



**COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE
(CVMP)**

**GUIDELINE ON
LIVE RECOMBINANT VECTOR VACCINES FOR VETERINARY USE**

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1. INTRODUCTION

The requirements for the production and control of immunological veterinary medicinal products (IVMPs) laid down in Directive 2001/82/EC as amended fully apply to live recombinant vector vaccines.

The objective of this guideline is to define what should be presented in the analytical, safety and efficacy part of the application taking into account the particular properties of live recombinant vector vaccines in the target and non-target species, including the natural host of the parental organism (*where relevant*). The guideline will particularly provide advice where the vector itself is an immunogen.

This guideline does not repeat the requirements for environmental risk assessment, already developed in the Note for Guidance (EMEA/CVMP/074/95) and as an updated version published in the Notice to Applicants, Volume 6B, Part II G and H.

Vaccines where a live micro-organism (*bacteria or virus*) has been modified to express entire genomes or a portion of foreign RNA or DNA sequences or proteins and where the replicative competent vector acts as a carrier and may itself act as a protective immunogen fall within the scope of this guideline.

In addition to the requirements of Directive 2001/82/EC as amended, the applicant has to provide the information relating to the genetically modified organisms (GMO) which are requested in Annex IIIA of Directive 2001/18/EC. As not all the points included in this Annex IIIA will apply to live recombinant vector vaccines, it is not expected that the applicant will address all the points of this Annex.

As the headings or titles and the definitions of starting materials differ slightly in Directives 2001/18/EC and 2001/82/EC, the required data on starting materials, construction of the recombinant organisms and the recombinant vaccine are regarded as part of the history of the master seed as defined in Directive 2001/82/EC, Annex I, Title II, Part 6.C.2.1.

2. DEFINITIONS

Vector/ Recipient:

A replicative competent micro-organism (*bacteria or virus*) into which the genetic sequence (s) of interest will be inserted.

Vector vaccines (according to PhEur monograph 0062):

Vector vaccines are liquid or freeze-dried preparations of one or more types of live micro-organisms (bacteria or viruses) that are non-pathogenic or have a low pathogenicity for the target species and in which have been inserted one or more genes encoding antigens that stimulate an immune response protective against other micro-organisms.

Recombinant live vector vaccines:

Recombinant live vector vaccines are preparations of one or more types of live bacteria or viruses. One or more DNA/RNA sequences have been inserted into these organisms. These organisms generally have a stable non or low pathogenic phenotype for the species the vaccine is intended for.

Recombinant live vector vaccines are expected to be attenuated and genetically defined live vaccines, which have defined, non-reverting mutations or deletions.

Homologous vector:

When the target species of the vaccine is a natural host for the vector, this is considered a homologous vector.

Heterologous vector:

When the target species of the vaccine is not one of the natural hosts for the vector, the vector is classified as a heterologous vector.

3. POINTS TO BE ADDRESSED FOR A LIVE RECOMBINANT VECTOR VACCINE

3.1. QUALITY

The vector, bacteria as producer of shuttle plasmids, any genetic material used in the construction, the inserted gene(s) and the final construct should be described in detail. In this context, the final construct is regarded as master seed. Bibliographical references for the source materials could be acceptable, provided they cover the material(s) used for the production of the final construct.

The identity and direct relation of the material described in the scientific publication with the master seed (s) of the vaccine should be justified.

The description of the starting materials shall include:

3.1.1 Substrates for production/parental organisms

A full description of the starting cells, bacteria or virus strains and plasmids should be provided. The description should cover the material to produce the master seed and the master seed (final construct).

3.1.2. Genetic material used in the construction of the vector (according to Directive 2001/18/EC: II.B.2. Sequence of transposons, vectors and other non-coding genetic segments)

For the plasmids used to construct the live recombinant vector vaccine, all the data available about the construction, the structure, the sequence and the properties should be provided.

The recombinant transfer plasmid and the bacteria used as carrier for the plasmid should be described in detail and the information presented should indicate their characteristics and their detailed origin. All the elements in the plasmid should be described, including promoters, enhancers and the selected foreign coding sequences. Information on the analysis conducted to confirm the structure of the transfer plasmid should be submitted.

As some plasmids are suspected to be insufficiently stable, plasmid instability must be excluded if it may have an impact on the final vector vaccine.

The use of antibiotic markers encoding resistance to antibiotics used for therapy should be avoided wherever possible. Art. 4 of Directive 2001/18/EC should be taken into consideration. Transfer of the encoding resistance to the final vector vaccine is unacceptable.

3.1.3 Vectors

The strategy of the construction of the recombinant vector vaccine should be presented as described in Directive 2001/18/EC: II.C. Characteristics of the modified organism. I. Information relating to the genetic modification.

Whenever possible, the virulence genes of the vector should be characterised. If applicable, knowledge about the function of deleted and added genes and proteins expressed in the vector has to be provided; a detailed description of markers which are recommended to be present should be provided.

