Moving from mAbs to rAAV: how long is the journey to assess product potency?

Focus on differences/challenges that spice up the potency road to Gene Therapy.

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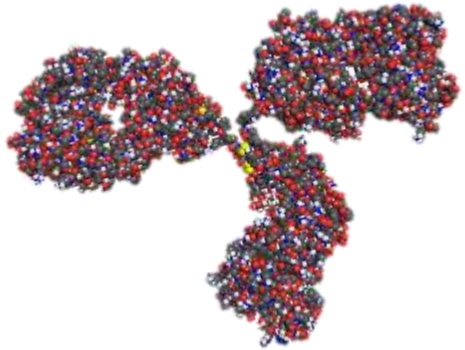
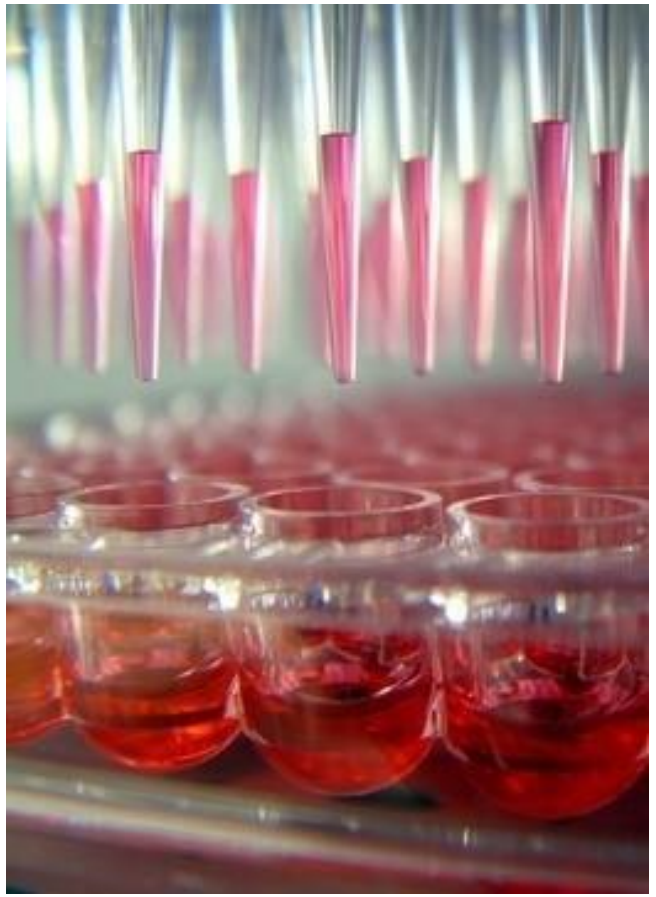
CMC Strategy Forum Europe

Stockholm, 17th Oct 2023

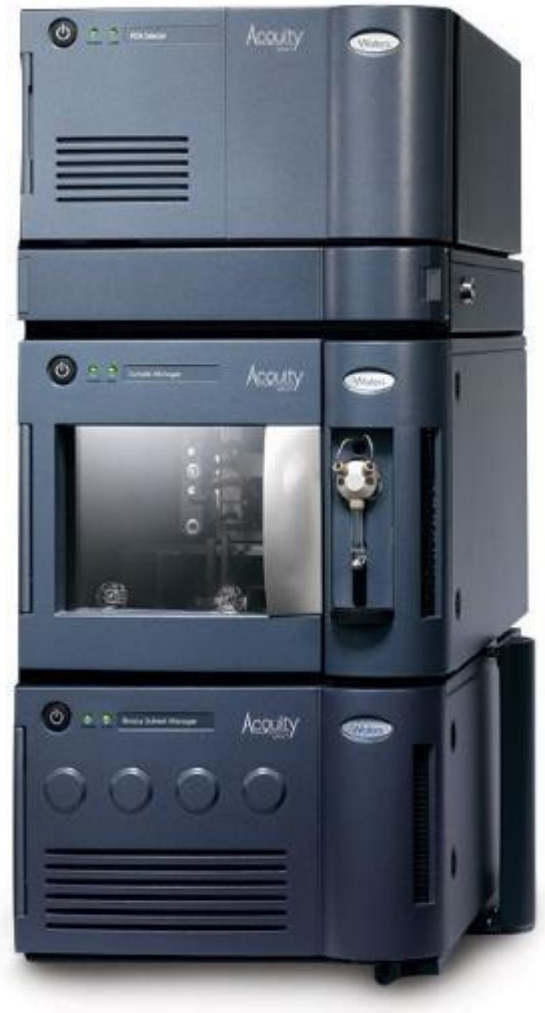
Inspired by **patients.**
Driven by **science.**



What makes mAbs relative potency assay so… unique?



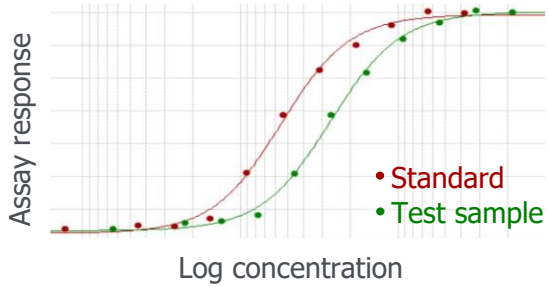
Vs



What makes mAbs relative potency assay so… unique?

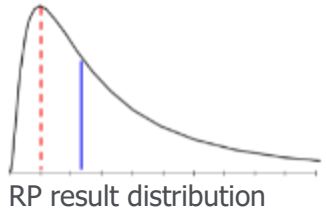
RPs are comparative measurements

Test sample and the Standard dose-response curves must share **similar functional parameters**



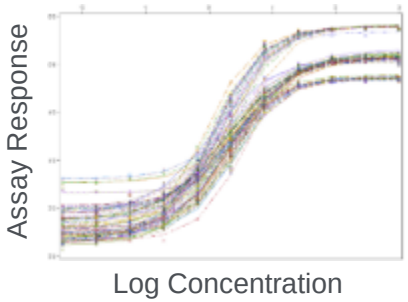
<u>Nonlinear</u> relationship between the response and the analyte concentration
(full dose-response relationship is preferred)

RPs are <u>log normally distributed</u>
impact on the result reporting (e.g., averaging) and on the specifications



