Expanding access to gene therapy by addressing seropositivity

Parent Project Muscular Dystrophy

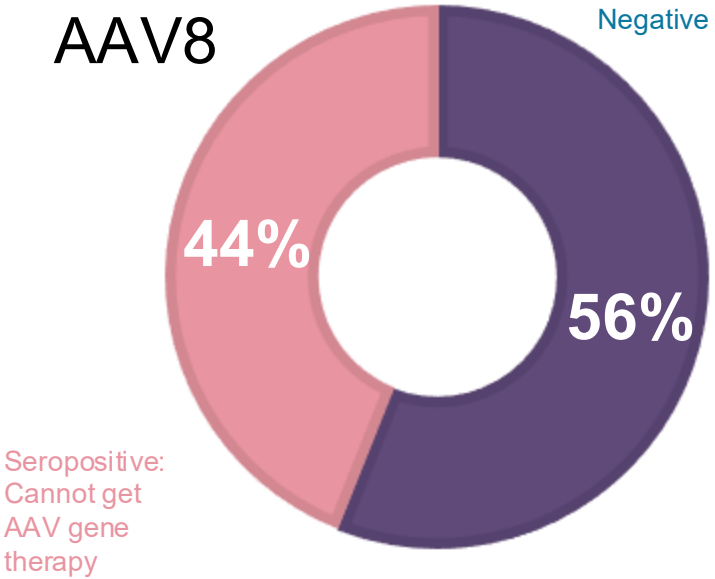
Michelle Rengarajan, MD, PhD

Associate Physician, Massachusetts General Hospital
Instructor in Medicine, Harvard Medical School
Associate Member, Broad Institute

Antibodies to AAV prevent nearly half of patients with DMD from accessing gene therapy.

- Current AAV-based gene therapy in DMD:
  - *Elevidys* (Sarepta, commercial use)
  - RGX-202 (RegenxBio)
  - SGT-003 (Solid Bio)
  - INS1201 (Insmed)
  - SRD-001 (Sardocor)
  - PBGENE-DMD (Precision Biosciences)

- AAV-based approaches include:
  - gene replacement
  - gene editing
  - suppressor tRNA



From: Leborgne et al. Cell. Immun. 2019

Seropositivity is an exclusion criteria because of both efficacy and safety concerns.

