

---

*This text was drafted by the EDQM in collaboration with Group of Experts 15 (vaccines for human use) of the European Pharmacopoeia (Ph. Eur.) Commission, composed of experts from licensing authorities, national control laboratories, academia and industry, from Europe, the USA, Canada and Australia. It is not an official text of the Ph. Eur. and is not binding.*

---

# RECOMBINANT VIRAL VECTORED VACCINES FOR HUMAN USE

This document is published for information in the context of the [COVID-19 pandemic](#).

It provides [analytical strategy options](#) for the control of recombinant viral vectored vaccines and is intended to support developers of candidate COVID-19 [viral vector-based vaccines](#). It includes suggested tests that may be conducted at each stage in the manufacture of viral vectored vaccines and can be used by COVID-19 [vaccine developers](#) as an [aid to building appropriate analytical strategies](#) during the development of their candidate vaccines. Additional tests may be considered on a case-by-case basis, depending on the nature of the vector backbone and/or the specific manufacturing process established for the viral vectored vaccine. The principles described in this document may also be used for other recombinant viral vectored vaccines.

The list of tests provided herein has been drawn up using available knowledge of the specific analytical strategies established for existing products and existing regulatory documents issued by the Ph. Eur., EMA, FDA and WHO as well as input from the experts involved in the drafting of this text.

References to official texts of the Ph. Eur. are made throughout this document. Selected key Ph. Eur. texts, including those mentioned below, have been compiled in a package for COVID-19 vaccine developers that is available on the EDQM website for free (<https://go.edqm.eu/Pheurvaccinespackage>).

The content of this document will be reviewed on a regular basis and updated as needed to adapt to the evolving situation, and/or to reflect further experience gained with new constructs or products. Stakeholders are welcome to contribute to this process by submitting relevant information for consideration via the mailbox:

[VaccinesTF@edqm.eu](mailto:VaccinesTF@edqm.eu)

## 1. DEFINITION AND SCOPE

Viral vectored vaccines are live viruses that are genetically engineered to express one or more heterologous antigens. This document covers both replication-competent and replication-defective viral vectored vaccines. Replication-competent viral vectored vaccines can replicate in humans and include viral vectors using yellow fever virus, measles virus, vesicular stomatitis virus (VSV), or influenza virus as backbone. Replication-defective viral vectored vaccines cannot replicate in humans and include viral vectors using adenovirus, modified vaccinia virus Ankara (MVA), or influenza virus as backbone.

## 2. SUBSTRATE FOR RECOMBINANT VIRAL VECTOR PROPAGATION

The vector may be propagated in human diploid cells (*Ph. Eur. 5.2.3*), in continuous cell lines (*Ph. Eur. 5.2.3*) or in chick-embryo cells or in the amniotic cavity of chick embryos derived from a chicken flock free from specified pathogens (*Ph. Eur. 5.2.2*). *Ph. Eur.* chapter 5.2.3 provides a list of tests that may be conducted on human diploid cells and continuous cell lines used as cell substrate for recombinant viral vector propagation. When the vector is propagated in a continuous cell line or in human diploid cells, a cell-bank system is established.

Tests for replication-competent viruses may be necessary for certain replication-defective viruses, for example replication-defective adenovirus vectors. The occurrence of replication-competent viral vectors may be significant when large regions of homology exist between the viral genome and the genome of the complementation cells. This occurrence may be minimised by minimising the homology between both genomes. The use of cells with no sequence homology with the vector is recommended for production.

## 3. RECOMBINANT VIRAL VECTOR SEED LOT

Production of the recombinant vector is usually based on a seed-lot system.

The strain of virus used is identified by historical records that include information on its origin and its subsequent manipulation, notably deleted or modified regions. A detailed description of the genetic insert(s) and the flanking control regions is established, including the nucleotide sequence. The origin of the genetic insert and the method by which it is introduced into the vector are documented.

Based on the nature of the viral vector and pre-clinical safety studies (*e.g.* biodistribution), a risk analysis is carried out. Where necessary, tests for viscerotropism and/or neurotropism or neurovirulence in a suitable animal model may be carried out on seed lots (*e.g.* for recombinant viruses using yellow fever virus, measles virus or VSV as backbone).

The following tests may be considered for the control of seed lots.

**3-1. Identification.** The vector is identified in the master seed lot and each working seed lot by suitable methods such as immunochemical methods (*Ph. Eur. 2.7.1*) or NAT (*Ph. Eur. 2.6.21*).

