viral vectors vaccine bioprocessing handbook

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Viral Vector Vaccines

A live vector vaccine is a vaccine that uses a weakened or harmless microorganism to transport pieces of the antigen in order to stimulate an immune response. Vectored vaccines show promise in their reliability to induce potent cell-mediated immunity, which is essential for complex disease like AIDS, malaria, and cancer among others.

Viruses and bacteria can both be used as vectors. Attenuated *Salmonella typhi* (Ty21a) and *Lactobacillus acidophilus* are two common bacterial vectors. Bacterial vector vaccines are administered orally for mucosal immunity. Common viral vectors are adenovirus, canarypox, lentivirus, and alphaviruses. They transfect their own DNA into the host cell, which is later expressed to produced new viral particles.

A Japanese encephalitis (JE) vaccine uses an attenuated yellow fever virus (YFV-17D) encoding the JE preM-

Typical Viral Vectors in Vaccine

Vector Name	Size (nm)	Cell Line	Used in vaccine
Adenovirus (Ad-5)	70–90	**HEK293, PER. C6**	**Malaria, HIV, Hep. B**
Canarypox (ALVAC)	200–300	**Chicken embryo fibroblasts**	**HIV**
Alphavirus (Sindbis, Semliki Forest, etc)	70	**Chicken embryo fibroblasts, vero**	**SARS-Cov, Ebola**
Lentiviral vector	80–100	**HEK293**	**HIV**
Modified Vaccinia Virus Ankara (MVA)	230	**Chicken embryo fibroblasts, EB66**	**HIV, smallpox**
Attenuated yellow fever virus (YFV-17D)	50–90	**Embryonated chicken egg, Vero**	**West Nile, Dengue**

Env protein. It is the first human viral vectored vaccine on market. There are also twelve viral vector vaccines currently in use for veterinary diseases. The approved vaccines include adenovirus, fowlpox virus, attenuated yellow fever (YFV-17D), and vaccinia virus vectors, all of which are relevant as potential human viral vectored vaccines.

Vectored vaccine elicits strong humoral and cell-mediated immune responses that result in immunological memory. They can be targeted by viral tropisms for particular cells such as intestine cells, brain cells, etc., inducing desired immunity. Vectored vaccines can encode for several antigens from different pathogens, introducing the possibility of a single vaccine for several diseases. They are relatively inexpensive, and some are easily transportable.

Since the live virus being used is an attenuated form of a human pathogen, there is always a risk of reversion to virulence. Some of the vectors under consideration, such as adenovirus, have the capability of transforming cells to a cancerous phenotype. Many large vectors (canarypox and vaccinia, for example) cannot be sterile filtered because they are too large to pass through 0.22 µm membrane. Completely closed or aseptic manufacturing practices must be followed for these large vectors.

There are several advanced molecular biology methods available for the design of the unique genetic make-up of vectors. The core of vectored vaccine innovation is in the molecular architecture and design of the vector. We will focus on the manufacturing process of adenovirus-based vaccine as a typical representative of vectored vaccine, but we will not cover vector design and development.

Adenoviruses are non-enveloped viruses with a ds-DNA, 70–90 nm in size. They are efficient at transducing target cells and can be produced at high titres (>10^{11}/mL). Adenovirus (AV) in its normal form is a pathogen that causes respiratory ailments such as conjunctivitis and the common cold. It is also capable of infecting neurons, damaging cells, and invoking strong immune responses. However, adenoviruses used as vectors are specially designed not to cause any disease and are generally regarded as safe.

The manufacturing process for adenovirus vectors is straightforward and fairly templated; a general outline is shown in Figure 8. Typical pilot scale bioreactor size for adenovirus production is 20 L cell culture, which becomes ~4 L after UFDF and ~500 mL post chromatography and final sterile filtration. Full-scale process volumes range from 100 to 200 L, and overall process yield is typically 65%.

Cell Culture

Efficient manufacture of adenovirus vectors can be accomplished using genetically engineered human cell lines that complement the deleted adenoviral genes required for replication (e.g., 293-ORF6 cells, HEK293, PER.C6). These cells have a well-characterized safety profile and can be adapted for growth in serum-free suspension. Production cells are grown in stirred-tank bioreactors with serum-free culture medium.

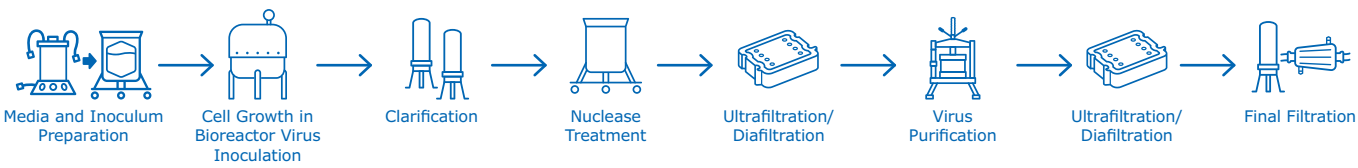

Figure 8: Generic adenovirus-based vectored vaccine process

During the adenovirus infection phase, the metabolic processes of the production cell line are significantly increased to support vector manufacture. In order to facilitate successful vector production, a medium exchange step is performed to remove spent medium containing metabolites such as lactic acid, which can be detrimental to virus production. The virus yield drops significantly when the media pH < 7. A 50–100% typical cell density during adenovirus infection is 0.5–9.0 x 10^6 cells/mL. The adenovirus titer during harvest generally ranges from 10^9 to 10^{11} pfu/mL.