Bioassays can be highly variable

… compared to other phys/chem methods



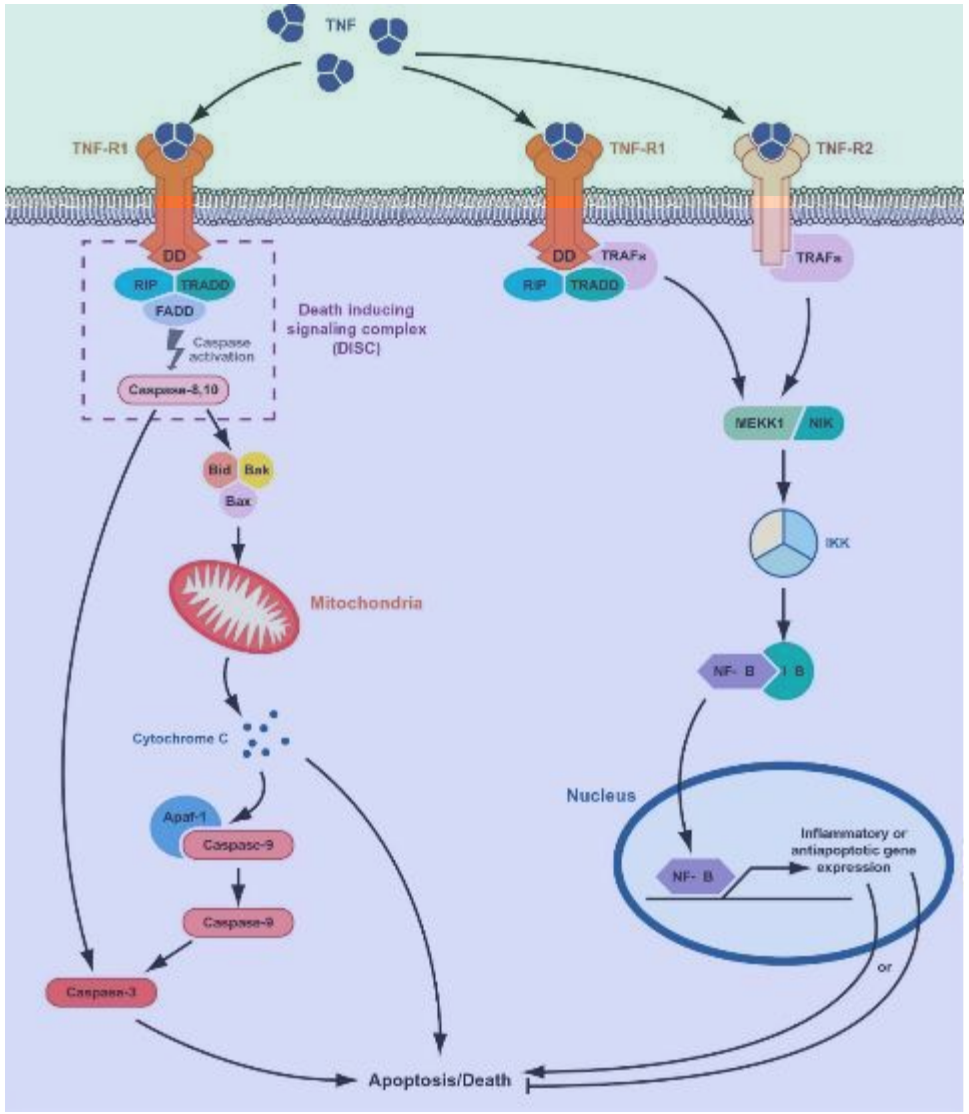
Phase-appropriate Bioassay strategy for MAbs

Early Phase

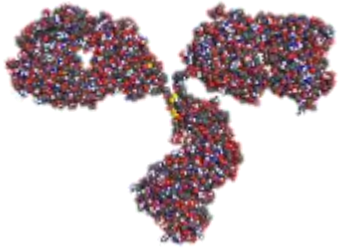
Late Phase

Drug/target binding activity
(e.g., ELISA)

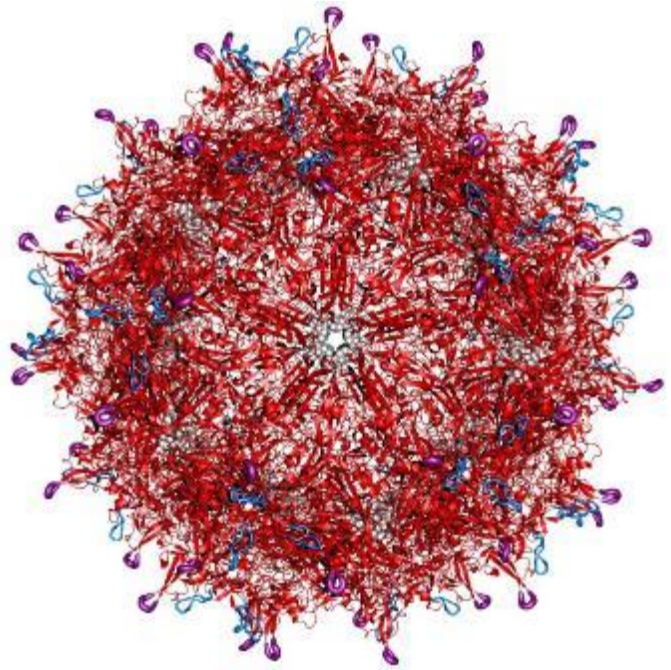
Functional impact on cell signaling pathway/metabolism
(e.g., CBA)



What makes GT products even more… different?



Vs



What makes GT products even more different?

1) Product is NOT the API per se

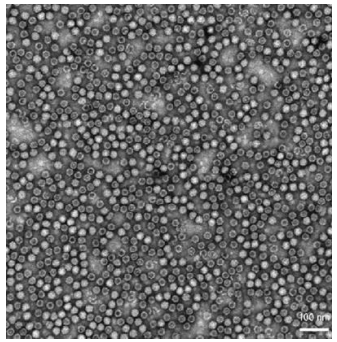
- Unprecedented complexity…

Mabs = ~20k atoms

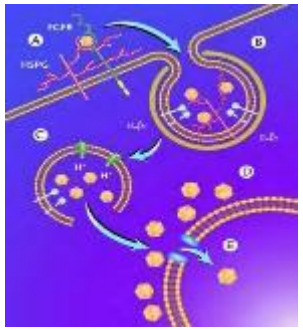
rAAV= > 190k atoms

- Raising new questions…

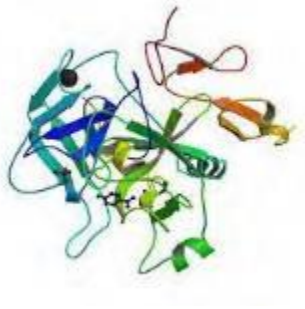
Is my input (plasmid DNA) of good quality?



