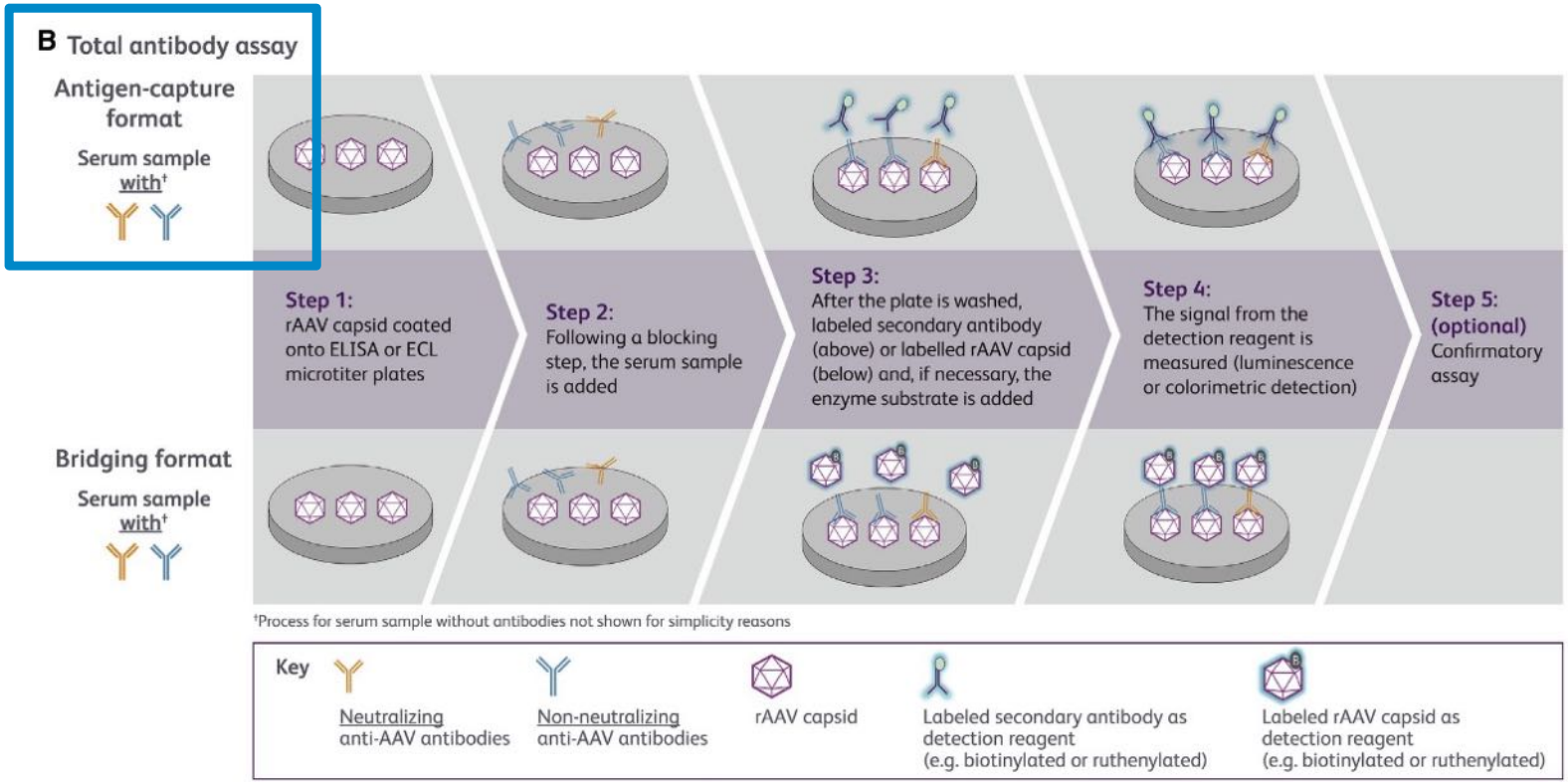
Platform: LBA; ELISA, MSD, etc

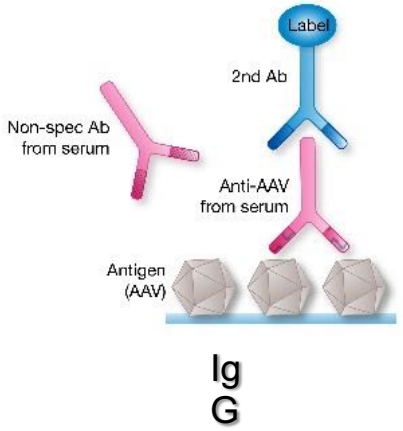
Transduction inhibition assay evaluates the capacity of the antibodies of blocking AAV transgene expression.

