The impact of AAVs and LVVs depends on a vector's ability to express sufficient levels of AAV or LV-mediated therapeutic proteins in the target cell types. As part of regulatory review and stability testing of clinical-grade vector candidates, you must assess transduction efficiency and functional titer. Flow cytometry is used to quantify transduction rates and determine the functional titer in a target cell line using an integrated fluorescence transgene or by staining viral proteins with fluorochrome-labelled antibodies. For that a permissive cell line is selected that is inoculated with defined MOIs of test material. The calculation of the MOI is based on the genome titer. After a defined incubation time the inoculated cells are harvested, stained if necessary and analyzed with flow cytometry. The calculation of the functional titer is based on the percentage of transduced cells in a sample. The gating to discriminate transduced from non-transduced cells must be performed during method development based on the fluorescence intensities of a negative cell population and a positive cell population. In the scope of fast track LVV analytics, the viral particle concentration can be determined directly with flow cytometry within 5 to 10 minutes per sample using devices that are equipped with an avalanche photodiode (APD) detector.

Finally, the utilization of adventitious virus testing (AVT) using next generation sequencing (NGS) can identify the presence of any unwanted viruses. This method identifies all nucleotide sequences in the product and assigns them to a corresponding virus. Though this methodology is widely used, regulatory authorities are beginning to specify quality standards of NGS and its accompanying software for use in AVT. As regulatory requirements increase, identifying a CDMO that offers GMP-validated AVT workflows is highly advantageous and to date, extremely rare. IDT Biologika's AVT workflow is fully GMP-validated, including the NGS analytics, bioinformatics algorithms, and all accompanying software according to Good Automated Manufacturing Practice documentation, GAMP-5. A validated AVT workflow will provide reliable results and a much higher safety profile. Upon completing your analytical portfolio, you can move to platform manufacturing. For more information see also: **"GMP – Validated Adventitious Virus Testing By Next Generation Sequencing"** on our IDT Biologika website.

A Platform Tailored to AAVs and LVVs

Typically, clients arrive at a CDMO with a product idea and plasmid design. A CDMO can place your product design onto an existing manufacturing platform and then manufacture clinical trial material on rapid timelines. If you are looking to manufacture AAV and/or LVV material, identify a partner with established manufacturing and analytical technologies that are prepared for AAV and LVV material production at R&D scale and have the capabilities for manufacturing under GMP qualified condition.

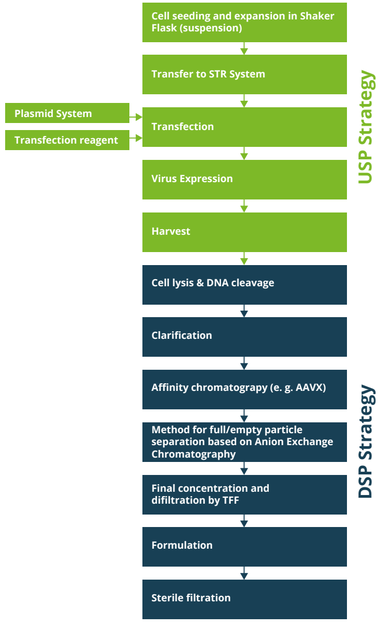

Illustration of a typical AAV Platform Manufacturing approach established at IDT Biologika. Current state of the Art is to generate AAV's via suspension cell culture in combination with a transient triple or dual plasmid transfection system. The USP platform can easily be scaled from 50 – 2000 L batches. Typically, the DSP concept is comprised of an initial enzymatic treatment for DNA reduction and clarification, followed by Affinity interaction chromatography (AIC) for Capturing. The enrichment of full AAV´s is performed by Anionen exchanger chromatography followed by a concentration and diafiltration into the formulation buffer. The final step is a sterile filtration.

The desired batch phase, indication, and analytical portfolio must be established in advance for clinical Phase 1 products. No matter the characteristics of the drug product, functionality, safety, and adventitious virus testing are always necessary. Feasibility testing at the R&D scale can be conducted to ensure that a construct fits to a platform and that parameters are detectable in a good range. With AAV and LVV manufacturing, a CDMO will need to conduct a tech transfer of the plasmid sequence to begin manufacturing. From there, all other components are established including material, cell line, and strategy, thereafter the team will move to clinical trial material production.

IDT Biologika's platform is based on the common AAV manufacturing design. We utilize a HEK-293 cell line and suspension cultivating technologies. For cell transfection, we established a polyethyleneimine (PEI) based transfection strategy to achieve stable and competitive yields. Our downstream processing design includes affinity chromatography, a full-empty separation step, and a final concentration step to reach greater than 10^{12} viral genomes/ml.