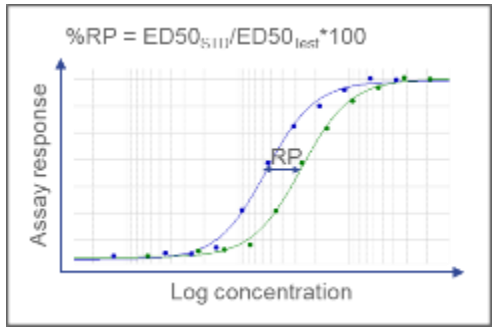
- How many viral particles are in my product?
- Are they full, partiallly filled or empty?



Is my rAAV product still infectious?



Does my rAAV product still produce the API?



Is the produced therapeutic compound biologically active?

… that require additional analytical tools

GTP specificities and impact on potency assessment?

2) Potency should be evaluated at various levels

Multiple Assays (Assay Matrix)* as stepwise approach to evaluate GT product potency

Step 1: Vector Genome titer assay

Measuring the number of viral vector particles containing the therapeutic transgene.

Step 2: Relative infectivity assay

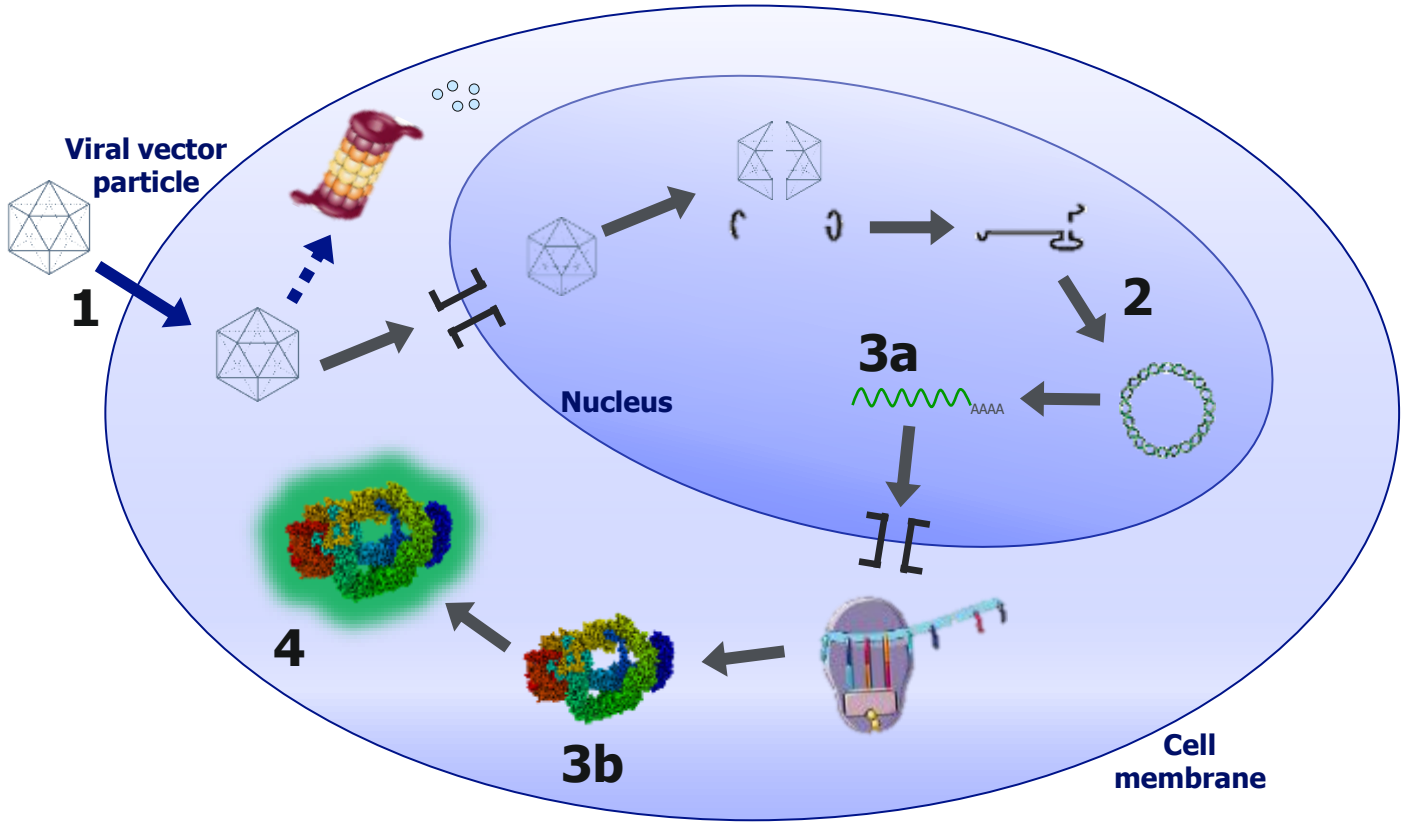
Measuring viral vector particles that can enter the targeted cell and translocate into the nucleus.

Step 3: Transgene expression assay

Measuring viral vector particles that can use the host cell machinery to express the therapeutic transgene at **a)** mRNA level and **b)** protein level.

