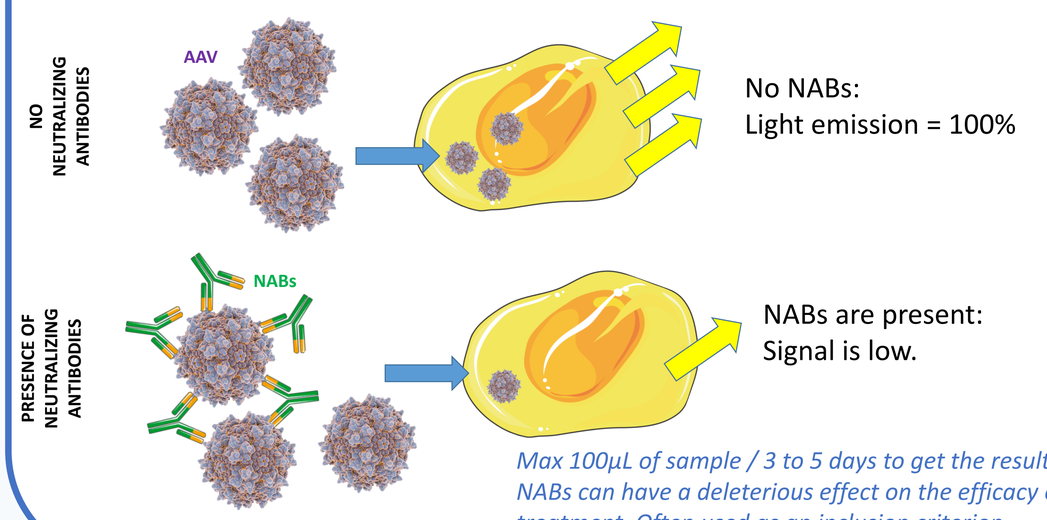
ANTI-DRUG ANTIBODIES ASSAYS FOR AAV GENE THERAPY PROGRAMS

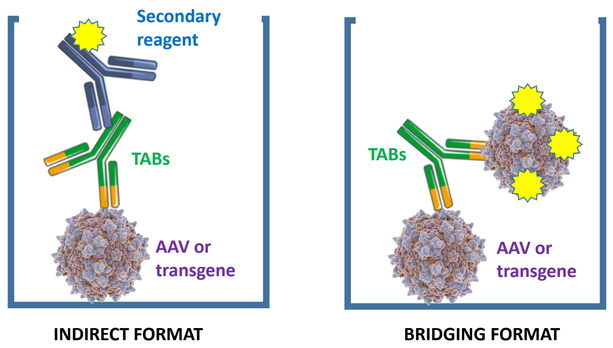
CHOOSE YOUR FORMAT

NEUTRALIZING ANTIBODIES (against the capsid): Cell-based transduction inhibition assay



Max 100µL of sample / 3 to 5 days to get the result.
NABs can have a deleterious effect on the efficacy of treatment. Often used as an inclusion criterion.

TOTAL ANTIBODIES (against the capsid or transgene): ELISA or ECLA (or Western blot)*



Indirect or bridging ?

Indirect: easier to validate, easier to maintain for a long term study, cheaper

Bridging: highly adaptable from a species to another, high specificity

ELISA or ECLA ?

ELISA: several suppliers for reagents, cheaper

ECLA: high sensitivity, less dilution required

1 day to get the result.
TABs may or may not have a biological effect on the treatment.