Since all the needed equipment are already in-house and our process is established, IDT Biologika doesn't have high preparation timelines. Depending on the product, we may adjust some specific process parameters but not the assay itself. Since materials for the platform approach are the same every time, we do not have new material to implement that is complex and time consuming for manufacturing under GMP conditions. When compared to creating a new product-specific process, this saves significant time and costs.

Conclusion

Although it may seem daunting at first, you can conquer the inherent challenges of AAV and LVV manufacturing for your clinical trial by collaborating with an experienced CDMO with established platform technologies. A reliable partner can build your process design and conduct a highly efficient analytical testing panel that will release your batches for trial faster. IDT Biologika, a CDMO with ample experience in AAV and LVV process design and manufacturing, has established a ready-to-use manufacturing platform in combination with trend-setting analytical methods e.g. ddPCR technologies that can provide crucial testing panel data for mycoplasma and mycobacteria, as well as a GMP-validated AVT workflow that utilizes NGS technology. Highly efficient processes such as these will help your drug product reach patients in need safely and efficiently.

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About the company

IDT Biologika is a global Contract Development and Manufacturing Organization for Innovative Vaccines, Cell & Gene Therapeutics and Aseptic Fill-Finish of Biologics & Sterile Injectables. At our sites in Germany and the USA, we offer seamless end-to-end solutions and the ability to nimbly scale projects from development through to commercialization. This includes process development, drug substance manufacturing, sterile dosage fill-finish, labelling & packaging, quality control & analytics for clinical and commercial batches.

Our commitment over more than 100 years on the advancement of vaccines, drove us to explore additional solutions in advanced therapies. Building upon our industry leading knowledge of virus and viral vectors, IDT Biologika's next generation AAV and LVV platforms expand on the company's success and partnerships with our clients.

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.

IDT BIOLOGIKA

Overcoming Challenges in AAV and LV Viral Vector Manufacturing

How to Design and Launch a Successful Clinical Trial Manufacturing

Mathias Kahl, Stephan Bauer, Dr. Daniel Köhler, Dr. Tobias Thom, Rico Hinrichs, Stefan Borutzki, Christina Pospisil, Dr. Rico Schmidt, Erik Arnold, Dr. Thomas Kreisig

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Overcoming Challenges in AAV and LV Viral Vector Manufacturing

How to Design and Launch a Successful Clinical Trial Manufacturing

Cell and gene therapies are growing exponentially across the pharmaceutical field due to their potential to treat and possibly cure a wide range of devastating diseases, including cancer. As these therapies are becoming more wide spread, many drug sponsors are approaching trials with limited knowledge of how to design and launch a successful clinical trial manufacturing (CTM) process. This includes how to strategically investigate and manufacture adeno-associated virus vectors (AAVs) and lentiviral vectors (LVVs), two of the leading viral vectors used for gene therapeutics. Sponsors are faced with a stark learning curve that, without intervention, may lead to high costs, delays, and a failure to bring their drug to patients.

The key to avoiding process failures is leveraging innovative testing and manufacturing platforms that can reduce timelines and increase cost-savings for viral vector production. Collaborating with a contract development and manufacturing organization (CDMO) with AAV and LVV manufacturing expertise will help your team design a seamless and scalable process while also avoiding expensive mistakes.

Develop of a Process and Partnership to overcome challenges

Often, when they begin their CDMO search, emergent drug sponsors have not yet had to factor process scalability and manufacturing of clinical trial material under GMP conditions into their project plans. CDMOs with an understanding of AAV and LVV manufacturing workflows can build a process that conserves resources due to established platform applications. An experienced partner will make thoughtful recommendations on the upstream and downstream technologies, analytical testing methods, and formulation strategies that are best suited for your product.

Typically, Inventors are confronted with a relatively unclear risk and cost situation when it comes to clinical trial material manufacturing, since production costs per batch are generally high. Frequently inventors tend to have a specific manufacturing concept for their production, especially for the process design. In combination with limited budget and timelines for their process development Inventors are challenged with the setting up of the process, which increases the risk for a successful first time production of their product. To lower these risks, experience in the field of AAV and LVV manufacturing is mandatory. Therefore, it is beneficial for both the Inventor and CDMO companies to work with a well-established and characterized manufacturing platform where the processing risk and additional production costs can be reduced.