Step 4: Functional *in vitro* relative potency assay

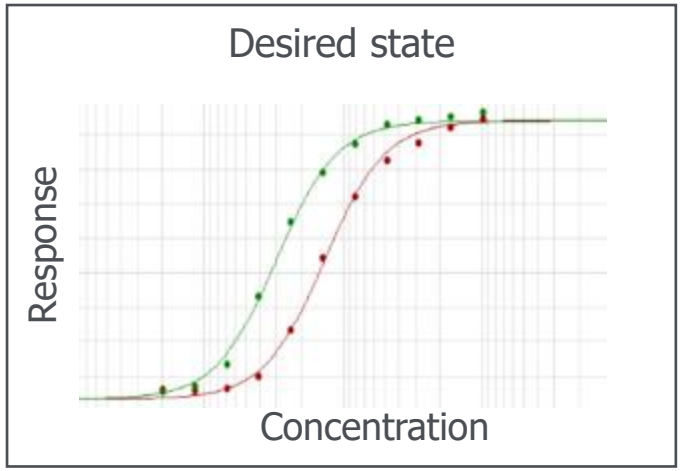
Measuring the biological activity of the expressed therapeutic protein.

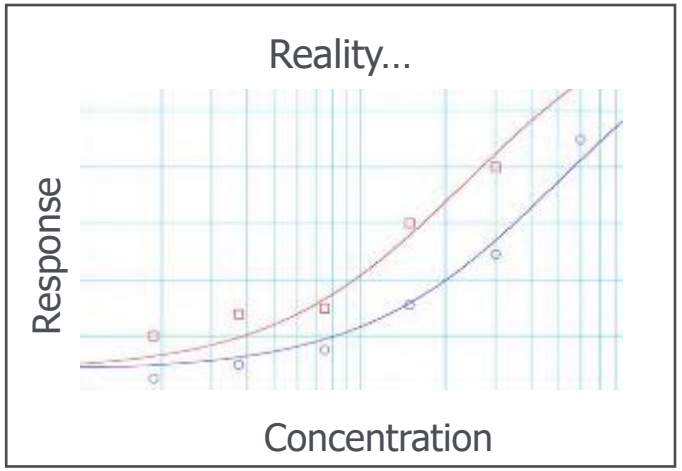


* Assay Matrix terminology adopted from FDA Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products 1/2011.

GTP specificities and impact on potency assessment?

3) It is difficult to have a nice sigmoid dose-response





Whilst 4- PL fit is the typical fitting model used in bioassay*, it clearly does not work in this example…

Asymptote not clearly really defined (especially upper asymptote)
Impossible to evaluate curve parallelism

Using 4-PL fit in this case would have a direct impact on method performance (accuracy and precision)

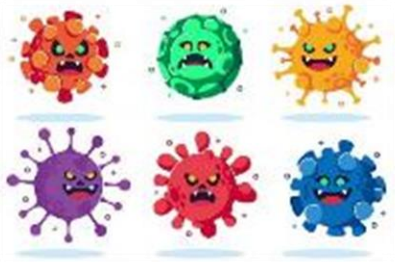
Avenue to explore:

- Alternative fitting models
- Increased concentration ranges (but impact +++ on the "QC Tax")
- Method optimization to better control sources of variability
- …

* As described in White *et al*., Biotechniques 2019

Case study on the assessment of infectivity for a rAAV-based product

Virus infectivity



- Infectivity = capacity of a virus to enter host cells and hijack their transcriptional/translational machinery to produce infectious progeny particles.

- TCID50 = traditional virology method to measure infectivity:
  - A permissive cell line is infected with serial dilutions of the virus preparation and viral replication is monitored through visual examination of the cytopathic effect (CPE). The TCID50 value corresponds to the viral dilution where 50% of the cells are infected.

- rAAV = non-replicative & does not produce any cytopathic effect ➔ TCID50 method has to be adapted:
  - Use of HeLa RC32 stably expressing the AAV Rep-Cap genes necessary for replication
  - viral replication triggered by Co-infection with a helper virus (wtAd5)
  - No CPE ➔ viral replication is monitored via PCR amplification of the rAAV DNA

CPE= CytoPathic Effect; TCID 50 = 50% tissue culture infectious dose. Adapted from ATCC protocol "Taqman TCID50 for AAV2 (VR-1616) Vector Infectious Titer Determination"

TCID50 is the gold standard infectivity assay

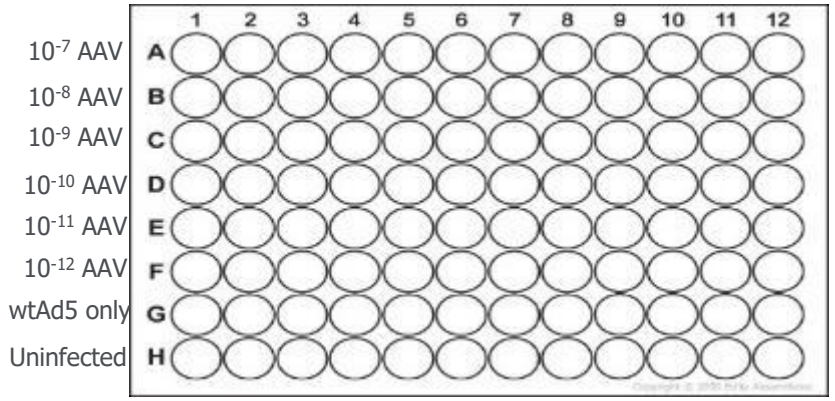
Principle:
RC32 HeLa cells (expressing AAV2 rep/cap genes) are infected with serial dilutions or rAAV. Viral replication is monitored after 72h via PCR. The TCID50 value corresponds to the viral dilution at which 50% of the cells are infected and is directly representative of the virus infectivity.