Platform: Cell-based (HEK293, HeLa, and Huh7 cell lines)

Assays for Gene Therapy

HUMORAL RESPONSES – TAb





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Assays for Gene Therapy

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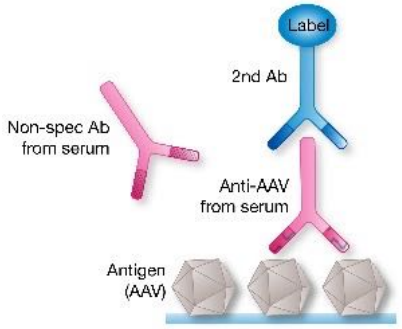
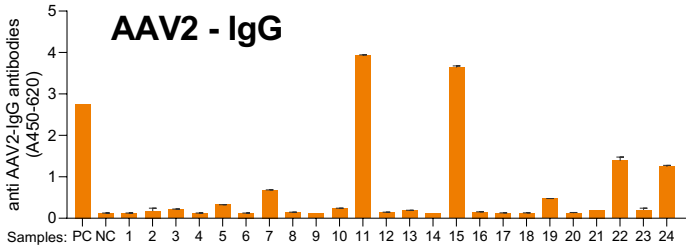
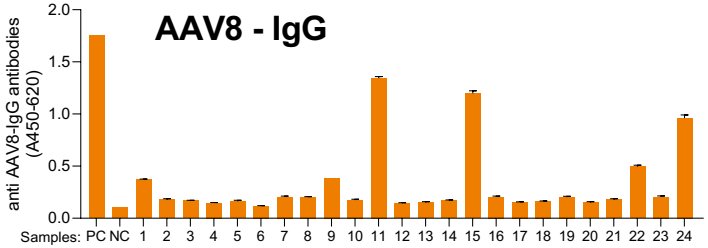
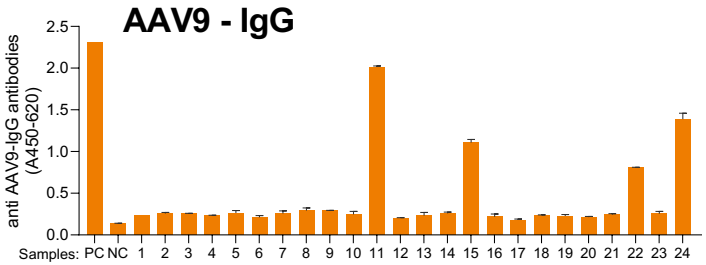