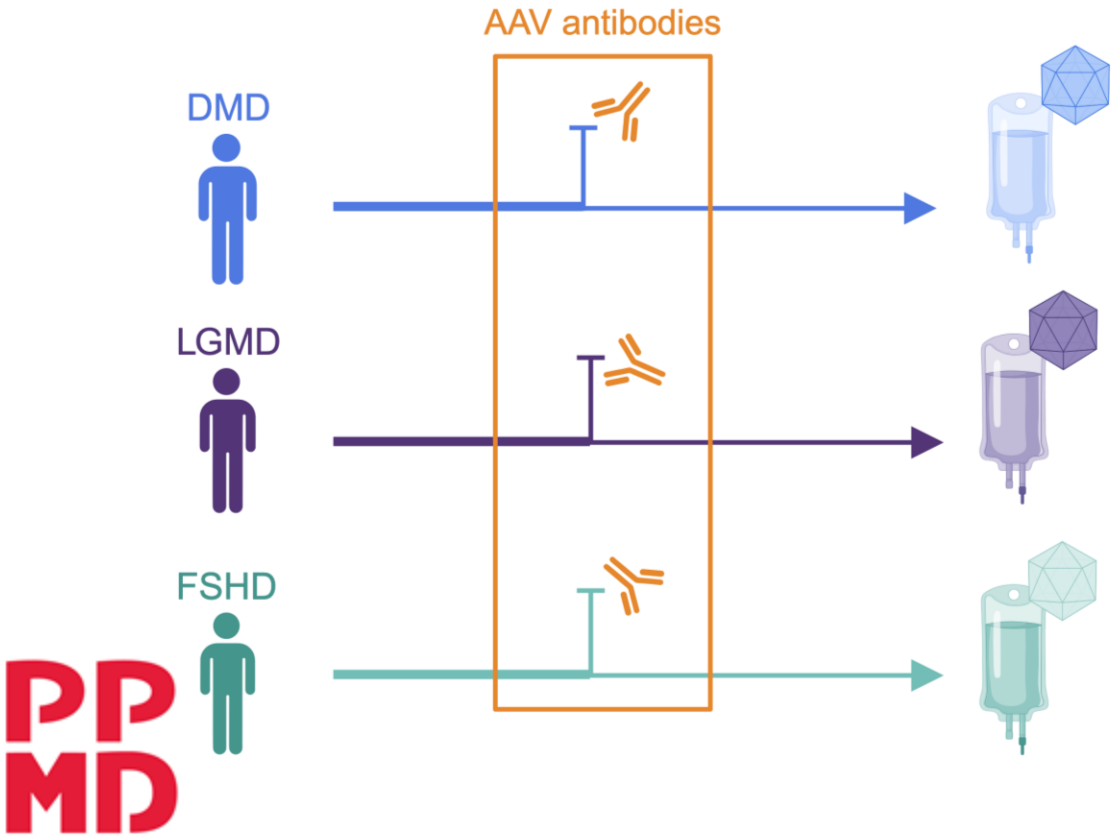
AAV

Antibody

Ineffective therapy

Adverse events

ALLiance for the study of AAV Immunity (ALL in AAV)



- PPMD sponsored a meeting at the Broad Institute in November 2024
- How can we address barriers to treating seropositive patients?

ALL in AAV: A cross-disease, industry/academic consortium focused on AAV immunology.

- Focus on pre-competitive areas to increase AAV access and safety across therapies
- Collaboratively addressing:
  - Immunological adverse events
  - Seropositivity
  - Redosing
- Housed in a new MA-based 501(c)3 public charity

ALL in AAV: A cross-disease, industry/academic consortium focused on AAV immunology.

DEFINITIONS:

Seropositivity

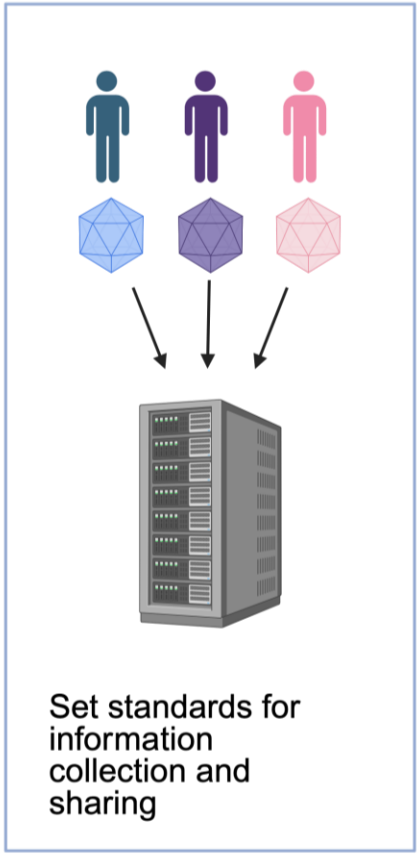
- Antibodies are present PRIOR to AAV dosing ("natural immunity", "environmental exposure")
- Goal: Decrease antibodies to enable a 1st AAV gene therapy treatment

Redosing

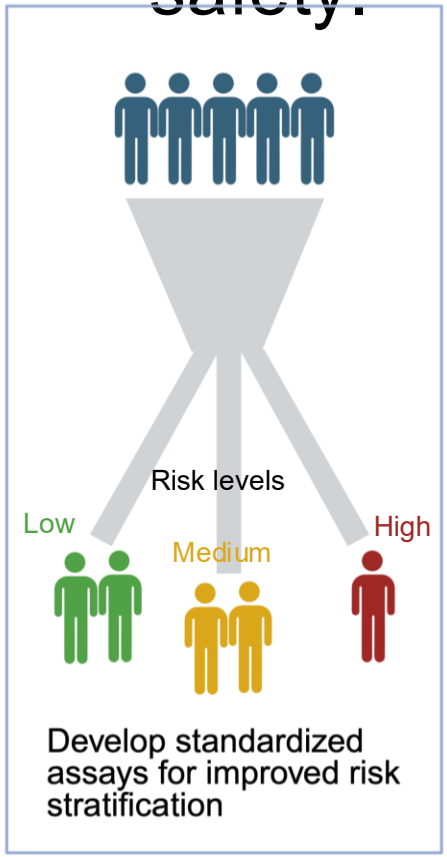
- Re-dose someone who has received a first dose
  - Goal: Decrease high level antibodies that develop AFTER AAV treatment to enable a 2nd AAV treatment
- Pre-treating patients at their 1st AAV gene therapy to enable a future repeat dose
  - Goal: Prevent high level antibodies from developing

ultragen
REGENER
SO BIOSC
DYNC THERAPEUTI
NG NabG
GEMMABio
SAREPTA THERAPEUTICS
UMass Chan MEDICAL SCHOOL
Massachusetts General Hospital Founding Member, Mass General Brigham
UCL GREAT ORMOND STREET INSTITUTE OF CHILD HEALTH
Boston Children's Hospital