RC32 HeLa cells plated in 96-well plate

O/N incubation @37°C 5% CO_2

Co-Infection with 10-fold dilutions of rAAV sample and fixed quantity of Ad5

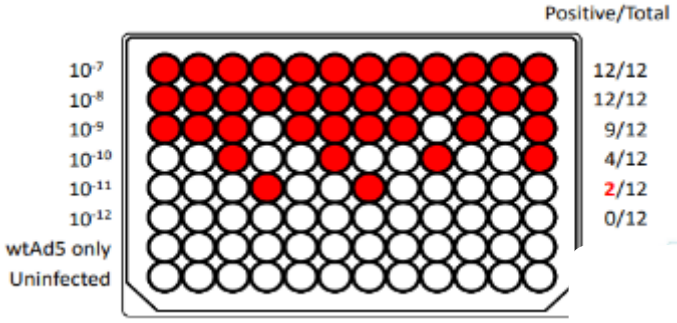
12 replicates



Incubation @37°C 5% CO_2 for 72h

Cell lysis + qPCR amplification of rAAV DNA

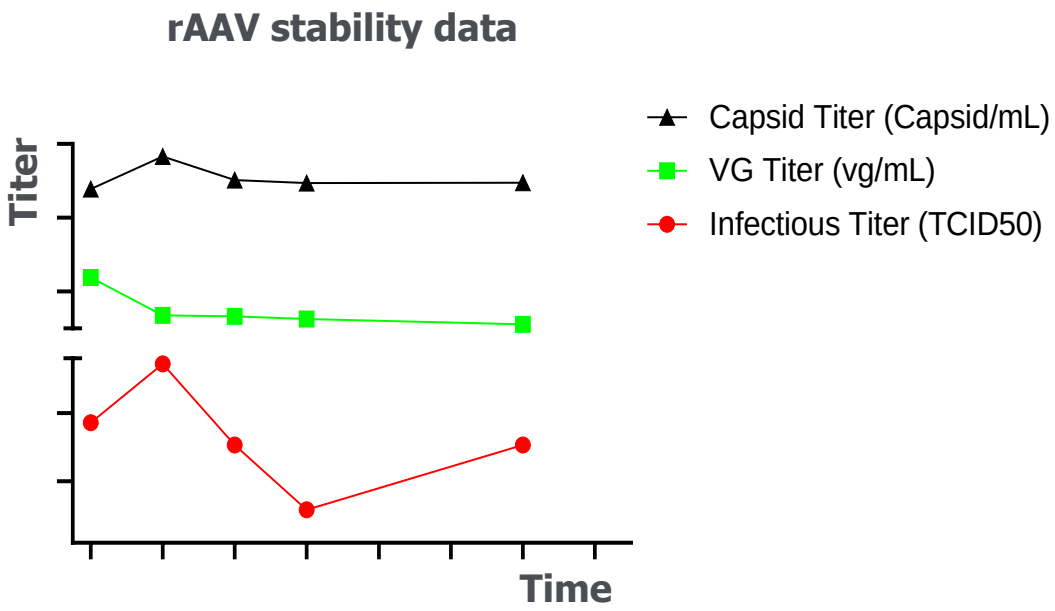
TCID50 titer is calculated with Spearman-Karber's method (titer expressed in IU/mL)



TCID 50 = 50% tissue culture infectious dose. Adapted from ATCC protocol "Taqman TCID50 for AAV2 (VR-1616) Vector Infectious Titer Determination"

Challenges/Limitations of the TCID50 for rAAV

- Not predictive of *in vivo* efficacy (viral replication is not part of the MoA)
- Not QC friendly:
  - Time-consuming (5 days)
  - Error-prone due to complexity
- Requires steady supply of purified and well characterized Ad5 (already challenging)
- Fixed choice of target cells (HeLa RC32) ➔ performance of the assay could be impacted by the intrinsic transducibility of the considered AAV serotype
- High variability*: → very limited added value for release/stability purposes



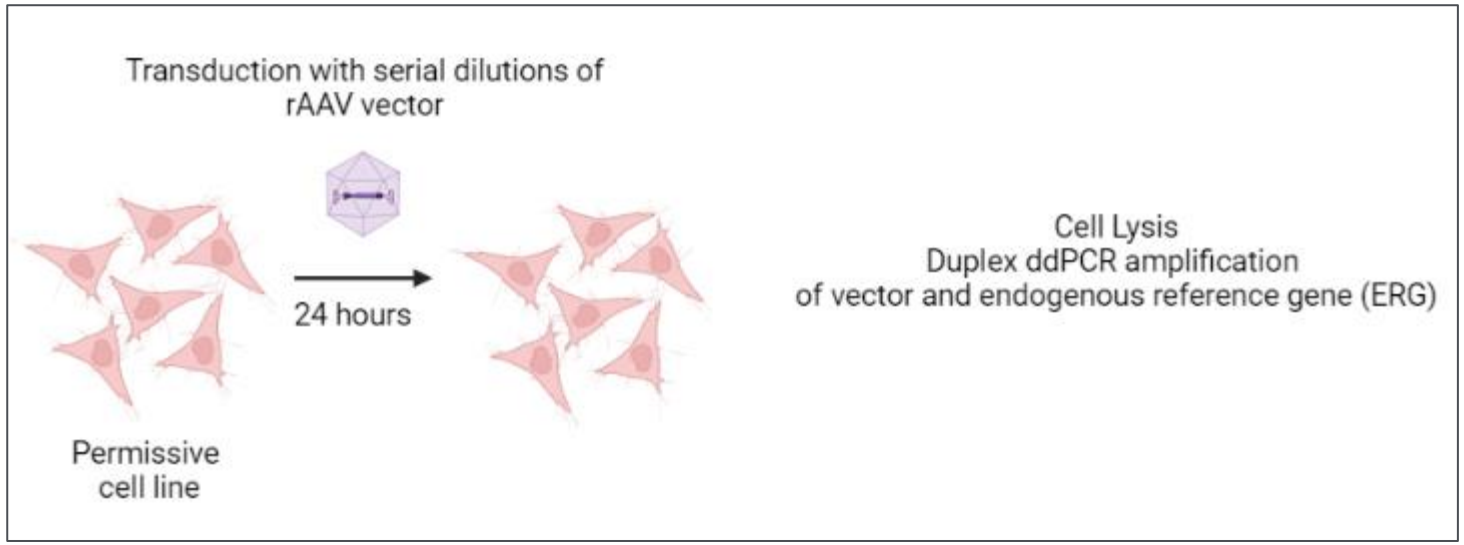
* Also reported by Win Den Cheng Relative Infectivity as a Reliable Alternative to the TCID50 Assay (casss.org)

Looking for alternatives: the Relative Infectivity assay

Initial postulate:

- rAAV = non-replicative ➔ infectivity can be defined as the **ability of the vector to enter cells and deliver its transgenic payload**
- rAAV infectivity = number of rAAV genome copies found intracellularly after infection of a permissive cell line

Assay Principle:



- ERG used for normalization (fixed copy of ERG per cell)
- Infectivity is expressed relatively to a reference standard (% relative infectivity)

