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Guideline on plasmid DNA vaccines for veterinary use

Draft

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This guideline replaces 'Note for guidance: DNA vaccines non-amplifiable in eukaryotic cell for veterinary use' (CVMP/IWP/07/98-FINAL).

Comments should be provided using this template. The completed comments form should be sent to Vet-guidelines@ema.europa.eu

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Executive summary

The aim of the guideline is to outline the information which should be included for plasmid DNA vaccines in the marketing authorisation application (MAA) dossier of veterinary vaccines as required in Section IIIb (Requirements for immunological veterinary medicinal products) of the Commission Delegated Regulation (EU) 2021/805 amending Annex II to Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products (repealing Directive 2001/82/EC), referred to as Annex II to Regulation (EU) 2019/6 throughout the document.

The guideline discusses aspects to consider for veterinary DNA vaccines and provides guidance on the information which should be included in Parts 2, 3 and 4 of the MAA.

This guideline replaces the 'Note for guidance: DNA vaccines non-amplifiable in eukaryotic cell for veterinary use' (CVMP/IWP/07/98-FINAL).

1. Introduction (background)

The use of DNA for vaccination has progressed in the last few years and several trials using products of this type for vaccination are in progress. DNA vaccination involves the inoculation with (a) gene(s) encoding (a) relevant antigen(s) against which an immune response is desired. The gene(s) can be under the control of a promoter, which will permit its expression in the vaccinated animal. This gene construct may be contained, for manipulation and manufacturing purposes, within plasmid DNA (bacterial plasmid DNA or de novo synthetic DNA).

DNA vaccines have potential advantages over the direct inoculation of the antigen itself, e.g., they may provide a much wider stimulation of the immune system, including the stimulation of a cytotoxic T cell response. DNA vaccines can also have advantages over the use of a live attenuated microorganism, e.g., the avoidance of the potential risk of reversion to virulence. Furthermore, the manufacture of a plasmid DNA vaccine may in some instances be simpler, quicker, more adaptable, and cost efficient than more traditional forms of vaccines as well as providing wider scope to encompass other modes of delivery.

DNA vaccines have the potential to be used as a vaccine platform technology. In case the use of a vaccine platform technology master file is pursued for authorisation, then reference should also be made to the guidance on data requirements for vaccine platform technology master files.

The 'Note for guidance: DNA vaccines non-amplifiable in eukaryotic cell for veterinary use' (CVMP/IWP/07/98-FINAL) came into effect in 2001. Taking into account the scientific developments and experience gained in the meantime, the revision and the development into a guideline were recommended by CVMP.

2. Scope

This document provides advice to manufacturers seeking marketing authorisation for nucleic acid vaccines for use in animals when the vaccine consists of (a) bacterial or a synthetic DNA plasmid(s).

This document is applicable to DNA vaccines, as defined in the document, consisting of plasmid DNA non-amplifiable in eukaryotic cells. Plasmid DNA vaccines may be composed of more than one plasmid coding for different immunogens isolated from a single pathogen (virus, bacterium or parasite) or different pathogens or synthesised de novo.

Developments involving plasmid DNA delivered by live vectors (e.g. bacteria/viruses) or capable of amplification in the vaccinated animal by any mechanisms are not within the scope of this document.

However, this guideline can be used as a reference for other DNA vaccines under development e.g. lentiDNA, minicircle DNA, minimalistic expression constructs (e.g. MIDGE DNA and Doggybone DNA) and delivery vehicles (e.g. lipid micelles).

3. Legal basis

This Guideline should be read in conjunction with the introduction and general principles of Annex II to Regulation (EU) 2019/6 and all other relevant EU and VICH guidelines as well as relevant European Pharmacopoeia (Ph. Eur.) monographs.

Commission Delegated Regulation (EU) 2021/805 of 8 March 2021 amending Annex II to Regulation (EU) 2019/6 of the European Parliament and of the Council

Ph. Eur. 0062 Vaccines for veterinary use

Ph. Eur. 0784 Recombinant DNA technology, products of

Ph. Eur. 5.2.5 Management of extraneous agents in immunological veterinary medicinal products

Ph. Eur. 5.2.6 Evaluation of safety of veterinary vaccines and immunosera

Ph. Eur. 5.2.7 Evaluation of efficacy of veterinary vaccines and immunosera

4. General considerations

There are several aspects of the use of a plasmid DNA vaccine, which have to be considered:

1. The plasmid DNA which is internalised by the cells of the vaccinated animal may integrate into the chromosomes of the vaccinated animal and disrupt the normal replicative state of that cell, causing uncontrolled cell division and tumorigenesis:

   After the injection of DNA into an animal, a small proportion of the DNA molecules enters cells. The probability of any DNA molecule integrating into the chromosome is low and given that oncogenesis is a multi-factorial event, the risk of insertional mutagenesis is exceedingly low. The use of supercoiled DNA (scDNA) can reduce the potential risk of insertional mutagenesis. It is recommended that the pDNA should be supercoiled to more than 80% to help prevent the potential risk of insertional mutagenesis (Lee et al, 2018). Additionally, the use of minicircle and ministring DNA reduces the risk further as the unmethylated CpG (cytosine-phosphate-guanine) repeats are removed (Lee et al, 2018). So far, the integration of plasmid DNA into chromosomal DNA of a vaccinated animal has not been observed (EFSA, EFSA Journal 2017). However, integration (e.g. into the muscle cells surrounding the vaccination site or into germ line cells in the gonads) cannot be discounted.

   The current testing methods are not sufficiently sensitive to routinely detect actual integration that may be orders of magnitude below the limits of detection of the methods. Therefore, each product must be assessed on a case-by-case basis, taking into consideration the specific limits of detection, the route of administration, the target tissue, the amount of plasmid administered, and the age of the vaccinated animal. The information should be compiled in a risk assessment.

2. The immune response to antigens which are expressed due to injected DNA also raises concerns about possible adverse effects on the immune system, including auto-immune reactions. Molecular mimicry and bystander activation can be attributed to vaccination in general; however, there is little further evidence that this risk is increased in response to DNA vaccines specifically.

Although DNA generally has a very low immunogenic potential, bacterial DNA can have a mitogenic or immunostimulatory effect. Double stranded (ds)DNA alone can induce both innate and T cell-mediated immune responses via a number of signalling pathways. While this may be of benefit due to an adjuvant effect, there is a possibility of this triggering autoimmune activation. The specific incorporation of immunostimulatory sequences in any form should be applied with care and should be adequately risk assessed and justified.

3. Additional genes encoding co-stimulatory molecules (cytokine/chemokines) may pose additional risks:

   The introduction of genes encoding co-stimulatory molecules, aimed at enhancing the immune response triggered in the vaccinated animal, could have detrimental effects especially, for example, if the cytokine has been introduced in a plasmid whose expression cannot be terminated. Any introduction of co-stimulatory genes would have to be thoroughly justified from both safety and efficacy perspectives

4. The expressed antigen may itself have undesirable biological activity:

   An encoded antigen may exhibit undesirable biological activity. In such a case, appropriate steps may have to be taken (e.g. deletion mutagenesis) to eliminate the activity while retaining the desired immune response.

5. In principle, the presence of (functional) antibiotic resistance genes in the finished product is not acceptable.

5. Data requirements

The data requirements for veterinary vaccines have to be addressed in the application dossier. The information must be presented in accordance with the format set out in Annex II to Regulation (EU) 2019/6). The following are points to be addressed, as appropriate, in the various sections of the dossier.

5.1. Data requirements for Part 2 Quality

Qualitative and quantitative composition (IIIb.2.A1.)

The name of the active substance, the quantity per dose, the function and reference to standards should be given (in a tabular format). "DNA" is not an adequate description of the active substance. The full title (identity) of the plasmid encoding for the antigen/protein of interest should be provided. The minimum concentration of plasmid used to establish efficacy should be given (if supercoiled DNA is used, the amount of supercoiled plasmid as a measurement of the potency should be stated).

Product development (IIIb.2.A2.)

A detailed description of the source and development of the vaccine plasmid and its characterisation should be provided.

The rationale for the selection of the gene(s) encoded in the plasmid(s) should be discussed and the sequence of the wild-type gene and the antigenic properties of the encoded protein in its natural state should be described and justified. This should include details of the gene(s) encoding the protein(s), against which the immune response is sought, information on the construction of the entire plasmid(s) and the host bacterial cell(s) or, if a synthetic plasmid DNA is used, the details of the synthesis of the plasmid should be provided.

For bacterial DNA plasmids, the origin of the gene of interest should be described in detail, such as the name of the micro-organism or cell from which the gene was derived, source of origin, its species, passage history, subtype and isolation strategy followed.

For a synthetic DNA plasmid, the steps involved in the synthesis should be provided; de-novo gene synthesis, and the cloning and mutagenesis techniques used should be described. Details of sequence optimization and oligo design should be provided, where relevant.