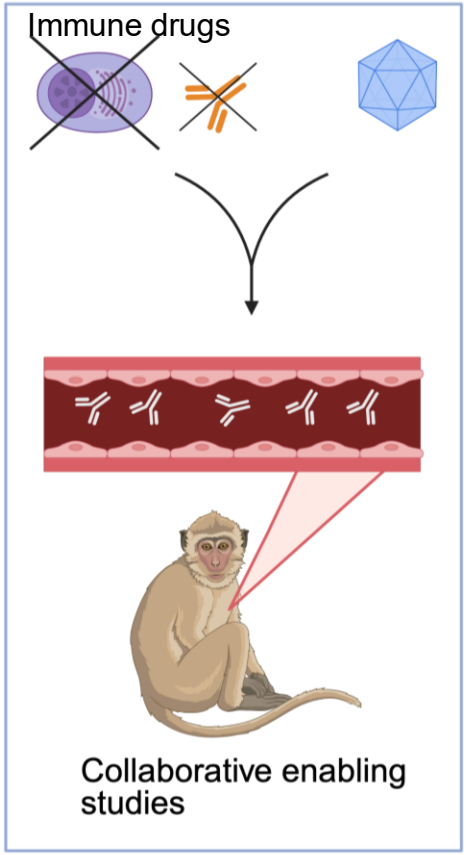
We are taking a collaborative, cross-disease approach to address 3 types of gaps in seropositivity and AAV safety.



Set standards for information collection and sharing



Develop standardized assays for improved risk stratification



Collaborative enabling studies

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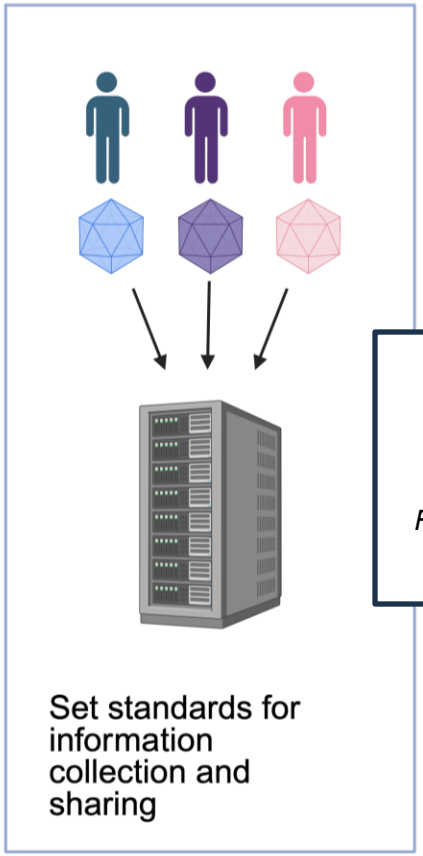

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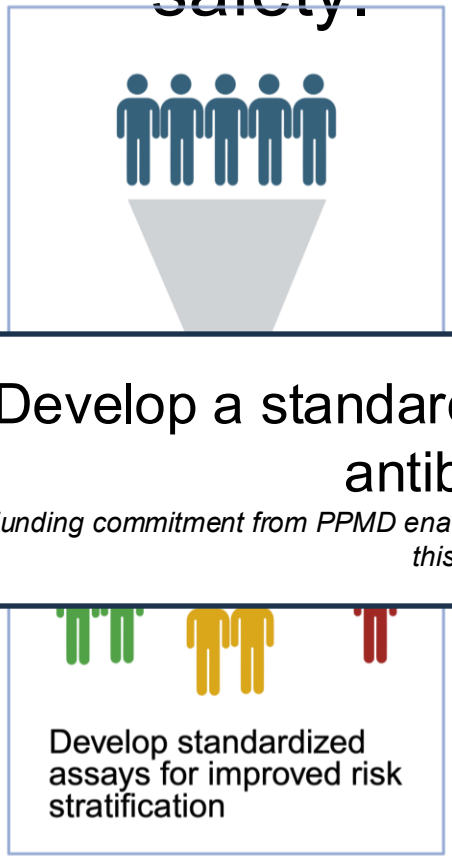