The effect of the gene deletion causing a change in the biological properties of the live vector should be investigated. Details of the integration of plasmid DNA into the vector should be presented and should address the impact of the gene insert on the expression of the neighbour genes in the vector, whenever possible.

The genetic characterisation of the vector should be presented. This should include at least the sequencing of the regions flanking the insertion sites and the sites themselves.

3.1.4 Sequences to be inserted

Characterisation of the inserted sequence with appropriate methods should be performed.

The sequences to be transferred to the vector should be clearly defined and sequenced as far as it appears to be necessary to evaluate quality, safety and efficacy of the product.

3.1.5 Characteristics of the final vector vaccine (Directive 2001/18/EC: II.C.2. Information on the final GMO)

Information should be presented on the genotype and phenotype of the live recombinant vector and on the methods used for its screening and identification. Data on its genotypic and phenotypic stability, virulence, tissue and host tropism should be submitted as part of the safety package. If the strain is deemed to be replication abortive in the target species, the applicant may confirm this *in vitro* using an appropriate range of cell types from the target species.

During licensing, it should be demonstrated as part of validation of the production process that the recombinant vector vaccine is stable throughout the manufacturing process to the finished product and that the integrated sequences have not undergone any rearrangements or mutations.

The antigen expressed by the recombinant vector vaccine should be characterised by biochemically, molecularly and/or immunologically relevant methods, to demonstrate the quality of the final product.

The applicant should provide techniques that allow differentiation between the parent strains of the vector and the vector vaccine.

3.2. SAFETY

3.2.1. Target species

The safety of the live recombinant vector vaccine should be investigated in the target species for the vaccine according to the requirements of Directive 2001/82/EC, Annex I, Title II, Part 7.

The following points in particular deserve attention:

3.2.1.1. Spread of the recombinant vector vaccine

If the live recombinant vector vaccine has been shown capable of spreading to target and non-target species, adequate evaluation should be performed. For that purpose, safety studies should be performed for relevant species sharing the same ecosystem as vaccinated animals and focussing on species known to be susceptible to the vector, in particular the natural host species of the parental vector. The range of species to be addressed should be justified.

Three steps should be undertaken:

- Transmission from vaccinated target animals to non-vaccinated target animals.
- Transmission from vaccinated target animals to non target animals. This includes the most common domestic and, if relevant wild species, which live in the same environment as the

vaccinated target species or may have direct or close contact with them. A risk analysis concerning the extent of the exposure should be performed.

- Transmission from vaccinated target animals to humans.

If there is a reason to suppose the live recombinant vector vaccine is able to spread to humans, a risk analysis of the pathogenicity of the recombinant vector and of the parent strain in humans should be performed.

If the GMO can be transmitted to animals or humans, the conditions for use should be described in detail.

3.2.1.2. Dissemination in the vaccinated animals

The applicant should investigate possible changes of tissue tropism by comparing the behaviour of the vector and the recombinant vector vaccine.

If the recombinant vector is deemed to be replication abortive the applicant should demonstrate this in the target species. A sensitive, validated detection system for the recombinant vector vaccine should be available.

3.2.1.3. Reversion to virulence

Like conventional vaccines, recombinant vector vaccines must be non pathogenic or low pathogenic to a level that will ensure that the vaccine is safe for the intended target species.

It has to be demonstrated that the insertion of foreign gene(s) does not lead to an increase in virulence or a modification in tissue tropism.

3.2.2 Ecotoxicity

Ecotoxicity should fully rely on the requirements of Directives 2001/82/EC and 2001/18/EC (Annex II).

The possible ecotoxicological effects of the vector vaccine have to be assessed as follows:

- Study of virulence to target and non-target species at risk see also 3.2.1.1.
- Horizontal transmission and potential of recombination of the vector vaccine or part of it.
- Host-range specificity
- Potential for establishment in the environment (e.g. dissemination of baits in the environment, persistence of the product in the environment at various climate conditions).
- A validated method (e.g. identification of molecular structure) should be provided to differentiate the vector vaccine from the wild type microorganism/antigen and/or inserted sequences and to detect the vector vaccine in the field. (see also Directive 2001/18/EC, Annex 3A, Part V.1), especially if diseases caused by the wild strains are subject to eradication and control programs).
- Data from other assessments performed with the same vector but other inserted sequences could be used as well as long as the new insert does not change the characteristics and specifications of the final construct.

3.3. EFFICACY

The requirements of Directive 2001/82/EC, Annex I, Title II, Part 8 fully apply. Unless a specific monograph for live recombinant vector vaccines exists the most relevant monograph should be taken into account to define the type of studies which have to be performed.

For each claim, the immunogenicity test described in relevant PhEur monographs may be used.

For recombinant vector vaccines for which no claim is made for the vector, the applicant should document the immune response which is induced after vaccination and consider the possible impact on current vaccination schedules.

The effect of pre-existing immunity to the vector and/or the foreign antigen(s) expressed by the vector should be studied.

The possibility to boost the induced immunity against the vector antigen and/or the foreign antigen(s) within a claimed/intended vaccination schedule should be investigated if booster vaccinations are deemed necessary.