Ig
G

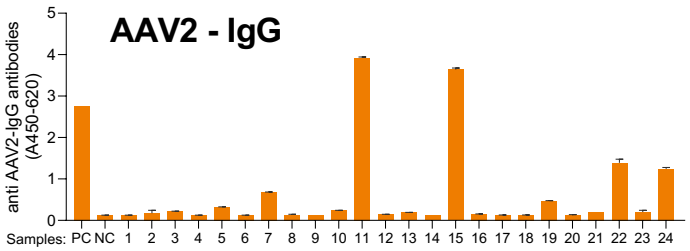


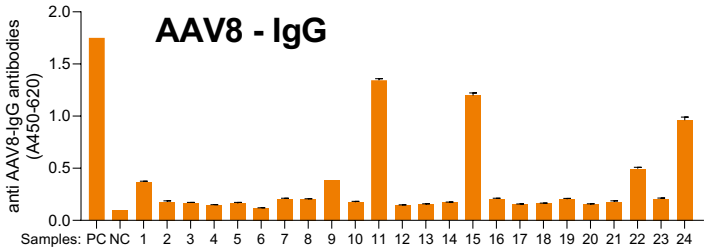


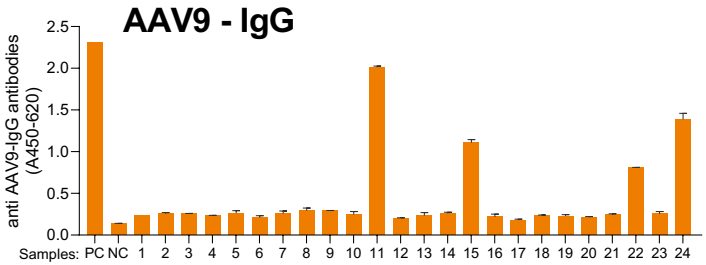


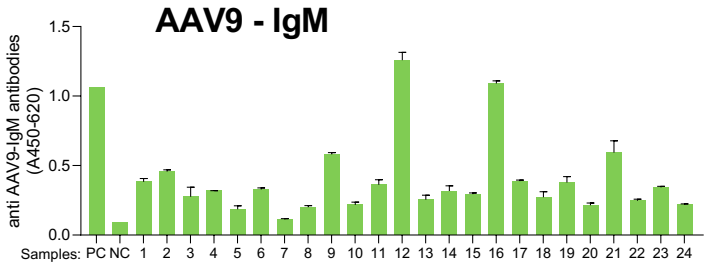
Assays for Gene Therapy

HUMORAL RESPONSES – TAb

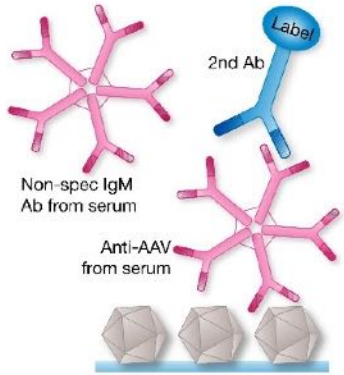


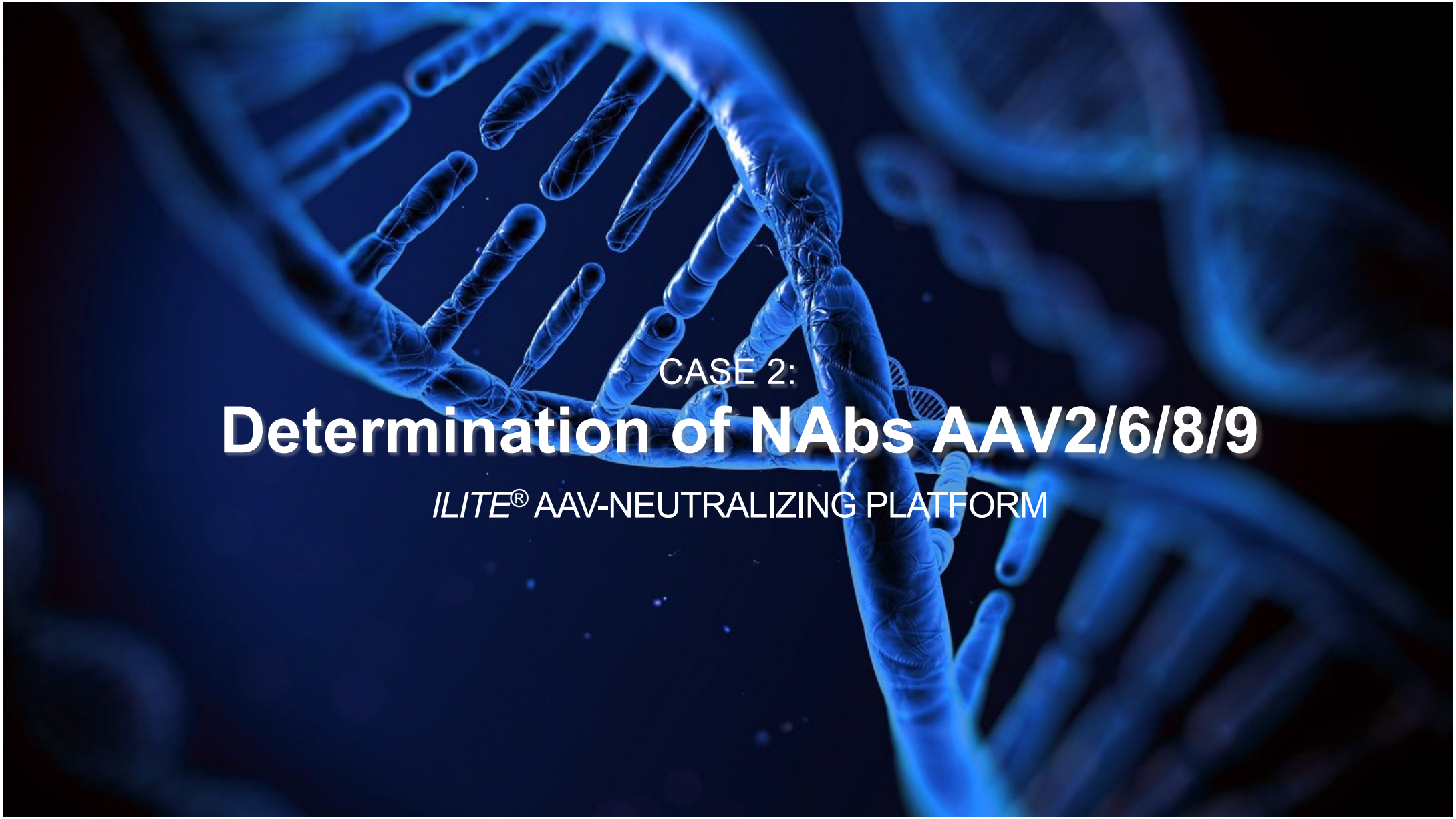






IgM





CASE 2:

Determination of NAbs AAV2/6/8/9

ILITE® AAV-NEUTRALIZING PLATFORM

Assays for Gene Therapy

HUMORAL RESPONSES

Total binding assay measure the total amount of binding antibodies.