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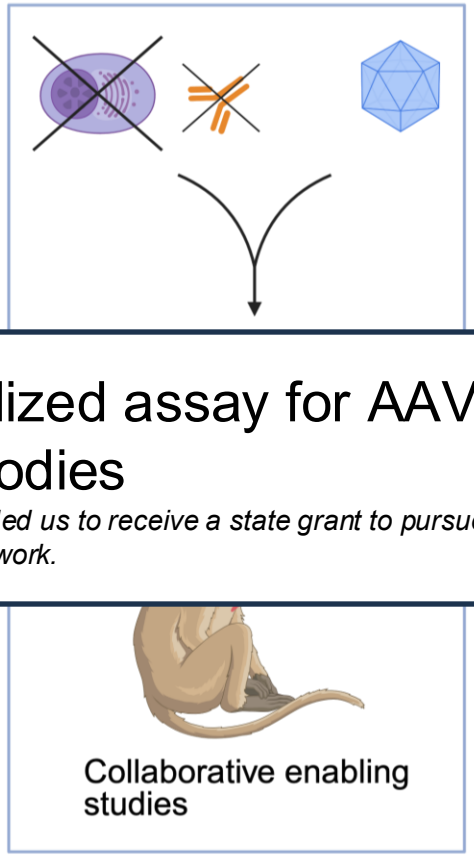


Collaborative enabling studies

Develop a standardized assay for AAV antibodies

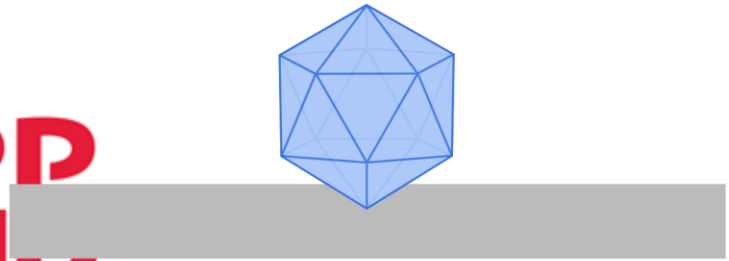
Funding commitment from PPMD enabled us to receive a state grant to pursue this work.

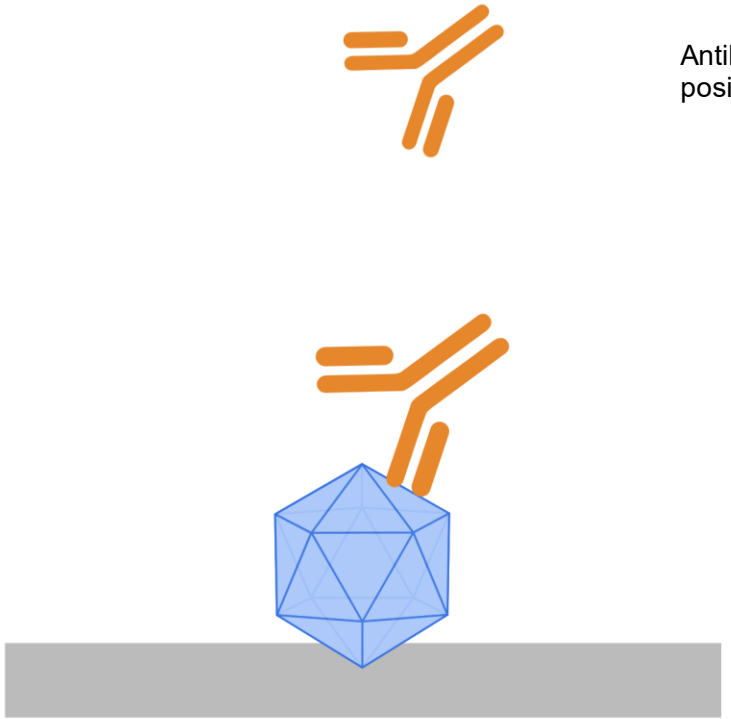
Why do we need a standardized antibody assay

- Assay development and regulation is complex
- Antibody assays and exclusion cutoffs vary widely among sponsors.
- Lack of standardization make is challenging to translate results across different studies
- Clinical antibody testing is linked to a specific product
- This is a critical industry-wide gap in addressing seropositivity

Binding antibody assays measure antibody binding to AAV capsid.