**3-2. Genetic and phenotypic characterisation.** The following tests may be considered.

- The entire genome of the vector is sequenced at a passage level comparable to a production batch and the analytically determined sequence is compared to the theoretical sequence based on vector construction and available databases.
- A suitable number of isolated sub-clones are tested for expression of the genetic insert product(s) at a passage level comparable to a production batch. Sub-clones giving lower levels of expression need further characterisation. For recombinant vectors where a loss of the insert is known to occur (*e.g.* adenoviruses), the percentage of clones expressing the genetic insert is evaluated.

**3-3. Infectious vector titre and vector particle concentration.** The titre of infectious vector, alone or in combination with the concentration of vector particles, in the master seed lot and each working seed lot are determined.

**3-4. Extraneous agents** (*Ph. Eur. 2.6.16*). The master seed lot and each working seed lot comply with the tests for extraneous agents. *Ph. Eur.* chapter 2.6.16 provides a list of tests that may be conducted based on a risk assessment

to design an appropriate analytical strategy to ensure the absence of extraneous agents.

**3-5. Absence of replication-competent viral vectors.** For some non-replicating viral vectors *e.g.* adenoviruses, replication-competent viral vectors may be generated by homologous recombination between the recombinant viral DNA and the viral vector sequences integrated into the genome of the complementation cells.

Detection of replication-competent viral vectors is generally performed by an infectivity assay on sensitive detector cell lines, which are not able to complement for the genes deleted from the vector. Other indicators of viral replication may be used as appropriate.

When replication-competent viral vectors are not supposed to be present in the test sample, considering vector construction and cell lines used, at least 2, but preferably 3 or 4 successive passages are performed on the detector cell line, where applicable. Detection of a cytopathic effect at the end of the passages reveals the presence of replication-competent viral vectors in the preparation. Positive controls are included in each assay to monitor its sensitivity.

## 4. PROPAGATION AND HARVEST

It is preferable to have a production free from antibiotics. Unless otherwise justified, at no stage during production is penicillin or streptomycin used. A portion of the production cell cultures is set aside as uninfected cell cultures (control cells). If the virus is propagated in embryonated eggs, a portion of the production eggs is set aside as uninfected control eggs.

The following tests may be considered for the control of each single harvest.

**4-1. Identification.** The recombinant vector including the genetic insert is identified by suitable methods such as immunochemical methods (*Ph. Eur.* 2.7.1) or NAT (*Ph. Eur.* 2.6.21)

**4-2. Vector particle concentration.** For some vectors, for example adenoviruses, the concentration of vector particles in single harvests is determined by a suitable method (*e.g.* qPCR (*Ph. Eur.* 2.6.21)).

**4-3. Infectious vector titre.** The titre of infectious vector in single harvests is determined following inoculation into cell cultures using a suitable technique for example plaque assay or CCID<sub>50</sub> assay, which may employ immunostaining or a molecular readout such as qPCR, or by flow cytometry (*Ph. Eur.* 2.7.24), or fluorescent focus assay.

**4-4. Extraneous agents** (*Ph. Eur.* 2.6.16). The single harvest complies with the tests for extraneous agents.

**4-5. Control cells or eggs.** The control cells and control eggs comply with the tests for extraneous agents (*Ph. Eur.* 2.6.16). If human diploid cells or continuous cell lines are used for production, the control cells also comply with a test for identification (*Ph. Eur.* 5.2.3).

## 5. PURIFIED HARVEST

Several single harvests may be pooled before the purification process. The purification process is typically validated to demonstrate the satisfactory removal of impurities.

The following tests may be considered for the control of purified harvests.

**5-1. Identification.** The recombinant vector including the genetic insert is identified by suitable methods such as immunochemical methods (*Ph. Eur.* 2.7.1), NAT (*Ph. Eur.* 2.6.21) or liquid chromatography (*Ph. Eur.* 2.2.29).

**5-2. Vector particle concentration.** For some vectors, for example adenoviruses, the concentration of vector particles in purified harvests is determined by a suitable method (*e.g.* qPCR (*Ph. Eur.* 2.6.21)).

**5-3. Infectious vector titre.** The titre of infectious vector in purified harvests is determined following inoculation into cell cultures using a suitable technique for example plaque assay or CCID<sub>50</sub> assay, which may employ immunostaining or a molecular readout such as qPCR, or by flow cytometry (*Ph. Eur.* 2.7.24) or fluorescent focus assay.