In the beginning of your collaboration with a CDMO, all necessary deliverables will be established, including necessary batch sizes of your product for clinical studies. Based on these specific indications, further criteria (including a safety profile, relevant analytics, and/or model systems) may be available to start building a process from. Once this information has been gathered, the CDMO's process design team will determine the upstream and downstream activities necessary to generate the required material. Often process staff in the CDMO industry have adapted to the repeated operations of commercial production; this can make it difficult for staff to utilize a procedure that has not been well characterized. However, working with a CDMO that has a specific CTM process design team who is well-accustomed to adapting to new protocols will help circumvent any unforeseeable events.

One challenge and often risky component within the manufacturing of new products is the tech transfer, especially when different stakeholders are involved (e.g. external analytical services, R&D sites and manufacturing companies). Transfer activities increase the risk of something getting lost in translation; they also require documentation throughout each phase, comprehension from the faction receiving the transfer, and time to provide feedback and ask questions. Opting to work with a CDMO whose requisite departments are in-house including analytics, process development, and manufacturing, helps avoid common transfer obstacles or failures. At IDT Biologika, we provide all the necessary activities for new product manufacturing. Our workflow is aligned between each department, which allows us to offer fast timelines and reduced costs.

Another aspect that needs to be taken into account during manufacturing is the final filling of the drug product. In contrast to common viral vaccines, which are mainly applicate intramuscular, the route of administration for CGT can be very different. Based on the routes of administration, the administered volume and therefore the filling volume of the respective therapeutics can vary. At IDT Biologika filling of different volumes and primary packaging systems is possible for example, the filling of 0.5 – 3 mL syringes and 2R up to 25R vials can be performed. Chemical interactions like binding of (viral) proteins on surfaces of primary packaging material can be a difficulty which leads to a decrease in product stability. Improvement of AAV stability was obtained by the use of COP (cyclic olefin polymer) vials. Besides the lower chemical interactions to product components, the benefit of COP vials is that these also break resistance. COP vials are implemented at IDT Biologika and can be used as primary packing material.

IDT Biologika Core Service

Process Development	Clinical Trial	Commercial Manufacturing
• Small scale Feasibility testing • Process preperation for Scale Up • Final Parameter adjustments • Starting point for Scale-Up to GMP facility • Analytical Development	• CTM-1 – 3 Production under GMP control • Suspension and Fixed Bed Bioreactor Technologies in place • Trained and experienced CTM staff • Automated filling capacities (DS / DP) • Assay prevalidation (APMP)	• Commercial Production Scale and Filling capabilities • High experience commercial manufacturing systems • Risk Assessment (FMEA) • Development of supportive process chracterization and risk assesments • Process and Assay Validation
STR: 3 – 40 L FBR: 4 m²	STR: 50 – 200 L FBR: 30 –500 m²	STR: 2000 L FBR: 500 m²

IDT Biologika Core service comprises the complete value chain of biopharmaceutical product development and manufacturing. Starting with development, using established platform technologies and ending up at commercial manufacturing scale. Different installed platform technologies allow for the specific production of the client's demands. All necessary activities can be done completely at IDT Biologika avoiding high costs, risks and additional time for tech-transfer.

Utilize High-Efficiency Analytical Technology to Improve Timelines

High-throughput, rapid analytical technology ensures patient safety, high-quality standards, and fast timelines. Your testing panel must meet regulatory requirements, GMP guidelines, and necessary product- and process-specific analytics. When manufacturing AAVs and LVVs, typical safety tests include assessments for mycobacteria, mycoplasma, adventitious viruses, and endotoxins. Many of the traditional safety-related analytical tests are limited by time; however, modern techniques are faster and more sensitive, providing information rapidly for batch releases.

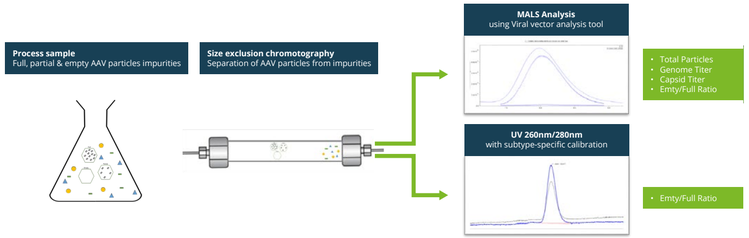
In addition to the test panel for approvals, characterization assays are just as important to guarantee a functional vector as well as keep the impurity profile as optimal as possible. These include capillary electrophoresis, mass spectrometry, particle analysis and cell-physiological analysis.