Antibody negative





Antibody positive

Binding antibody assays measure antibody binding to AAV capsid.

Antibody negative

Antibody positive

Why do we need a standardized antibody assay

What is a titer?

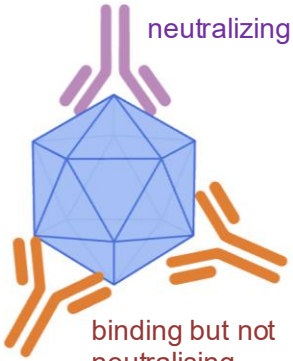
- The most a blood sample can be diluted while still detecting antibodies to AAV.
- Negative: Less than 1:5
- Low: 1:10 to 1:100s
- High: Greater than 1:1000

Capsid	Type of Assay	Cutoff
AAVrh74	Binding	1:400
AAV9	Neutralizing	Not disclosed
AAV8	Binding	Not disclosed “Detectable”
SLB101	Not disclosed	Not disclosed
AAV9	Binding	1:50
AAV1	Neutralizing	Not disclosed
AAV9	Not disclosed	Not disclosed

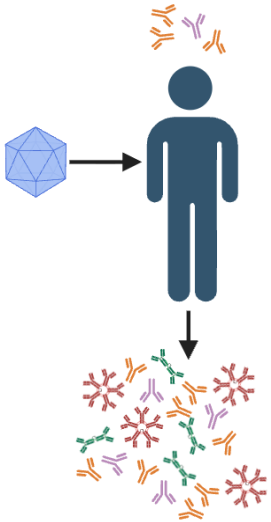
This standardized assay has several requirements