- 5-4. Ratio of vector particle concentration to infectious vector titre.** If the vector particle concentration is measured, a ratio of vector particle concentration to infectious vector titre is specified.
- 5-5. Ratio of infectious vector titre to total protein concentration.** For vectors less amenable to purification, for example poxviruses, the total protein concentration is determined by a suitable method (*Ph. Eur.* 2.5.33). The ratio of infectious vector titre to total protein concentration is calculated.
- 5-6. Expression of the genetic insert product.** For non-replicating viral vectors, the expression of the genetic insert product(s) is determined wherever possible, following inoculation of cell cultures with the particular preparation at a predetermined multiplicity of infection, by suitable immunochemical (*Ph. Eur.* 2.7.1) or biochemical assays or by flow cytometry (*Ph. Eur.* 2.7.24).
- 5-7. Residual host-cell protein.** The concentration of residual host-cell protein is determined by a suitable method (e.g. immunochemical method (*Ph. Eur.* 2.6.34)), unless the process has been validated to demonstrate suitable clearance.
- 5-8. Residual host-cell DNA** (*Ph. Eur.* 2.6.35). The content of residual host-cell DNA is determined using a suitable method, unless the process has been validated to demonstrate suitable clearance. qPCR is recommended for its sensitivity and specificity, but other suitable techniques may also be used.
- 5-9. Vector aggregates.** When vector aggregation has been shown to occur during characterisation studies, vector aggregates are determined by suitable methods (e.g. light-scattering).
- 5-10. Residual reagents.** Where reagents are used during the production process (e.g. benzonase), tests for these substances are carried out on the purified harvest, unless the process has been validated to demonstrate suitable clearance.
- 5-11. Residual antibiotics.** Where antibiotics are used during the production process, their residual concentration is determined by a microbiological assay (adapted from general method of *Ph. Eur.* 2.7.2) or by other suitable methods (e.g. liquid chromatography *Ph. Eur.* 2.2.29), unless the process has been validated to demonstrate suitable clearance.
- 5-12. Absence of replication-competent viral vector:** Reversion to replication competency must be evaluated carefully for replication-incompetent viral vectors. Detection of replication-competent viruses is performed by a suitable method (see section 3-5). No replication-competent viruses are found.
- 5-13. Microbiological control.** Depending on the preparation concerned, it complies with the test for sterility (*Ph. Eur.* 2.6.1) or the bioburden is determined (as described in *Ph. Eur. general monograph 0153*).
- The use of alternative microbiological methods for sterility testing could be possible, provided that a comprehensive validation package including the demonstration of the equivalence with the compendial test is available (*Ph. Eur.* 5.1.6).
- 5-14. Bacterial endotoxins** (*Ph. Eur.* 2.6.14): The level of bacterial endotoxins is monitored to ensure that it is compatible with the specification in the final lot.

## 6. FINAL BULK

Several purified harvests may be pooled during preparation of the final bulk. A stabiliser and other excipients may be added.

The following test may be considered for the control of the final bulk.

- 6-1. Microbiological control.** The microbiological quality of the final bulk is controlled either through a bioburden test (provided that a sterilising filtration is performed later in the production process i.e. at the filling stage) or through a sterility test (*Ph. Eur.* 2.6.1 when the formulated product was sterile filtered or when sterility is assured by aseptic conditions). Certain viral vectors such as vectors using poxvirus as backbone are not filterable and require processing under aseptic conditions.

**6-2. Antimicrobial preservative.** For multidose liquid preparations, the need for effective antimicrobial preservation is evaluated, taking into account likely contamination during use and the maximum recommended period of use after broaching of the container (in-use period).

**6-2-1.** If an antimicrobial preservative is used, it should not impair the quality of the vaccine. During development studies, the effectiveness of the antimicrobial preservative throughout the shelf-life and the in-use period is demonstrated (*Ph. Eur. 5.1.3* and *Ph. Eur. general monograph 0153*).

The preservative content is tested on the final bulk (section 6-2-1-1), on the final lot (section 7-7) and during stability studies for final lot.

*6-2-1-1. Antimicrobial preservative content.* The amount of antimicrobial preservative is determined by a suitable chemical or physico-chemical method. The content is not less than 85 per cent and not greater than 115 per cent of the intended amount.

**6-2-2.** If no antimicrobial preservative is used (e.g. if the matrix is not growth promoting), the quality of the vaccine needs to be ensured over the shelf-life and the in-use period.

## 7. FINAL LOT

The following tests may be considered for the control of the final lot.