Sequence verification including a detailed plasmid map is required.

The steps in the construction of the entire vaccine plasmid should be described, including the source of the plasmid(s) used, and subclones generated during the cloning procedure. Flow diagrams of all intermediate recombinant DNA cloning procedures should be provided.

Functional components such as regulatory sequences (e.g. origins of replication, viral/eukaryotic promoters, enhancers, introns, termination sequences) and selection markers (if used) should be clearly indicated and information on the source and function of these elements should be provided. Sequence data, including a sequencing certificate of analysis, on the entire plasmid (or all of the plasmids in the case of multivalent vaccines) will be required and the use of all specific elements or regions of DNA should be justified. DNA sequence homology checks of the plasmid with all published DNA sequence data of the target species should be performed and the information given in the application dossier. An informative restriction map of the vaccine plasmid should be presented. Special attention should be given to the nature of a selection marker, if used. The use of certain selection markers such as resistance to antibiotics as well as certain sequences such as retroviral-like long terminal repeats (LTRs) and oncogenes should be avoided.

The rationale for the choice of the host bacterial cell used for production of plasmids should be provided along with a description of its source, phenotype and genotype. It should be demonstrated that the host cell is free from bacteriophage and other extraneous agent contamination in line with Ph. Eur. requirements. The identity of the vaccine plasmid after transfection into the bacterial cell to be used for production and the phenotype of the transfected cell should be confirmed. Since rearrangements of the plasmid are unacceptable, data on the stability (plasmid retention and sequence homology) of the plasmid within the bacterial cell will be required. The expression of prokaryotic genes, such as a selection marker, in a eukaryotic cell line should be investigated. A risk assessment should be provided to justify the inclusion of a particular marker gene, where relevant. The likelihood of any cross-contamination e.g. by recombination with endogenous sequences in the cell substrate used during the construction or production of the DNA plasmid should be evaluated.

Description of the manufacturing method (IIIb.2B.)

Procedures and materials used in general should be described in detail, for example, in the fermentation (and /or culturing) and harvesting process**.** A comprehensive flow chart of the manufacturing process should be provided. Information on process parameters (e.g. fermentation and harvesting conditions) should be presented, relevant in-process controls should be identified and acceptance criteria should be established. These may include, but are not limited to: number of passages, culture growth rates and viability, bioburden and endotoxin, identity (desired transgene and plasmid), purity and plasmid yield.

For fermentation processes, the minimum and maximum level of cell growth to be accepted during production should be defined and should be based on information concerning the stability of the host cell/plasmid system up to the maximum level of fermentation used. At the end of fermentation and harvesting, bacterial cell/plasmid characteristics should be investigated. This may include restriction fragment analysis, and the yield of both cells and plasmid.

Any methods used to extract the plasmid DNA and remove and/or reduce the concentration of process- and product-related contaminants or impurities must be described in detail and the process explained and validated.

Clearance capacity for the removal of contaminants will be established for the purification process by the difference in contaminant levels before and after each purification step. Batch acceptance will be established based on compliance with the upper acceptance limits defined for each contaminant. Validation studies for clearance capacity will be required.

Suitable in-process controls for any potential contaminants of concern should be developed and routine batch test upper acceptance limits established, based on data from tests showing the safety of that concentration.

Production and control of starting materials (IIIb.2C.)

Cell seed

The production of plasmid DNA vaccines should be based on a well-defined master cell seed (MCS) and working cell seed (WCS) system, wherever possible. The cloning and culturing procedures used for the establishment of the MCS should be described. The origin, form, storage and use must be described in detail for all cell seeds. The MCS should be fully characterised and specific phenotypic features which form a basis for identification should be described. Potential testing conducted on producer cell lines (organised in a cell bank system) includes: identity, purity, cell number, viability, strain characterisation, genotyping/phenotyping, and if appropriate verification of the plasmid/transgenic/helper sequence structure (e.g. restriction analysis or sequencing), genetic stability, copy number, identity and integrity of the introduced sequences, as relevant.

The sequence of the entire plasmid should be confirmed at the stage of the MCS and WCS or at critical stages of de novo synthesis. Plasmid stability should be demonstrated throughout the process to the finished product, i.e. sequence alignment of DNA sequence results at each stage from MCS/starting molecules of de novo synthesis up to and including the finished product should be provided, and should be 100% identical (the consistency batches can be used to demonstrate this). WCSs/intermediate products should be adequately characterised also and meet established acceptance criteria.