Any serotype

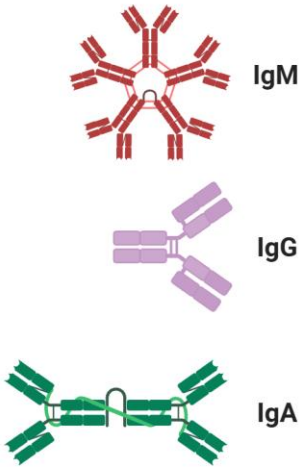
Total binding antibodies detected



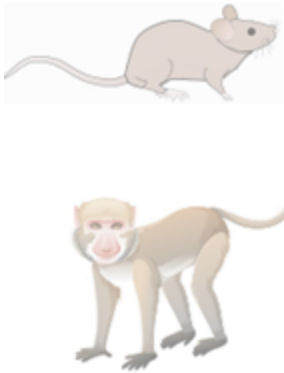
Broad dynamic range



Recognize all antibody classes/subtypes



Nice to have: Easily transferred to other species

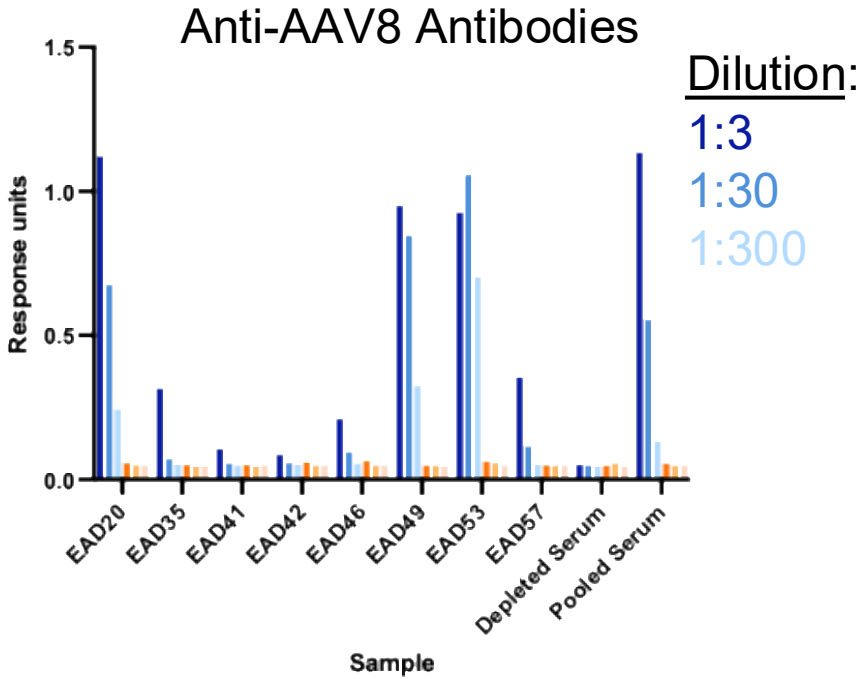


Other practical requirements have contributed to deciding the final assay format

- **Turnaround time**
  - Hands on
  - Hands off
- **Machine Access**
  - Instrumentation distribution
  - Single vendor vs multiple vendor instrumentation and reagents
- **Cost per test**
  - Input capsid (+ labelling)
  - Assay-specific reagents
  - Patient sample volume
  - Batch vs individual runs
- **Consistency**
  - Within 1 experiment
  - Across days
  - Between users
  - With other assays
- **Sensitivity**
  - Monoclonal antibody
  - Serum

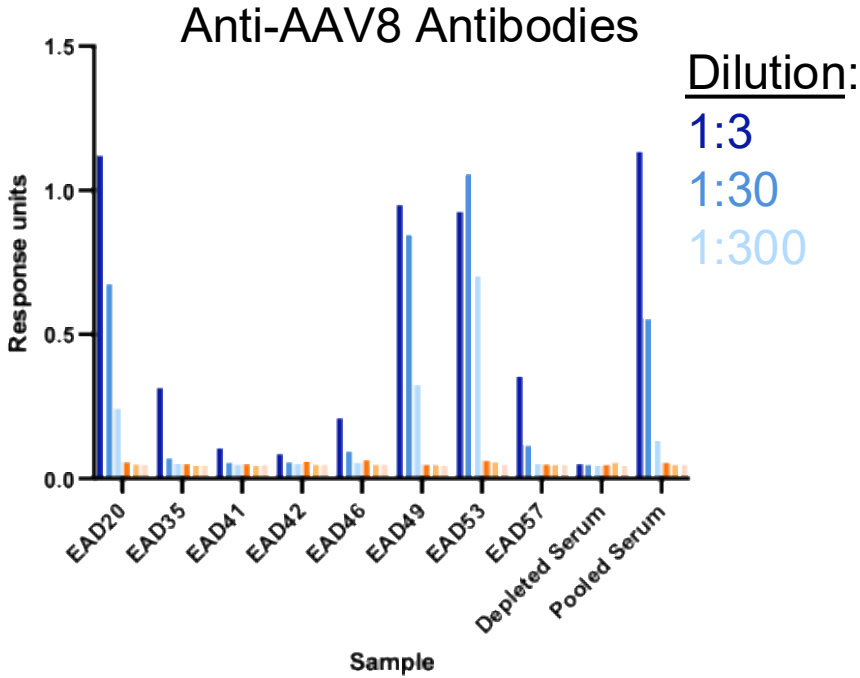
We will use a standard indirect ELISA to detect total binding AAV antibodies.

- We compared 2 different formats (bridging and indirect) in each of 3 different ELISA assays (standard, GyroLab and MSD).
- All 6 versions met regulatory standards for sensitivity
- We selected indirect ELISA based on background noise, access, and cost per test.
- We are generating new positive control standards.

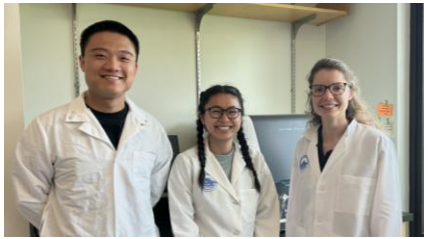


We will use a standard indirect ELISA to detect total binding AAV antibodies.

- We are performing larger-scale assay testing
- We will compare the assay to other available testing in upcoming preclinical studies.
- We will transfer the test to a clinical lab.
- We will initiate regulatory interactions.



Acknowledgements



Camille Wouters
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Kristine Le
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Wendy Heywood
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Qin (Helen) Huang
Simon Pacouret

Contact: rengarajanlab@mgb.org