One of the key analytical technologies for gene therapy is digital PCR. In this technique, the PCR reaction is divided into separate partitions. Each partition represents one specific space where PCR reactions can take place. This approach yields significantly more accurate results than qPCR and the reduced time requirement leads to faster batch releases. dPCR is a high-throughput and sensitive technology, which provides accurate qualitative or quantification results of DNA and RNA samples.

Digital PCR can be used for several quantitative or qualitative questions. Whenever a quantification of the genome copy number (in general) or specific targets (e.g. helper plasmid /-virus) is needed, dPCR could be the best choice for quantification. Since there is no requirement for a standard curve (absolute quantification), the development or adaption of assays for a specific AAV or LentiVirus target can be done without the time consuming standard curve modifications. Only the exchange of the specific primer/probe system (target specific) is needed to adapt this key technology to a new target/product. Beside the quantification aspect of the assay, dPCR can also be used for Identity or Purity testing of the product. Due to the absolute signals (positive vs. negative signal), the results can be used to identify a product based on the specific primer design or observe the absence of a specific target region which was deleted from the product.

Another analytical question that can be solved with dPCR is Mycoplasma and/or Mycobacteria testing using PCR. Currently, one of the most common testing methods on the market for mycoplasma and mycobacteria is cultivation-based testing. For mycobacteria, sample material requires a 56-day cultivation period. For mycoplasma, cultivation takes 28 days. The indicator cell culture method, another common test, lacks sensitivity and reliability. To combat the limitations of these tests, IDT developed an approach using state-of-the-art digital polymerase chain reaction (dPCR) technology for detection of mycobacteria and mycoplasma contamination. These approaches enormously reduce the necessary time for the detection of contaminations. In addition it allows for the detection of mycobacteria in one week and mycoplasma detection in one day. Molecular detection using dPCR has many advantages in contrast to other PCR-techniques, especially the high robustness which leads to a high reliability.

Another key quality attribute that should be analyzed as part of the testing panel is the full-empty ratio. A full-empty determination assesses the proportion of full capsids to empty or partially filled capsids. Empty and partially filled capsids lack the needed genomic material or only contain fragments, whereas a full capsid contains all genomic material required and is thus the desired AAV product. Anion-exchange high-performance liquid chromatography (AEX-HPLC) is one method for determining full-empty ratios. However, since the difference between the isoelectric points of full and empty capsids is very small, it can be a difficult assessment to utilize. Furthermore, this assay requires many AAV-product-specific adaptations and may not be suitable for every kind of AAV. A more robust and generic approach for full-empty determination is size exclusion chromatography combined with MALS detection (Multi Angle Light Scattering). The MALS detector is a valuable instrument for acquisition of data in the scope of method development and process characterization, however its use is quite complex and implementation in routine QC processes might be challenging. As a way out, the obtained data can be used for establishment of a method that uses the different absorption maxima of virus capsid proteins (280 nm) and the corresponding viral DNA (260 nm). For a given vector system, the ratio between peak areas for HPLC-UV detection of both wavelengths is fixed for a certain full to empty ratio bearing the advantage of a simplified determination of this parameter with an ubiquitously available HPLC system.



Flow chart AAV analytics via SEC-MALS/UV for empty-full ratio determination via MALS and UV 260nm/280nm ratio.

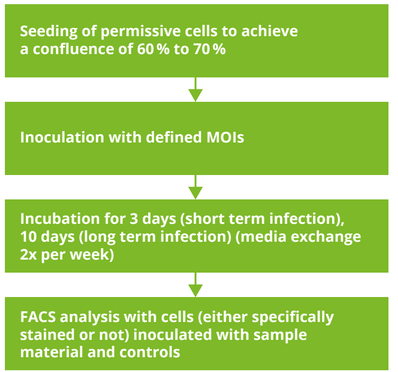


Fig. A

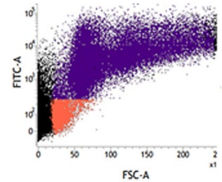


Fig. B

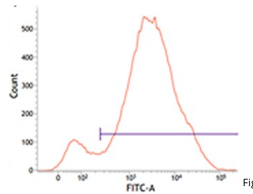


Fig. C

A: Overview on an exemplary assay procedure for a flow cytometry-based functional titer assay. B: dot plot of transduced cell population, cell size (FSC) is plotted against the fluorescence intensity (FITC-A), transduced cells (violet), non-transduced cells (orange). C: Histogram plot, fluorescence intensity (FITC-A) is plotted against the event count. P1 (orange): population of gated cell population excluding cell debris and aggregates. P2 (violet): fluorescence intensity at which cells are considered transduced.