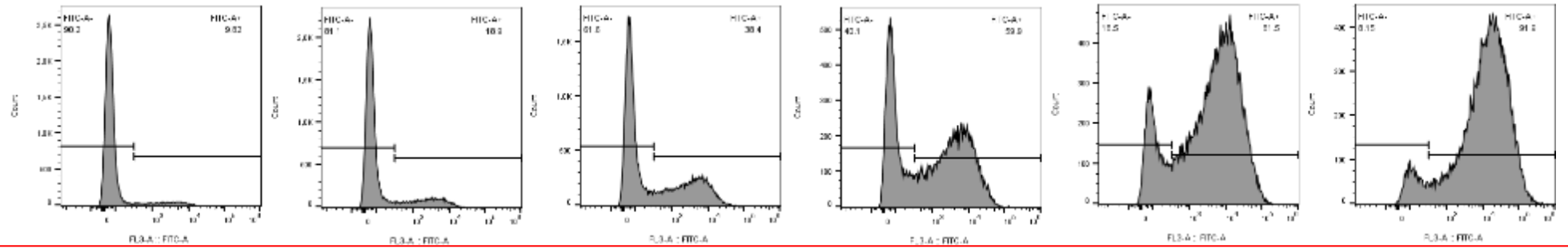
ERG = Endogenous Reference Gene

Step 1: Find the best Permissive Cell Line (using a rAAV GFP-expressing vector)

Depending on the rAAV serotype, *in vitro* transducibility might be challenging ➔ Identifying an appropriate cell model is key!

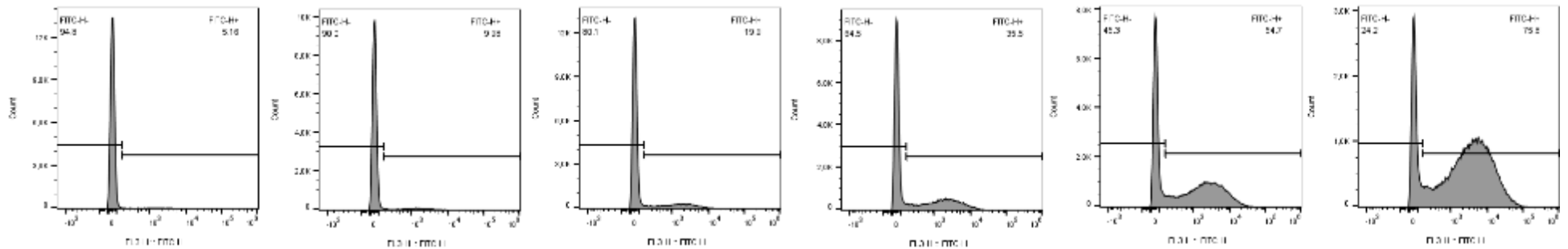
MOI

Cell line #1



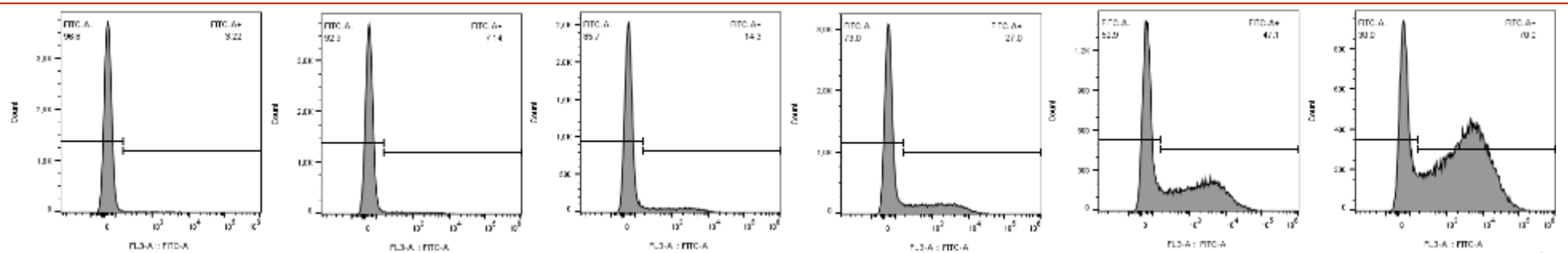
92%

Cell line #2



76%

Cell line #3



70%

GFP fluorescence

Cell line #2 selected for the transducibility and drug MoA-specific representativity

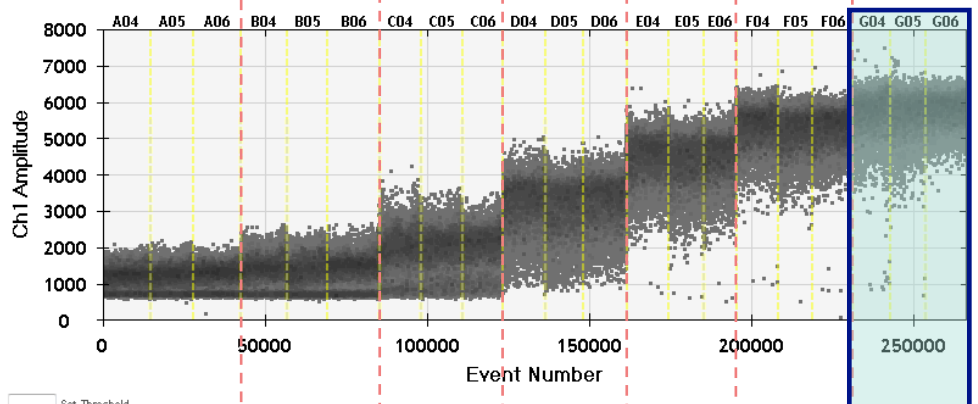
Step 2: Find optimal conditions for duplex amplification of rAAV DNA and reference genes