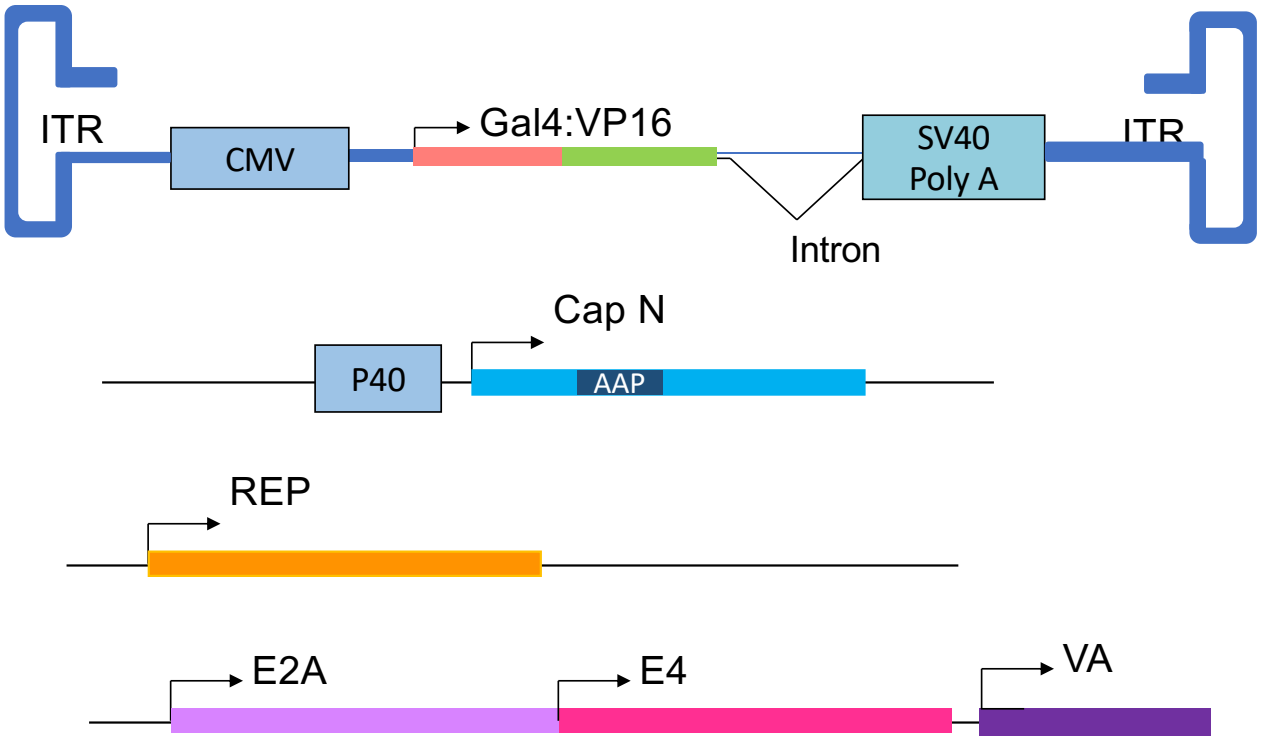
Platform: LBA; ELISA, MSD, etc

Transduction inhibition assay evaluates the capacity of the antibodies to block AAV transgene expression.

