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Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP Risk Management Strategy - Managing the risk of the potential presence of replication competent endogenous retrovirus RD114 in starting materials and final products of feline and canine vaccines

1. Introduction

The Committee for Veterinary medicinal products (CVMP) established an ad hoc expert group (AHEG) in 2015 to assist in the development of a risk management strategy for the potential presence of replication competent RD114 in feline and canine vaccines.

The AHEG was requested to reflect on any proposed risk mitigation measures and perform an impact assessment on the effect of these measures upon the availability of feline and canine vaccines.

The risk management strategy has been elaborated in the light of newly available regulatory guidance and takes account of the most recent scientific data provided by manufacturers of canine and feline vaccines and in context with published literature.

The Risk Management strategy should be read in conjunction with the Scientific Summary on RD114 (Annex 2) where key publications are discussed and referenced.

2. Scope

The presence of replication competent RD114 retrovirus in cat and dog vaccines on the EU market is a consequence of the use of starting materials of feline origin containing the RD114 endogenous retrovirus (ERV) in its replication competent form e.g. RD114 containing feline cell lines used during the manufacturing process of the vaccine or through seed viruses/*Chlamydia felis* passaged on such cell lines.

The risk management strategy is limited in scope to existing EU authorised feline and canine vaccines as well as new EU marketing Authorisation (MA) applications for such vaccines, manufactured using starting materials containing or susceptible to infection with replication competent RD114. Bacterial vaccines have not been included in the scope as the risk of RD114 contamination is considered negligible because cell cultures are not used during their propagation or during the establishment of seed materials. The risk of cross-contamination of cell lines and seed stock of species other than dogs

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and cats is not included within the scope of the risk management strategy. EU GMP compliant facilities should ensure that the risk of such an event occurring is negligible.

The risk management strategy includes the potential for off-label (cascade) use recognising that other species of the order Carnivora (see Annex 1) are administered EU registered dog and cat vaccines to protect against disease such as canine distemper.

3. Risk management strategy for the presence of replication competent RD114 in feline and canine vaccines

The points to consider for developing a risk assessment for the presence of replication competent RD114 in canine and feline vaccines are not exhaustive and should be considered in conjunction with the CVMP Scientific Summary on RD114 (see Annex 2).

3.1. General information on RD114

- RD114 related sequences or loci (RDRS) are present in the genome of all domestic cats (*F. catus*) and wild cats (*F. sylvestris*) whether vaccinated or not. Sequencing of the cat genome indicates the cat has undergone multiple insertions of RD114 during the evolution of the species.
- Endogenous RD114 or RD114-RDRS are **not** present in the dog genome, but cell lines derived from dogs will support replication of RD114.
- There is no published evidence that replication competent RD114 productively infects or replicates in cats or dogs, but cell lines from these and other species (e.g. human or mink) will support replication of RD114. Furthermore, RD114 defective particles have been isolated from peripheral blood mononuclear cells (PBMCs) of domestic cats. In addition, a proportion of these samples had reverse transcriptase activity suggesting that RD114 may continue to replicate in the native animal.
- *In vitro* studies have demonstrated that replication competent RD114 virus can emerge from *de novo* synthesis through recombination events, likely to occur during reverse transcription, in feline cell cultures. The exact mechanism of this *de novo* conversion to the replicative form is unknown. However, the frequency of conversion is likely to increase as the number of *in vitro* passages increase.
- Not all RD114 isolates replicate with the same efficiency *in vitro*, therefore different RD114 isolates may behave with unpredictable levels of pathogenicity in different species or categories of animals.
- There are few published references on the immunological responses to RD114. Sera from cats have not been shown to contain antibodies to the envelope protein of RD114. Similarly, dogs challenged with relatively large quantities of one strain of replication competent RD114 did not seroconvert. However, replication competent RD114 virus may elicit innate, humoral or cell mediated immune response to antigens of the virus undetectable by current techniques. Further immunological studies would be necessary to establish the potential of RD114 to elicit such immune responses in animals.
- In the study referred to above where dogs were challenged with a replication competent strain of RD114, no general clinical signs or increases in rectal temperature or abnormal blood

biochemistry were noted. Additionally, RD114 sequences could not be identified in the genomic DNA isolated from peripheral blood leukocytes, lymph nodes, spleen or sternal bone marrow cells.