Where the tests for bovine serum albumin and ovalbumin (if applicable) have been carried out with satisfactory results on the final bulk or the purified harvest, they may be omitted on the final lot.

**7-1. Identification.** The recombinant vector including the genetic insert is identified by suitable methods such as immunochemical methods (*Ph. Eur. 2.7.1*), NAT (*Ph. Eur. 2.6.21*) or liquid chromatography (*Ph. Eur. 2.2.29*).

**7-2. Appearance.** The product is inspected for degree of opalescence (*Ph. Eur. 2.2.1*), degree of coloration (*Ph. Eur. 2.2.2*) and presence of visible particles (*Ph. Eur. 2.9.20*)

**7-3. Osmolality** (*Ph. Eur. 2.2.35*): within the limits established for the particular preparation.

**7-4. pH** (*Ph. Eur. 2.2.3*): within the limits established for the particular preparation.

**7-5. Extractable volume** (*Ph. Eur. 2.9.17*). It complies with the test for extractable volume.

**7-6. Residual moisture** (*Ph. Eur. 2.5.12*): within the limits established for the particular freeze-dried preparation.

**7-7. Antimicrobial preservative.** Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

**7-8. Bovine serum albumin:** not more than the limit established for the particular preparation, determined by a suitable immunochemical method (*Ph. Eur. 2.7.1*), where bovine serum has been used during production.

**7-9. Ovalbumin.** If the vector is produced in embryonated eggs, the ovalbumin content is within the limits established for the particular preparation.

**7-10. Vector aggregates.** When vector aggregation has been shown to occur during characterisation studies, vector aggregates are determined by suitable methods (e.g. light-scattering).

**7-11. Sterility** (*Ph. Eur. 2.6.1*). It complies with the test for sterility.

See also section 5-13 for information regarding the use of alternative microbiological methods for sterility testing.

**7-12. Bacterial endotoxins** (*Ph. Eur. 2.6.14*): less than the limit established for the particular preparation. Guidance on the risk assessment to rule out the presence of non-endotoxin pyrogens not detected in the text for bacterial endotoxins is provided in *Ph. Eur. 5.1.10*.

**7-13. Thermal stability test.** This test can be carried out to monitor the lot-to-lot consistency (*Ph. Eur. general monograph 0153*). Maintain samples of the vector final lot at a temperature (e.g. 37°C) and for a length of time that are adapted for the particular preparation. Determine the infectious vector concentration after heating, as described below under Potency. Determine in parallel the infectious vector concentration of a non-heated sample. The estimation of the difference between the infectious vector concentration without heating and after heating is within the limits established for the particular preparation.

**7-14. Vector particle concentration.** For some vectors, for example adenoviruses, physical titration is performed by a suitable technique (e.g. qPCR (*Ph. Eur. 2.6.21*)). Use an appropriate vector reference standard to validate each assay.

The vector particle concentration is within the limits established for the particular preparation.

## 7-15. POTENCY

*A test or a combination of tests to assess vaccine potency are performed. The following (7-15-1 to 7-15-3) may be considered:*

**7-15-1. Infectious vector titre.** The preparation to be examined is titrated for infectious vector following inoculation into cell cultures using a suitable technique (e.g. by plaque assay or CCID<sub>50</sub> assay, which may employ immunostaining or a molecular readout such as qPCR, or by flow cytometry/FACS, or fluorescent focus assay). Titrate an appropriate vector reference standard to validate each assay.

The infectious vector titre of the preparation during its shelf-life is not less than the minimum titre stated on the label.

**7-15-2. Ratio of vector particle concentration to infectious vector titre:** if the vector particle concentration is measured, the ratio of vector particle concentration to infectious vector titre is within the limits established for the particular preparation.

**7-15-3. Expression of the genetic insert product.** For non-replicating viral vectors, the expression of the genetic insert product(s) is determined wherever possible, following inoculation of human cell cultures with the particular preparation at a predetermined multiplicity of infection, by suitable immunochemical (*Ph. Eur. 2.7.1*) or biochemical assays or by flow cytometry (*Ph. Eur. 2.7.24*).

## 8. GLOSSARY, LIST OF ABBREVIATIONS

<b>CCID<sub>50</sub></b>	50 per cent cell-culture infectious dose
<b>FACS</b>	Fluorescence-activated cell sorting
<b>MVA</b>	Modified vaccinia virus Ankara
<b>NAT</b>	Nucleic acid amplification techniques
<b>qPCR</b>	Quantitative polymerase chain reaction
<b>VSV</b>	Vesicular stomatitis virus