Platform: Cell-based using the HEK293, cell line

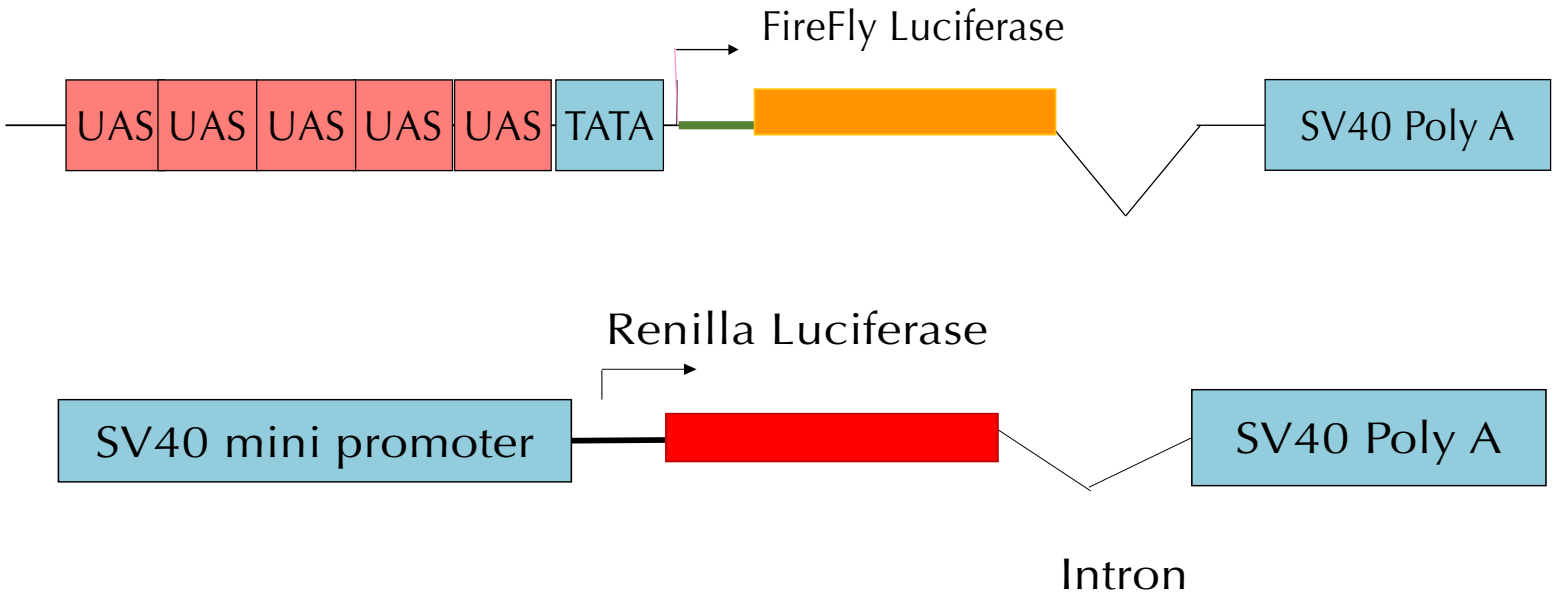
AAV Packaging Cell

MOLECULAR CONSTRUCTS

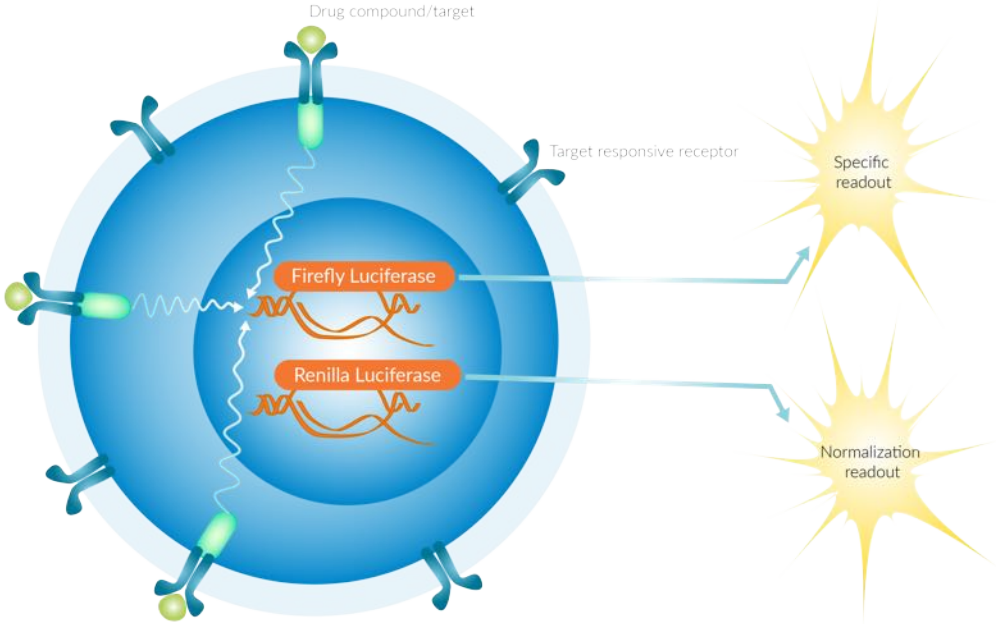


Normalized AAV Responsive Reporter Cell

MOLECULAR CONSTRUCTS



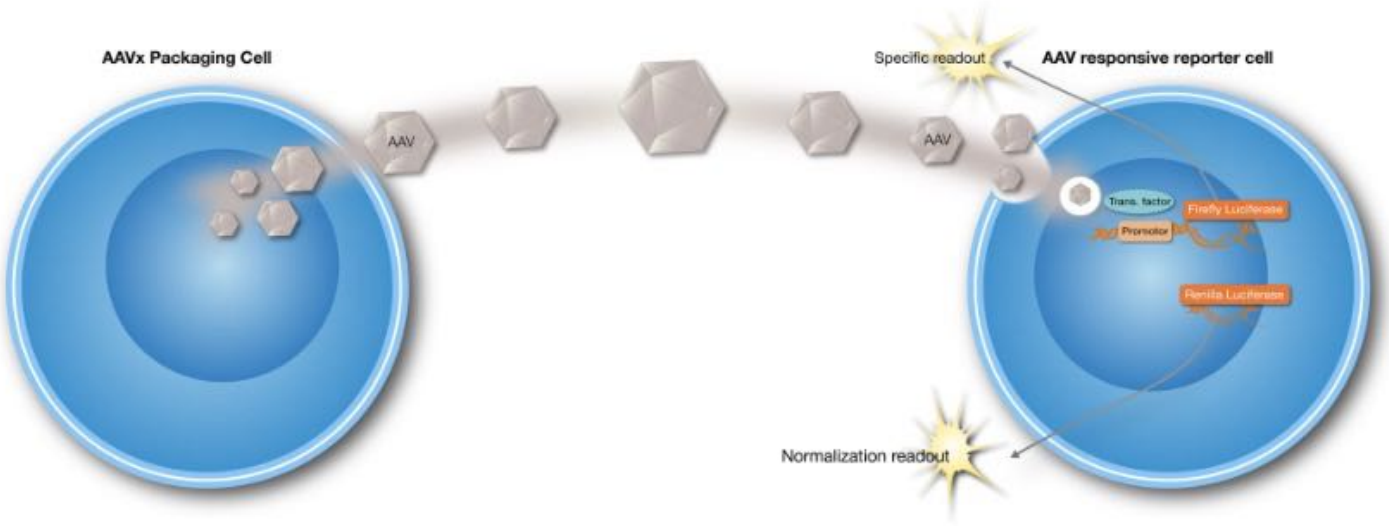
iLite® Reporter Gene Assays



- Assessment of **biological functionality** of a compound
- Reflects the **Mode of Action** (MoA) of a drug
- Can be designed & customized for individual use
- Cells are provided in ”Thaw & Use” **Assay-Ready Format**
  - Decreases **timeline and workload**
  - Offers reliable and **more robust results**
- **Normalization** gene, which eliminates unwanted matrix effects