Cell Lysis and Clarification

Cells are lysed either mechanically or by a chemical lysis agent (e.g., non-ionic detergent) for the harvesting of adenoviruses. Lysis with Triton™ X-100 solution is most common. Clarification is performed to remove the cells or cell debris and harvest adenoviruses. A depth filter is commonly used for primary clarification. Some manufacturers also use tangential flow filtration (TFF) at low shear conditions depth filtration or normal flow filtration (NFF) for clarification of adenoviral vaccine harvests. Filter capacities depend on cell density at harvest, the degree of lysis, and the particle size distribution. Typical lysate turbidity is >200 NTU. Similar to viral vaccine process, depth filter followed by bioburden reduction filter is commonly used for this application. TFF may also be an option in addition to the NFF options. Typical loading for TFF is 20-30 L/m^2. Some manufacturers also use centrifugation for primary clarification. Filtrate from secondary clarification has been, in some instances, filtered through 0.45 µm for bioburden reduction (~250–500 L/m^2). Post clarification turbidity is in the range of 5–10 NTU. This unit operation is conducted at room temperature.

Nuclease Treatment

Carryover nucleic acid from lysed cells is a key contaminant in adenovirus vaccine processes. Viruses propagated in nonhuman cells (i.e., HEK293, PER.C6) pose a greater risk of nucleic acid carryover. Regulations require that carryover host cell nucleic acid content should be below 10 ng/dose of attenuated viral vaccine. Nucleic acids are negatively charged; they are large molecular components that can interfere with virus purification. Virus harvest is treated with about 0.9 to about 1.1 U/mL of Benzonase®endonuclease at 30-34 °C for four to eight hours.

Ultrafiltration/Diafiltration

After Benzonase® endonuclease treatment, the harvest is diafiltered using TFF (100-300 kDa UF devices). The typical flux for 300 kDa is ~25–50 LMH at 5–10 psi TMP at 5–7 L/min/m^2 feed flow rate. Next, 4–10 X concentration and 5–8 N diafiltration are performed. More than 99% retention of adenoviruses is typical. Some manufacturers perform a vector concentration step to reduce overall volume before Benzonase® endonuclease treatment. Diafiltration is then performed to facilitate buffer exchange for further processing, such as downstream chromatographic processing. Sometimes an overnight hold step is employed prior to downstream purification. Consequently, a filtration step is performed to reduce the risk of bioburden and to protect the downstream chromatography columns.

Chromatography

Small-scale clinical lots are typically purified using CsCl-based density gradient ultracentrifugation. However, for large-scale production, column chromatography is employed. Two- or three-step column chromatography purification is normally used for adenovirus production. Purification methods commonly used are ion exchange and size exclusion chromatography (optional). Anion exchange is used to remove HCP, DNA, RNA, and other major contaminants. Size exclusion chromatography is used for trace contaminant removal. Typically, in anion-exchange columns, the adenovirus feed (5 × 10^{12} virus particles/mL of resin) is loaded at 75 cm/hour flowrate in 50 mM Tris-HCl, pH 8.0 in 5% glycerol and eluted in salt gradient. Adenovirus elutes at ionic strength of 40 mS/cm. Weak ion-exchangers are also proven to work for purification of adenovirus resulting in high purity and yield.

Sterile Filtration

Sterile filtration ensures the sterility of the final formulated product. A filter pore size of 0.22 µm or less is required to eliminate microbial contaminants.

Summary

Viral vectors are vehicles that deliver the genetic payload to target cells. Advancements have been made in the vector design to ensure safety of these types of vaccines. This section describes a template to manufacture of adenovirus-based vaccine used in the bioprocessing industry. Compared to the conventional vaccines, adenovirus vector-based vaccines can express a wide range of antigens from virus, bacteria, or protozoan. They elicit long-term immune responses against infectious diseases.

References

Brument et al., A Versatile and Scalable Two-Step Ion-Exchange Chromatography Process for the Purification of Recombinant Adeno-associated Virus Serotypes-2 and -5, Molecular Therapy, 2002, 6 (5): 678-686.

Burova and Ioffe, Chromatographic purification of recombinant adenoviral and adeno-associated viral vectors: methods and implications. Gene Therapy, 2005, 12, S5–S17

EMEA guideline on live recombinant vector vaccines for veterinary use

Lusky, M. Good manufacturing practice production of adenoviral vectors for clinical trials. Human Gene Therapy, 2005, 16(3), 281-291.

Lyddiatt and O'Sullivan, Biochemical recovery and purification of gene therapy vectors, Current Opinion in Biotechnology, 1998, 9: 177-185.

Zhou D and Zhang C, Adenoviral vector-based strategies against infectious disease and cancer, Hum Vaccin Immunother. 2016 Aug; 12(8): 2064–2074.

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