For fermentation processes the viability of the host-vector system in the MCS and WCS under storage and recovery conditions should be determined. The integrity of the plasmid DNA sequence should be demonstrated by validating the proposed storage and recovery conditions of WCS as the worst-case scenario.

It should be demonstrated that MCS and WCS/starting molecules for de novo synthesis are free from extraneous microbial agents in accordance with the Ph. Eur. requirements.

Bacterial plasmids should be demonstrated to be free from potential contamination, for example with endogenous viruses, including wild-type forms of any viral vectors.

The absence of bacterial and fungal contamination, as well as mycoplasma and spiroplasma where relevant, should be determined.

Control tests during the manufacturing process (IIIb.2D.) and control tests on the finished product (IIIb.2E.)

Specifications for the active substance and finished product should be established and justified. Descriptions of analytical methods and acceptance limits for in-process and finished product testing, including information on assay qualification or validation, should be provided. It is recommended that the specifications include an assessment of the identity, molecular form and quantity of the plasmid, purity, potency, endotoxin content and sterility. A justification of the specifications should be provided.

A summary of the results of the testing on all relevant batches produced should be provided. The appropriateness of performing tests on the bulk of purified plasmid versus the formulated vaccine should be considered on a case-by-case basis and justified.

At least three batches of vaccine, including the final dosage form, should be characterised as fully as possible to determine consistency of the manufacturing process and to demonstrate conformity with specifications. Any differences between batches should be noted.

Identity

The identity of each batch of purified plasmid should be confirmed by a suitable technique (e.g. PCR analysis, sequencing, restriction enzyme analysis), including confirmation of the molecular form of the plasmid (e.g. by agarose gel electrophoresis).

Confirmation of the identity of the expressed antigen should also be documented through the use of specific assays, such as Western Blot, immunofluorescence assay (IFA) and/or ELISA. For vaccines containing plasmids encoding non-antigenic biologically active molecules, confirmation of the identity of the expressed molecule should be assessed with an appropriate bioassay.

Other tests, as appropriate and depending on the method of production, purification and nature of the plasmid, should also be applied.

DNA quantification

A quantitative test for plasmid DNA content should be carried out on each batch of finished product. The amount of the active substance (supercoiled plasmid) should be determined and specifications set. These quantitative tests should be appropriately validated.

Potential degraded forms or non-functional forms of DNA should be taken into consideration, to ensure an efficacious plasmid DNA content.

Purity

The purity of each batch of plasmid vaccine must be assessed. Specified limits should be set to determine the level of contaminants of bacterial-cell origin which are considered acceptable. Additional tests may be required depending on the production process used, the results obtained from purification, process validation and safety studies (e.g. tests for the content of residual RNA concentration, residual host cell genomic DNA concentration, residual protein content).

Each batch of product must also be tested for endotoxin, unless the omission of endotoxin testing can be satisfactorily justified.

For all impurities, acceptance criteria based on consistency batches and safety studies should be established per quantity of total DNA or EU/ml for endotoxins (as per Ph. Eur. requirements).

Batch potency test

The potency of each batch of finished product should be established using a suitably validated test. The most appropriate approach will vary depending on the composition of the vaccine, the nature of the disease, the expressed antigen(s) and the immune response being sought. Thus, the design of a potency assay will require careful consideration by the manufacturer and will be assessed on a case-by-case basis.

Whatever the assay, an approved in-house reference preparation should be established, where possible, from an appropriately characterised batch of vaccine that has been shown to be efficacious (or of the same quality as the efficacious batches).

An appropriate level of functional activity of the DNA plasmid should be demonstrated at least during the development phase. A qualitative assay can be used to detect the expression of the protein of interest in a cell line of the target species origin, the assay may not be required to be performed on a routine basis for batch potency testing.

A quantitative assay to determine the amount of (sc) DNA, for example by HPLC or other suitably validated tests, needs to be established. This should be performed routinely and may suffice at release if a qualitative assay has been suitably verified during product development and linked to the expected functional activity of the DNA plasmid.

Information regarding the reagents used and replacement of those reagents for validated potency test methods should be provided.

Batch to batch consistency (IIIb.2F.)

Results from three consecutive batches of finished product from three production runs representative of routine production should be provided, or otherwise justified. Preferably, the three batches of vaccine should be prepared from three separate active substance batches. Manufacturing batch protocols for each of the batches should be provided.