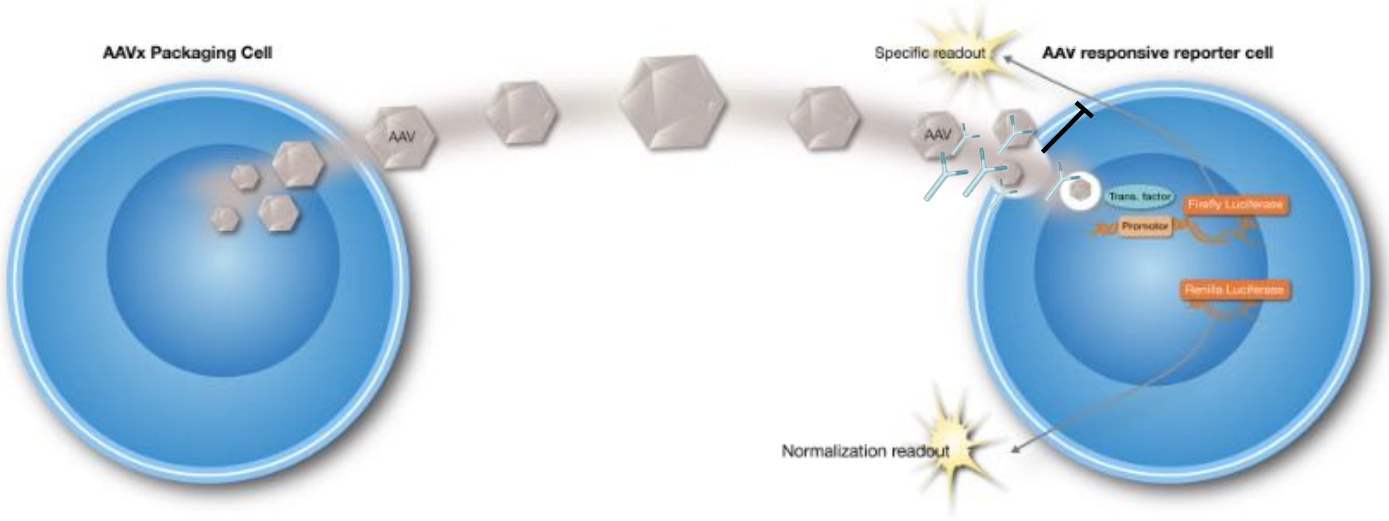
iLite® AAV Nab Platform

- The *iLite*® AAV-Neutralizing Platform for the assessment of anti-AAV NAbs is a novel tool for rapid and reliable detection of an inhibitory humoral response to the viral vector.
- Several serotypes available, including AAV2, AAV5, AAV6, AAV8 and AAV9.



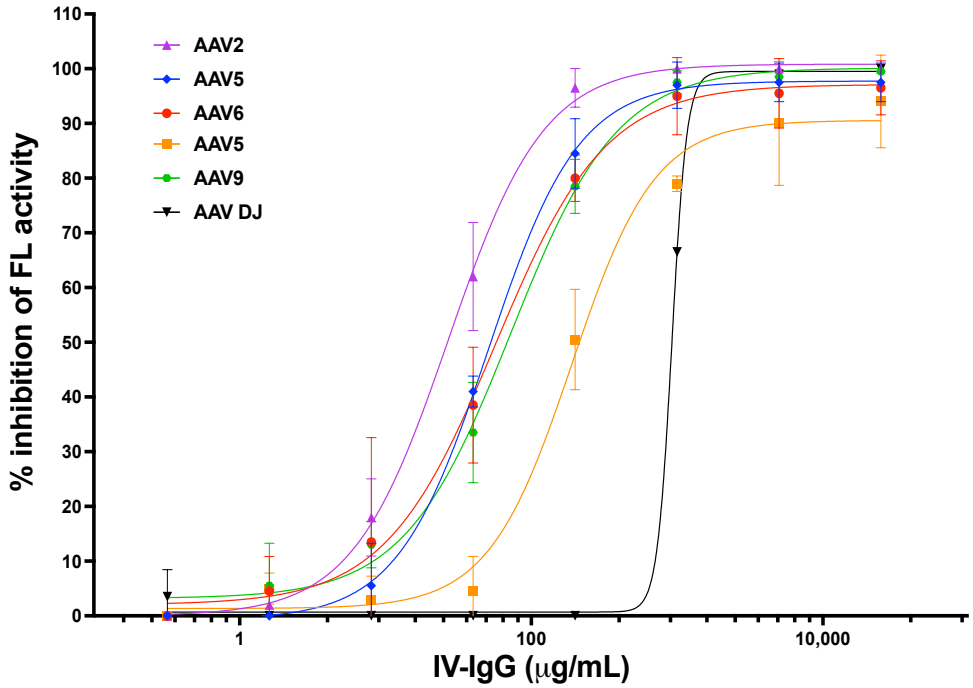
iLite® AAV Nab Platform

- Pre-existing neutralizing antibodies against the viral vector can impact the therapeutic potential of in vivo gene therapy