In case of difficulty with ELISA/ECLA, GenoSafe can develop a Western blot approach (capillary or classical)

CHOOSE THE VALIDATION LEVEL

R&D

Exploratory studies

- Positive control
- Negative control
- Subjective assay cut point

Typically available in less than 1 month

Qualified assay

Supportive data in regulatory studies

- 2 to 3 levels of positive controls
- Negative control
- Minimal Required Dilution
- Definition of preliminary acceptance criteria for assay controls
- Preliminary statistical assay cut point
- Preliminary assay sensitivity
- Matrix interference

Available in 2-3 months

Validated assay

Mandatory for regulatory studies

- 2 to 3 levels of positive quality controls
- Negative quality control
- Minimal Required Dilution
- Internal control (ISR if possible)
- Statistical cut point
- Evaluation of assay sensitivity, specificity, precision
- Matrix and drug interference if possible
- Stability
- Robustness
- Follow up of assay performance

Requires at least 4-6 months

STRATEGY OF ANALYSIS: 3 COMPLEMENTARY STEPS WITH 2 COMPLEMENTARY ASSAYS

SCREENING
Can I detect ADA in my sample ?

TITRATION
I wish to evaluate the amount of ADA in my sample

CONFIRMATORY
Can I confirm antibodies are responsible for the positive signal ?

Preclinical study

TABs (+/- NABs)

- Screen (all samples)
- Titration on positive samples

From preclinical study to the clinical trial: anticipate!

Some assays can easily be adapted on human matrix. Bridging and/or partial revalidation required.

Some assays require a full revalidation.

Clinical trial

Phases I/II

Confirmatory assay is NOT mandatory.
Validation on disease matrix is not mandatory

Inclusion step*:
- NABs (screen + titration)

Follow-up:
- NABs (screen + titration)
- TAB (screen + titration)

Is analysis of both NABs and TABs still required ?

Confirmatory assay validation

Gather a sufficient amount of matrix from patients : Revalidate screen/titration assays as appropriate

Phase III

The confirmatory assay is mandatory.
Validation on disease matrix is mandatory

Inclusion step*:
- Assess NAB or TAB (screen + confirmatory)

Follow-up:
- Assess TAB (screen + confirmatory)
- If positive, perform TAB titration +/- NABs (screen and titration)

**can be performed in real time*

These are example strategies. You can choose whatever scheme suits you best.

PRACTICAL QUESTIONS

ALREADY VALIDATED BY GENOSAFE

NABs (human): AAV2; AAV3; AAV5; AAV8; AAV9 + client-specific capsids

TABs (human): AAV2; AAV3; AAV5; AAV8; AAV9 + client-specific capsids + client-specific transgene

ADA assays on **multiple species**: NHP, dog, rat, mouse, pig

TYPICAL TURNAROUND TIMES

Real time analyses (for patient's inclusion)
- NABs: 7-10 days from receipt of sample to the signed CoA (and/or transfer file)
- TABs: max 3 days from receipt to the signed CoA (and/or transfer file)

DSMB or other deadlines
- Tell us your need in advance, we manage the planning!

Regular analyses
- If no specific requirement from the sponsor, results are typically transfered within 1 month from receipt

THE ASSAY I NEED IS NOT IN THE LIST OF ALREADY AVAILABLE ASSAYS. WHAT SHOULD I ANTICIPATE FOR ASSAY DEVELOPMENT / VALIDATION

- **Think about time first!!!** Several month are required to validate an assay once all reagents are available
- **AAV ADA assay**: if not a classical serotype of AAV, a batch of recombinant AAV (expressing the firefly luciferase or nanoluciferase transgene for NABs / empty capsids for TABs) – typically 2^E13 to 2^E14 vg
- 10 serum samples from individuals with the target disease (**matrix interference** assessment) – 500µL each
- Aliquot of the drug (for **drug interference** assessment); typically <1^E11 vg
- **Specific transgene ADA assay**: milligrams of the recombinant transgene (amount to be determined after assay development)
- **Preclinical studies**: serum from immunized animals, to be used as positive controls

WHY CHOOSE GENOSAFE ?

More than 20 years of experience in the gene therapy field. Specialized in the validation of multiple assays formats. Great experience in GLP preclinical studies. Validation of multiple assays for clinical trials from phase I/II to III. A team of specialists at your service. Highly adaptable planning. ADA, biodistribution, shedding, biomarkers: you need it, we validate it.