Overcoming Challenges in AAV and LV Viral Vector Manufacturing

The number of cell and gene therapies advancing toward commercialization continues to grow, increasing the demand for scalable, efficient manufacturing processes to ensure therapeutics reach patients with speed and safety. Building upon our industry leading knowledge of virus and viral vectors, our next generation AAV and LV platforms expand on the partnerships with our clients to advance your cell and gene therapeutics.

Having produced one of the first commercial CGT products approved by the FDA and EMA, we strive to bring the next generation of therapeutics to market.

View the webinar and listen to our expert Mathias Kahl, Director of Process Development at IDT Biologika, to learn more about how to overcome challenges in AAV and LV Viral Vector Manufacturing

https://idt-biologika.com/exp_content_download/watch-our-expert-talking-about-our-aav-and-lv-expertise-and-how-we-can-help-your-team-to-design-a-seamless-and-scalable-vector-production-process/

Q & A Session of the Webinar

QUESTION	ANSWER
What are the next steps for you to make this manufacturing process more efficient and safer?	In terms of efficiency, efforts are made to reduce costs on materials for production. The main costs are with transfection reagents and plasmids, so we try to reduce the quantities of these materials. Another aspect is to increase the productivity of the cell.
Apart from AAV and LV, is that possible to product live attenuated viral vector vaccine?	Yes, it is one of the core points of our expertise.
Is your NGS validated?	Yes, our methods are all validated according to the requirements. All equipment is qualified and software validated and accepted by the FDA. In addition, the bioinformatic software - which had been programmed by IDT Biologika - is validated, too.
You mentioned you have different HEK lines available at IDT to use for AAV production that is regulatory compliant. Can you comment on which HEK lines and any other information about the source of these cells?	IDT Biologika is licensee of a self-adapted HEK293 cell line in ADCF* medium providing comparable or better AAV and LVV productivity to commercially available HEK293 cell lines. ADCF* Animal derived component free.
What are the AAV and LV recoveries after each downstream purification step?	It is difficult to say as it depends on serotype and transgene.
What is the production yield AAV particles per ml 293T cells?	Typically we do not use a 293T cell line. Yield depends strongly on serotype and transgene. We have generated stable levels of 1E+11 to 1E+12 particles/mL.
What would you say are the advantage of the transient transfection over a stable packaging cell line?	Especially during the early clinical phases, transient transfection is much faster compared to cell line development.

QUESTION	ANSWER
Do you provide cell-based validation assay for cell-specific promoters? If so, what cells are used for?	We provide cell-based assays to demonstrate functionality of the construct, either by determination of the functional titer or determination of the potency by an in vitro cell-based potency assay. Choice of cell line hereby depends on the mechanism of action and the target tissue.
What is a typical or projected full vs. empty vector ratio?	It is difficult to say as it depends on serotype and transgene
Do you employ mass spec to measure viral capsid protein ratios and PTMs? Or is there another tool that is preferable?	We use cSDS-PAGE for viral capsid protein ratios. PTMs are not currently monitored at IDT Biologika, but would be possible via LC-MS in future projects with our inhouse LC-MS capabilities. Currently, analytics of PTMs are out of scope at IDT Biologika as they are for characterizational use which is usually performed by our customers. But we are open for any discussion on these methods in new projects.
How do you measure the viral genome integrity of AAV vectors? Is the technology charge detection MS, multiplex dPCR, HPLC, long read sequencing, or another?	Viral genome integrity will be proven using dPCR technologies. Therefore dPCR multiplexing with up to 5 different fluorescence dyes can be used to detect a wide range of genomes.We have implemented different technologies and are moving to next generation using NGS or ddPCR. Typical methods like agarose gel are also available. We are also open to other additional methods.
Roughly what % of the AAVs that you work with are natural serotypes versus engineered?	The majority of projects are still based on natural serotypes, however we are seeing an increasing number of projects based on engineered AAVs.
What method do you use to measure residual host-cell E1A testing?	We are using qPCR and are currently planning to switch to dPCR.
You mentioned increasing quality attributes being requested by the FDA and EMA- what are these CQAs and How do you measure them (i.e. what tool)?	Please refer to slide no. 19 of our webinar presentation. The used methods are NGS, dPCR, SEC-MALS, cSDS-PAGE, ELISA, DLS, FACS, infectious titer and many more.
Have you tested different AAV serotypes in your platform process? If so, did they all work equally well?	This depends on the serotype and can have an effect on virus packaging and titer.
Do you have any ongoing CGT contract/project?	We have different project ongoing in different statuses.
How many clinical trials for GT have you supplied?	We do have experience with clinical and commercial GT programs - covering DS as well as DP.
Where exactly does the CMC development take place (US or Germany)?	We do have CMC departments in Rockville, MA (US) as well as in Germany. AAV development work is done at both sites.
Does the plasmid ratio have an impact on the full/empty ratio? Have you done that comparison?	In establishing our platform, we looked at a lot of different parameters and also plasmid ratios. And of course we have seen influence on full/empty ratio. However, this ratio also depends on other factors such as serotypes and transgenes.
What is the % full particles at the end of your DSP process?	It is difficult to say as it depends on serotype and transgene and has a big impact on DSP performance.
What is your % recovery at the end of process?	This depends on the serotype and transgene.
Do you have plans to move to stable transfection using producer cell lines for AAV manufacturing?	It could be on option for a later phase. Current process allows us high flexibility in the use of transfection strategy.
What is the cost range to manufacture small lot of GMP grade AAV?	Price estimates should be discussed with our IDT Biologika Sales Representatives.

Learn more about Partnering with IDT Biologika

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