Detection of Neutralizing Antibodies

TO WILD-TYPE AAV SEROTYPES



	AAV2	AAV5	AAV6	AAV8	AAV9	AAV DJ
EC_{50} (µg/ml)	26,98	189,6	56,7	50,57	72,43	1274

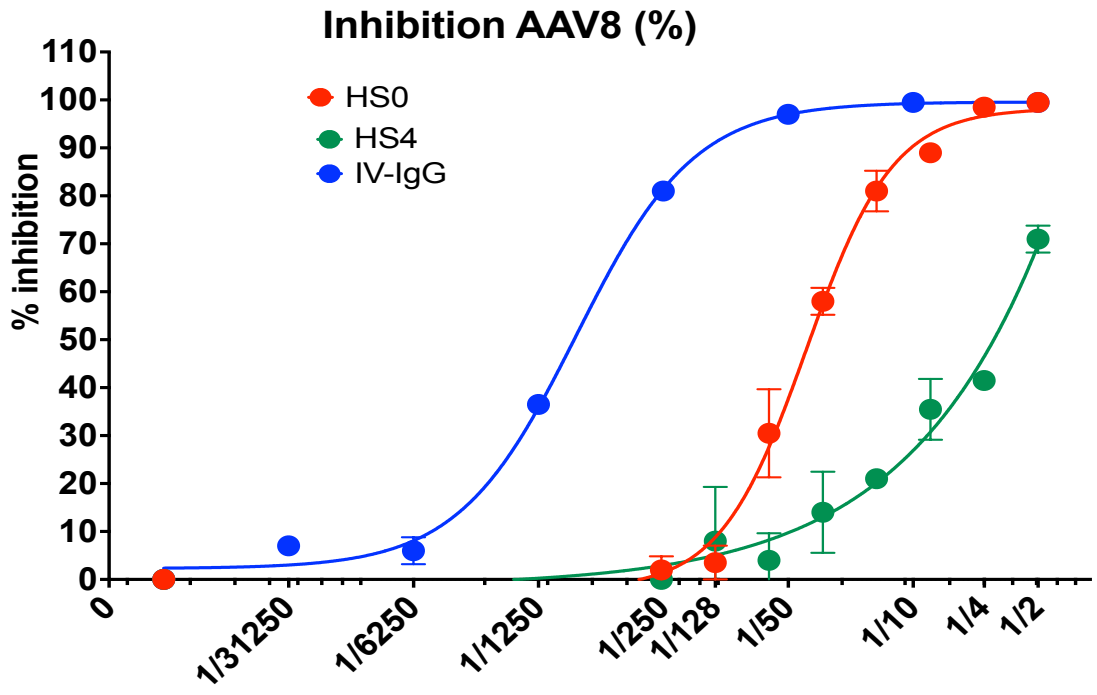
Characterization of Anti-AAV8 NAbs

ILITE® ASSAY

Serum	Neutralizing effect of the mAb	Cellular toxicity of the serum
Serum type 1	-	-
Serum type 2	+	-
Serum type 3	-	+
Serum type 4	+	+

