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4 **Reflection paper on the use of heat treatment to**  
5 **inactivate retrovirus RD114 in live immunological**  
6 **veterinary medicinal products (IVMPs)**  
7 **Draft**

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|----------------------------------------------|------------------|
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Reflection paper on the use of heat treatment to inactivate retrovirus RD114 in live immunological veterinary medicinal products (IVMP)

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## Executive summary

This guideline outlines the data requirements to be submitted by the marketing authorisation holder (MAH) to introduce a heat treatment to inactivate retroviruses in the active substance for the production of live viral vaccines for immunological veterinary medicinal products (IVMP) and to show the absence of negative impact of this treatment on the IVMP.

## 1. Introduction

Following the 2010 Journal of Virology publication [1] reporting the detection of retrovirus RD114 in some live attenuated feline and canine vaccines commercially available in the EU and Japan, a scientific assessment conducted by EMA/CVMP (EMA/CVMP/300321/2010) concluded that the presence of replicative retrovirus RD114 in existing feline and canine vaccines due to the use of feline origin starting materials (such as CrFK feline kidney cells, [2]) in their manufacturing processes could not be ruled out.

The EMA/CVMP assessment considered that immediate regulatory action was not necessary based on the history of use of these vaccines in the EU to date (e.g. millions of doses administered over a number of decades). The safety profile of authorised feline and canine vaccines was not considered to have changed rather the issue related to improvements in detection methods for retroviruses (e.g. use of molecular technologies). The benefit of the continued use of available vaccines against cat and dog diseases was considered to clearly outweigh the risk linked to the potential presence of replicative retrovirus RD114.

However IWP has also initially considered a risk management strategy for already existing veterinary vaccines in relation to RD114 and has identified some actions in order to have on the market only feline vaccines that have retroviral RD114 levels within an established acceptance limit, and for canine vaccines only products that are free of replicative retrovirus RD114.

One of the actions concerns adjustments to the manufacturing processes so that the retroviral RD114 content of the currently authorised vaccines is within the acceptance limit (e.g. use of inactivation steps/replacement of contaminated starting materials -e.g. seed materials, etc...). This document outlines the requirements with regard to the inactivation process that may be used to reduce the RD114 level.

## 2. Scope

The presence of retrovirus RD114 in vaccines is considered to be related to the use of feline origin starting materials (e.g. cells, viruses) in the manufacturing processes of feline and canine vaccines.

A meeting was convened on 8 February 2013 with industry to discuss and understand practical possibilities and proposals how reduce and/or to clear finished products from the presence of replicative competent RD114 and the timing of introduction of any such proposals. One of the methods discussed at the meeting was the use of the heat treatment to inactivate RD114 in live active substances.

Therefore this document considers the possible use of heat treatment for the inactivation of replicative RD114 applied to active substances of currently authorised vaccines in order to inactivate this extraneous agent in live vaccines.

This document indicates the requirements that need to be fulfilled to show that there is no negative impact of this treatment on the vaccines.

Before process changes such as heat treatment to remove / inactivate RD114 can be accepted, a standardised, validated retroviral RD114 detection test still needs to be developed and the acceptable limit for retroviral RD114 content has to be established (the term "limit" takes into account the detection limit of the test as well as the retroviral RD114 levels in current vaccines which to date are not considered to be associated with a significant risk).

### 3. Background

It is indicated in Annex I, Title II of Directive 2001/82/EC as amended *that the Seed materials, including cell seeds and raw serum for anti-serum production shall be tested for identity and extraneous agents.*

*If the presence of extraneous agents is detected or suspected, the corresponding material shall be discarded or used in very exceptional circumstances only when further processing of the product ensures their elimination and/or inactivation; elimination and/or inactivation of such extraneous agents shall be demonstrated.*

### 4. Validation of the heat treatment

#### Objective of the study:

Define the parameters of the heat treatment and provide evidence that this treatment will effectively inactivate the replicative retrovirus RD114.

#### Design of the study:

A spiking with a known amount of live RD114 retrovirus of the active substance that will be heat treated is performed. The active substance is produced as recommended in the analytical part of the marketing authorization dossier and once the heat treatment has been applied, a control of the residual RD114 is performed. The amount of retrovirus added to the active substance should be as high as possible in order to determine the ability of the heat treatment to inactivate the virus adequately and to calculate the reduction factor.

A validated quantitative infectivity assay should be performed to titrate the retrovirus before and after the treatment.

#### Limits:

The method of quantification of the RD114 retrovirus should have adequate sensitivity and reproductibility and should be performed with sufficient repetitions to ensure statistical accuracy of the results.

### 5. Possible impact of the heat treatment on the finished product

It is assumed that the heat treatment is intended to be applied to the live active substances of the vaccine during manufacture. The aim is to inactivate completely the replicative retrovirus RD114 with minimal impact on the live active substances.

The heat treatment may potentially have different consequences:

- there may be a necessity to increase the antigen content of the active substance in the finished product because the heat treatment has inactivated a part of the live active substance. In order to maintain the specifications, the applicant may need to adjust the formulation to keep the same content of live active ingredient as in the original vaccine. In practice this means that the vaccine will globally contain a higher amount of antigen (mixture of live and inactivated antigens of the same active substance).
- it may induce a selection of live virus mutants (quasispecies) that are more resistant to heat, potentially with different properties, and which then will be largely present in the finished product.

## 6. Requirements to demonstrate the absence of negative impact

If the heat treatment is introduced in the production process, the marketing authorisation holder has to demonstrate that this treatment has no negative impact on the quality, the safety and the efficacy of the finished product

In order to demonstrate the absence of negative impact of the heat treatment, the following points have to be taken into account:

- with regard to the quality of the finished product, except for the heat treatment and the possible adjustment of the content of the live active substances in order to keep the specifications unchanged, the manufacturing process of the vaccine should not be modified. The results obtained for the titration performed on the live active substances in the finished product have to remain within the range established for the original vaccine.
- with regard to the safety of the vaccine, the safety of an overdose administration has to be demonstrated in laboratory in compliance with the requirements of Directive 2001/82/EC as amended and with the Ph. Eur. General monograph "*Vaccines for veterinary use*" referring to the Ph. Eur chapter 5.2.6 "*Evaluation of safety of veterinary vaccines and immunosera*". The results obtained should be similar to those obtained with the original vaccine.
- the efficacy of the vaccine containing the heat treated active substance has to be tested in laboratory conditions according to the requirements of the immunogenicity test described in the Ph. Eur. monograph corresponding to the active substance. The results should be in compliance with the threshold defined in this Ph. Eur. monograph.

If no Ph. Eur. monograph exists, the immunogenicity test should comply with the requirements of the Ph. Eur. General monograph "*Vaccines for veterinary use*" referring to the Ph. Eur chapter 5.2.7 "*Evaluation of efficacy of veterinary vaccines and immunosera*", and should be the same as the one performed to demonstrate the efficacy of the active substance of the original vaccine and the results obtained should be similar.

## 7. References

- [1] Miyazawa T et.al., Isolation of an infectious endogenous retrovirus in a proportion of live attenuated vaccines for pets. J Virol. 2010 Apr;84(7):3690-4.
- [2] Baumann J et al., CrFK Feline Kidney Cells Produce an RD114-Like Endogenous Virus That Can Package Murine Leukemia Virus-Based Vectors. J virol, 1998 Sep;72(9):7685-7687.