Sample: cell line #2 infected in triplicate with the highest MoI for 24h

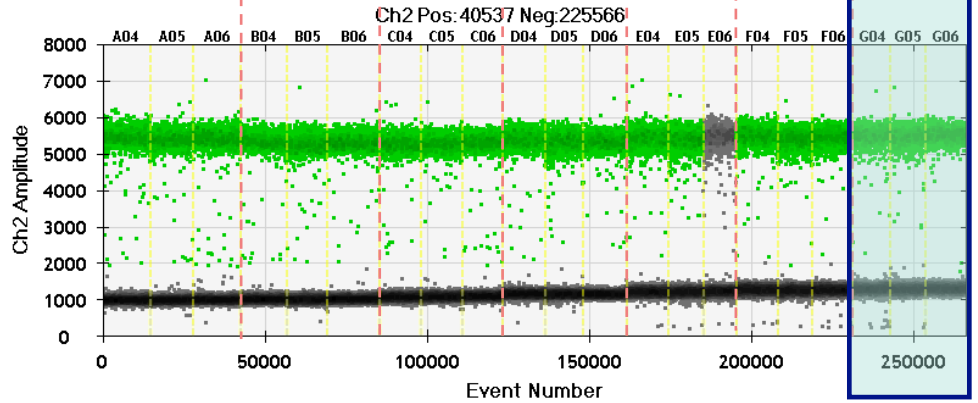
Annealing/Extension temperature

60°C | 59.7°C | 59°C | 58.1°C | 57°C | 56.1°C | 55.4°C

rAAV DNA



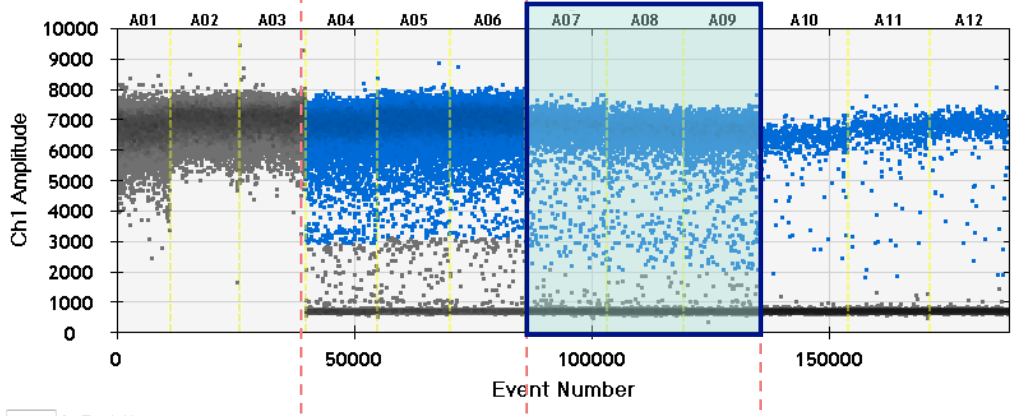
ERG

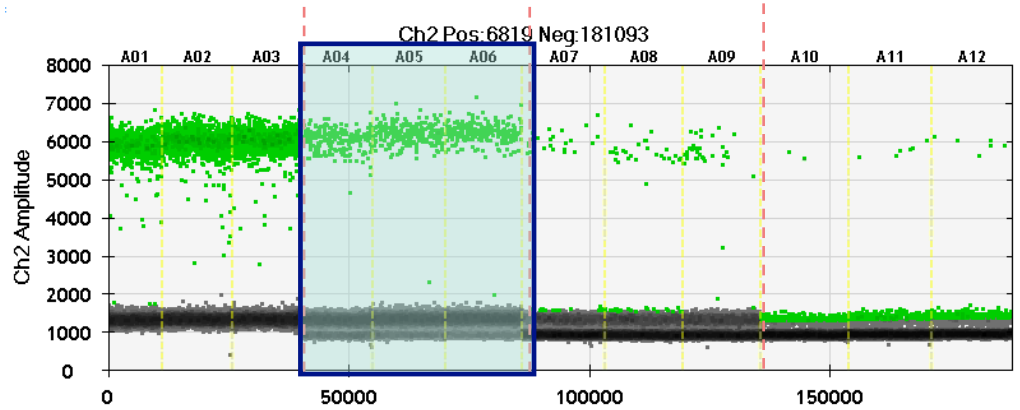


55°C

Pre-dilution

10x | 100x | 1000x | 10.000x



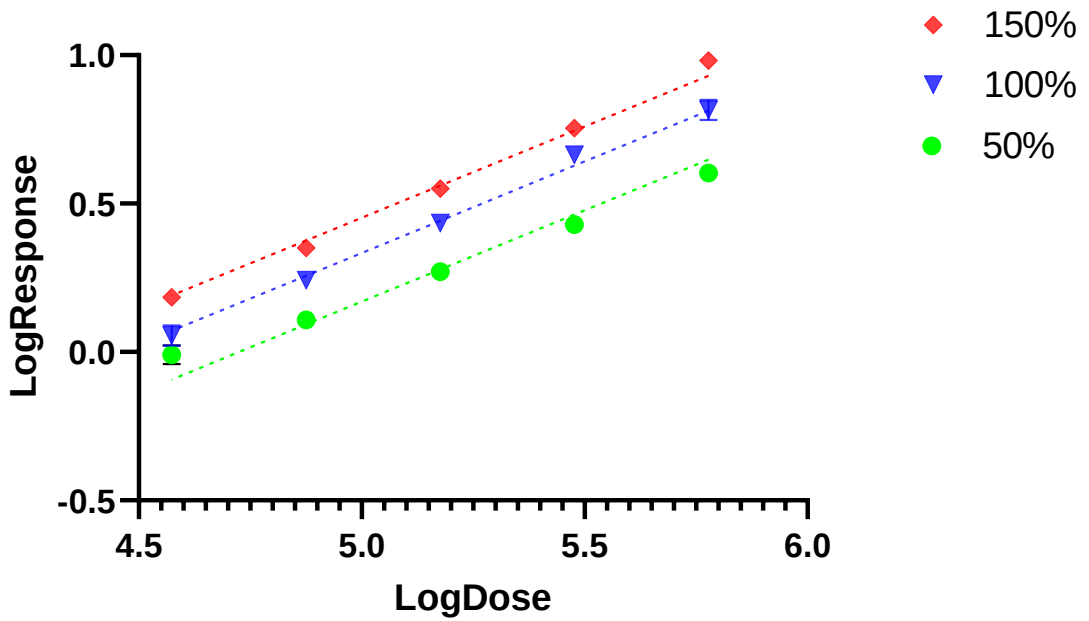


Samples are processed at two dilutions in the ddPCR plate to be able to determine vector and ERG copy number in optimal conditions with a primer annealing temperature at 55°C

Preliminary Relative Infectivity Data

PRELIMINARY

rAAV tested @ 3 theoretical relative infectivity levels (50, 100% (reference) and 150%) to mimic more/less infectious product