Characterization of Anti-AAV8 NAbs

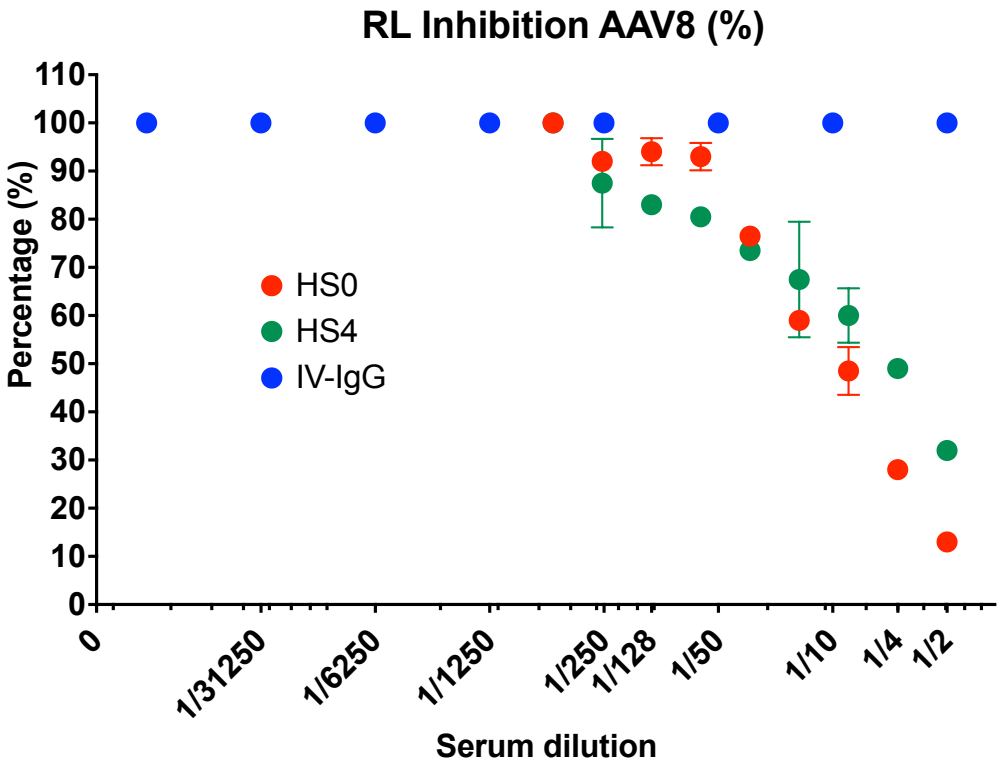
ILITE® ASSAY



Serum	Neutralizing effect
HS0	+
HS4	+
IV-IgG	+

Characterization of Anti-AAV8 NAbs

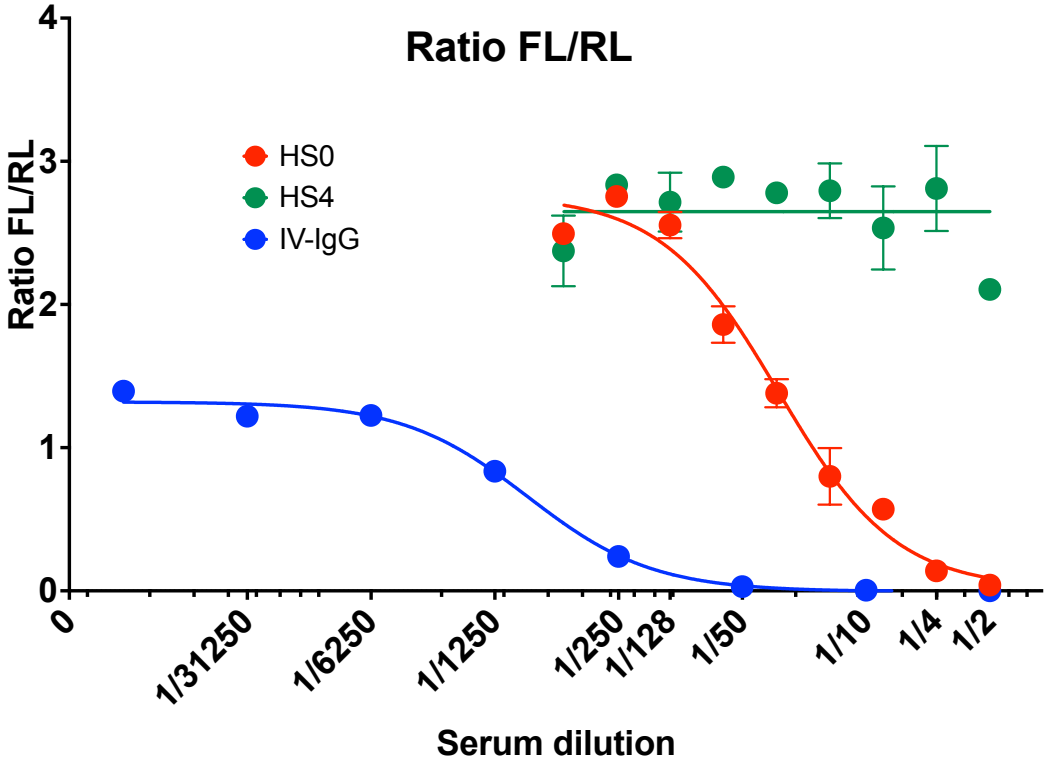
ILITE® ASSAY



Serum	Neutralizing effect (without RL)	Cellular toxicity
HS0	+	+
HS4	+	+
IV-IgG	+	-

Characterization of Anti-AAV8 NAbs

ILITE® ASSAY



Serum	Neutralizing effect (without RL)	Cellular toxicity	Neutralizing effect (with RL)
HS0	+	+	+
HS4	+	+	-
IV-IgG	+	-	+

iLite® Normalized AAV Responsive Reporter-Gene Assay

- The i*Lite*® AAV transduction inhibition assay can unambiguously distinguish between neutralizing antibodies and other matrix factors that can inhibit virus transduction
- Highly sensitive one-step overnight assay employing thaw & use cells
- Can detect NAbs against WT serotypes, or recombinant AAV vectors, including vectors with rationally designed capsids

Summary

An orthogonal assessment of both total(Tabs) and neutralizing antibodies (Nabs) has the potential to give a clearer picture of the therapeutic outcome.

However – developing and validating AAV TI and Tab assay present technical challenges, and assays can be cumbersome to perform.

We here present assays that can be used in more standardized settings, with robust results and transferable methods.

In addition to WT serotypes,SVAR assays present, for each individual AAV vector, a tailored and versatile customization option for both TI and Tab,

SVAr

Answers in Life Science

THANK YOU FOR YOUR ATTENTION!

We are looking forward to continuing our discussions at our booth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