



EUROPEAN MEDICINES AGENCY  
SCIENCE MEDICINES HEALTH

10 September 2015  
EMA/CVMP/IWP/37924/2014  
Committee for Medicinal Products for Veterinary Use (CVMP)

## Reflection paper on the use of heat treatment to inactivate endogenous retroviruses in live immunological veterinary medicinal products

|  |                   |
|--|-------------------|
| Draft agreed by Immunologicals Working Party | October 2014      |
| Adopted by CVMP for release for consultation | 11 December 2014  |
| Start of public consultation                 | 19 December 2014  |
| End of consultation (deadline for comments)  | 31 March 2015     |
| Agreed by Immunologicals Working Party       | 18 June 2015      |
| Adoption by CVMP                             | 10 September 2015 |



# Reflection paper on the use of heat treatment to inactivate endogenous retroviruses in live immunological veterinary medicinal products (IVMPs)

## Table of contents

|   |          |
|---|----------|
| <b>EXECUTIVE SUMMARY .....</b>  | <b>3</b> |
| <b>1. INTRODUCTION.....</b>   | <b>3</b> |
| <b>2. SCOPE .....</b>   | <b>3</b> |
| <b>3. BACKGROUND.....</b>   | <b>4</b> |
| <b>4. VALIDATION OF THE HEAT TREATMENT .....</b>                              | <b>4</b> |
| <b>5. POSSIBLE IMPACT OF THE HEAT TREATMENT ON THE FINISHED PRODUCT .....</b> | <b>4</b> |
| <b>6. REQUIREMENTS TO DEMONSTRATE THE ABSENCE OF NEGATIVE IMPACT .....</b>    | <b>5</b> |
| <b>7. REFERENCES.....</b>   | <b>6</b> |

## Executive summary

This guideline outlines the data requirements to be submitted by the marketing authorisation holder (MAH) to introduce a heat treatment to inactivate endogenous retroviruses in the active substance for the production of live viral for immunological veterinary medicinal products (IVMPs) 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 inactivate RD114. The requirements in this reflection paper are also considered to be applicable to the use of heat treatment to inactivate endogenous retroviruses in general, in live viral vaccines.

## 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 on how to reduce and/or to clear finished products 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 during production or at the active substances level for the inactivation of replicative RD114 and more generally endogenous retroviruses 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 a retrovirus can be accepted, a standardised, validated detection test needs to be developed and the acceptable limit for the relevant retroviral agent has to be established (the term "limit" takes into account the detection limit of the test as well as the levels of the retroviral agent 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 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 endogenous retrovirus.

#### Design of the study:

The active substance to be heat treated is spiked with a known amount of the live endogenous retrovirus (e.g. RD114) or other relevant virus. 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 test for the amount of residual spiked retrovirus 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 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 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 unless justified by corresponding data. 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 of the heat treated live active substance has to be demonstrated in the laboratory in compliance with the requirements of Directive 2001/82/EC. Where the concerned active substance is part of a combined vaccine(s), and the heat treatment does not require an increase of its antigen content, it is sufficient to demonstrate the safety of a monovalent vaccine containing the heat-treated live active substance under assessment. The results obtained should confirm that the heat treated live active substance is safe for use.
- where the in vitro and manufacturing data generated to support the heat inactivation of the retrovirus indicate that the heat treatment does not affect the viability of the live vaccine organism, and there is no need to adjust the manufacturing specifications, there is no need to confirm efficacy of the heat treated substance in the target species. Whenever there is an indication that the efficacy of the concerned active substance may be impacted by the heat treatment, 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.