- Dashed lines: multiple linear regression fitting model
- Symbols: average response +/- SD from the 3 replicates

Linear dose-response
Good relative accuracy
Limited inter/intra assay varability @ 100%

Accuracy

Relative Potency		
Target	**Measured**	**Accuracy (relative bias)**
50%	54%	+8%
150%	156%	+4%

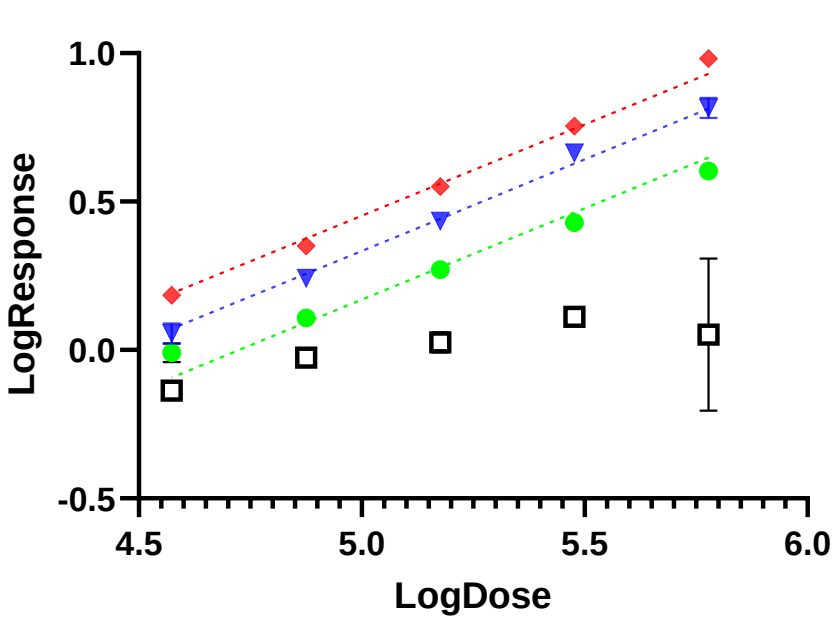
Variability of response @ 100%

MOI Level	Intra-assay %CV (3 replicates)		Inter-assay %CV (n=2)
	Assay #1	Assay #2	
5	8%	4%	10%
4	6%	3%	12%
3	3%	6%	7%
2	2%	1%	3%
1	8%	2%	5%
0	5%	3%	9%

Preliminary Data

PRELIMINARY

Degraded sample: rAAV product heated at 65°C for 10min



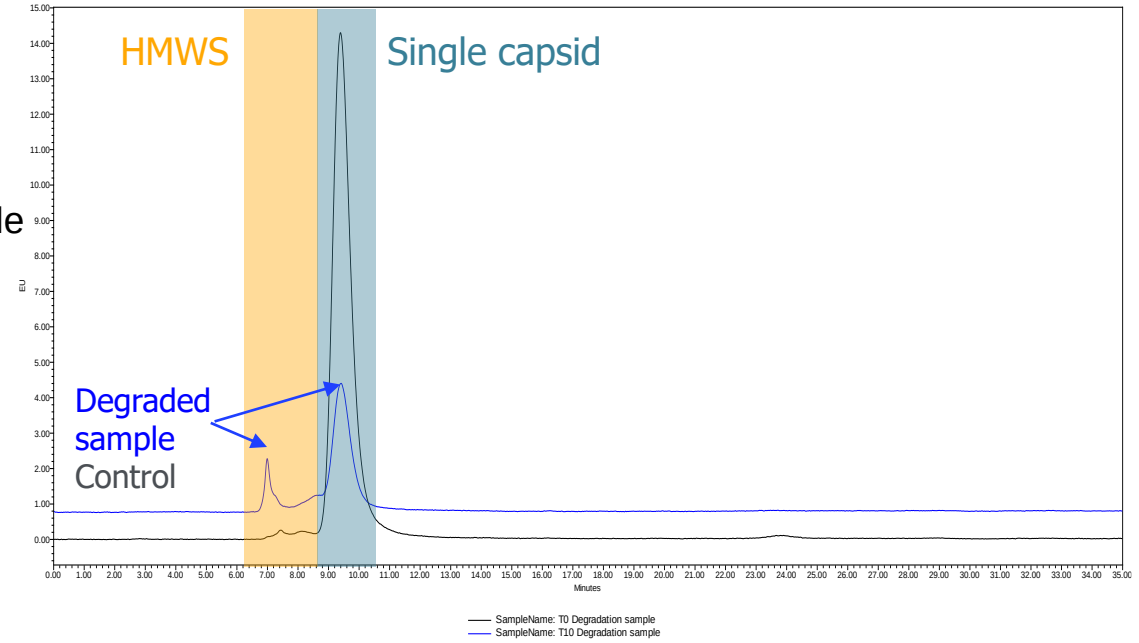
150%

100%

50%

Degraded sample (10 min - 65°C)

Confirmation in SEC-FLD



Stability indicating!

Loss of single capsid species + increase of HMWS observed in the degraded sample.

Next steps

- Confirm model suitability on multiple rAAV batches on the same product
- Deep dive the stability indicating ability of the relative infectivity assay by testing additional degraded conditions
- Definition of parallelism equivalence limits (e.g., upper/lower PSS limits)
- Validation according to USP1033 and extensive evaluation of precision, relative accuracy, linearity and range
- Assessment of the method « platformability » on different rAAV products
- Link relative infectivity results with the other matrix assay (e.g., transgene expression assay)

PSS= Parallelism Sum of Square

Take Home Message

- GT analytical world is a fast-moving area… consequently, no standardization of techniques, lack of reference material
- GT potency assay performance seems to be below the standards we currently have with mAbs
    - Dose-response relationship observed in GT potency assay is not sigmoid ➔ impact on the way to calculate the relative potency and potentially on method performance
    - TCID50 approach is clearly suboptimal (variability) and not stability indicating ➔ new approaches required
    - No clear guidance on how to design/characterize in-house reference standard
- Early engagement with HA is crucial to discuss the phase-based potency assay matrix approach (e.g., seeking for feedback on replacing TCID50 by relative infectivity assay)

Special thanks!

Annemie Wielant

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The GT A-TEAM

Wallonie