It should be demonstrated that the plasmid is stable throughout the production process to the finished product, i.e. sequence alignment of DNA sequencing results. A justified control strategy up to and including the finished product should be provided and results shown to be identical.

Stability tests (IIIb.2G.)

Stability of active substance

Data from batches of the bulk harvest (production culture) stored at a specified temperature demonstrating no detrimental effect of proposed storage on the plasmid DNA, should be provided.

Stability of the finished product

Stability data from at least three batches of finished product manufactured according to the proposed process should be provided to determine an appropriate shelf life for the product. To assess the physical integrity of the supercoiled plasmid DNA content in the finished product over time the supercoiled plasmid DNA content can be measured using a combination of different methods: e.g. agarose gel electrophoresis and chromatography (e.g. HPLC). The potency test should be used to determine if the product is efficacious (functional) over the proposed shelf life of the product.

5.2. Data requirements for Part 3 Safety

Safety testing should be carried out in accordance with the requirements of 'Section IIIb.3. Part 3: Safety documentation (Safety and residue tests)' of Annex II to Regulation (EU) 2019/6 and according to the requirements of Ph. Eur. 5.2.6 'Evaluation of safety of veterinary vaccines and immunosera'. The following are examples of specific points, which should be addressed as appropriate under the headings indicated. Unless otherwise justified, batches with the maximum dose should be used.

Distribution studies

Distribution data should be generated for the DNA vaccines.

The route of DNA inoculation as well as the amount of DNA administered may influence the distribution of the DNA in the body. Localisation studies should be designed to determine the distribution of the DNA after administration via the proposed route and employ the proposed method of inoculation. Using the most sensitive methods available, the extent of DNA distribution to and the cellular uptake by the

target and the surrounding tissues including the draining lymph nodes should be analysed at various time points (e.g. day 1, day 7 and one month after vaccination or at a longer time, when appropriate). The timing of sampling should take into account information on the duration of gene expression and the persistence of the DNA in the body of the vaccinated animal.

Distribution data obtained with one type of plasmid should also be applicable to all other plasmids sharing the same backbone and differing only by the cloned antigenic gene provided that the inserts are approximately the same size.

Integration and tumourigenesis

These studies, where relevant, should be undertaken with the finished product.

A step-by-step analysis should be carried out. Investigations should be undertaken for the presence of plasmid DNA at the site of administration and draining lymph node. If, or when, plasmid DNA is detected, the most sensitive methods available should be used to investigate integration of plasmid DNA into the host genome. If integration is detected or suspected, and if there is a risk of tumourigenicity due to the life expectancy of target animals, a test for tumourigenicity in a susceptible laboratory animal system should be carried out. The incidence of tumours in the target species, particularly at the site of injection and in the target tissue, should be recorded at the end of each experiment in the safety and efficacy studies. Post marketing, any reports of tumours in the target species should be carefully monitored, as part of pharmacovigilance.

Reproductive toxicity

Standard studies on impact on reproductive performance should be conducted for DNA vaccines as for other types of vaccine.

The possibility of migration of the DNA to gonadal tissues and potential DNA transfer into germ line cells of vaccinated male and female animals and thus potential transmission to offspring must be considered. If necessary, the distribution studies mentioned above should be extended to provide information on this point.

Examination of immunological functions

Specific studies should be conducted to address the possibility of adverse effects on the immune system, particularly if cytokine or other immunomodulatory genes are used as adjuvants.

5.3. Data requirements for Part 4 Efficacy

Data should be submitted in accordance with the requirements of 'Section IIIb.4. Part 4: Efficacy documentation (pre-clinical studies and clinical trials)' of Annex II to Regulation (EU) 2019/6 and according to the requirements of Ph. Eur. 5.2.7 'Evaluation of efficacy of veterinary vaccines and immunosera'.

The standard requirements for efficacy testing of veterinary vaccines are applicable to these products. Efficacy also should be demonstrated with batches containing the minimum efficacious amount of the active substance.

Definitions

Plasmid DNA vaccine:

A vaccine in which the active substance is a genetically modified plasmid(s) containing DNA sequences encoding for antigen(s) against which an immune response is sought.

References

Lee et al., 'Engineering DNA vaccines against infectious diseases' Acta Biomaterialia 2018, 80: 31-47

EFSA (European Food Safety Authority), Houston, R et al. 'Assessment of the potential integration of the DNA plasmid vaccine CLYNAV into the salmon genome', EFSA Journal 2017;15(1):4689, 15 pp