- To date there have not been any reports of an association of RD114 with any disease in dogs or cats. Furthermore, no long term health risks associated with RD114 have been identified. However, establishing a causal relationship or correlation with disease or health risk is limited by the quality of epidemiological data to investigate such hypotheses. This is especially true if potential adverse events were restricted to particular breeds or sub-categories of dogs and cats. Absence of evidence is not evidence of absence.
- Historical use of authorised canine and feline vaccines to date has not been associated with any identified disease or health risk linked to the presence of RD114 in some vaccines. Whether or not there are any long term health risks associated with RD114 is unknown but taking account of the number of booster doses most vaccinated animals receive over their lifetime would suggest the risk is very low.
- The sensitivity of detection methods for potential contaminants has improved significantly through the application of molecular techniques. The detection of RD114 in authorised vaccines was the result of an extremely sensitive molecular assay. The products continue to be manufactured and tested to the same standards and specifications that met the safety and efficacy requirements at the time of authorisation.
- No hazard has been identified from the presence of RD114 in authorised vaccines. However, the risk associated with administration of replication competent RD114 through repeated vaccinations, whilst apparently negligible, remains uncertain given the paucity of scientific data on this virus. It is, therefore, prudent to adopt a precautionary approach to minimise the exposure of dogs and cats to replication competent RD114.
- Scientific data exist that demonstrate replication competent RD114 can be inactivated by heat treatment and that retroviruses are sensitive to chemical inactivants used in the production of inactivated veterinary vaccines. It is, therefore, highly likely that the risk for the presence of replication competent RD114 in chemically or heat inactivated vaccines is less than for live vaccines.

3.2. Objective for EU authorised canine and feline vaccines manufactured using materials of feline origin

To ensure that there is negligible risk for animals administered dog and cat vaccines and for users of the products by ensuring that the vaccines comply with current quality requirements. The regulatory objectives apply equally to currently authorised products as for new applications for marketing authorisation.

3.2.1. Steps involved in RD114 risk assessment

A risk assessment consists of the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards. Risk identification is a systematic use of information to identify hazards where such information can include historical data, theoretical analysis, informed opinions, and the concerns of stakeholders. To date there have been no adverse events or diseases associated with RD114 in dog and cat vaccines and therefore the identification of hazards are only

theoretical based on the behaviour of related endogenous viruses and their potential to proliferate in and integrate into the genome of infected animals.

A risk assessment for RD114 in starting materials and final vaccine can only be a qualitative process of linking the likelihood of occurrence with potential theoretical hazards. For RD114 the ability to detect the harm should also factor in the assessment of risk given the relative paucity of published literature on the biological properties of the virus and the difficulty in associating infection with longer term sequelae in potentially specific breeds or categories of dogs or cats.

The first step is to identify the presence or not of replication competent RD114 in starting materials:

- A validated *in vitro* test is carried out to detect the presence of RD114 in starting materials of animal origin (cell substrates, seed viruses, substances of animal origin) e.g. detection of retroviral reverse transcriptase (RT). If the presence of retrovirus is known or established by testing such as an endpoint product-enhanced reverse transcriptase (PERT) assay, then infectivity assays should be carried out to establish the presence of replication competent virus in the starting material (Ph. Eur. 5.2.4). A PERT assay may be suitable to detect infective retrovirus after passage on permissive cells (Ph. Eur. 2.6.21) followed by an RD114 specific assay.

  Responsibility: Manufacturers.

- Where replicative retroviral RD114 is detected in starting materials consideration should be given to replacement of the starting material with an alternative material free of RD114 (For regulatory requirements for this type of change to an EU authorised vaccine refer to "Replacement of cell lines used for the production of immunological veterinary medicinal products (IVMPs)" EMA/CVMP/IWP/37620/2014).

  Responsibility: Manufacturers.

- Where replacement of the starting material is not feasible consideration should be given to downstream processing to remove or inactivate replication competent RD114 (For regulatory requirements for this type of change to an EU authorised vaccine refer to "Use of heat treatment to inactivate endogenous retroviruses in live immunological veterinary medicinal products" (EMA/CVMP/IWP/37924/2014).

  Responsibility: Manufacturers.

- Where starting materials have been identified as positive for replicative retroviral RD114 qualitative and/or quantitative tests should be performed for the presence of replication competent RD114 retrovirus in final vaccines or in active ingredients derived from the concerned starting material.

  Responsibility: Manufacturers.

- If a manufacturer considers there is no feasible alternative to the continued use of a starting material positive for replication competent RD114 a risk assessment should be performed for the presence of replication competent RD114 in the final product. The results of the risk assessment should demonstrate that the risk associated with the presence of replication competent RD114 in the final product is negligible. Some of the criteria that should be considered for inclusion are:

- Results from previous investigations including, the extent of replication competent RD114 in starting materials (cell cultures and seed viruses), intermediate products and active bulk antigens used in the production of the vaccine.
- The quantitative titre of replication competent RD114 in the final vaccine. Higher titres of RD114 represent a potentially greater risk due to the increased probability of replication in the vaccinated animal and integration into the genome of the vaccinated animal.
- The strain of RD114 and its biological properties including *in vitro* characteristics.
- Inherent susceptibility or resistance of vaccinated species to ERV infection e.g. dogs appear to have escaped retrovirus infection with no identified exogenous infection from this family of viruses and a very low level of ERV-like sequences in their genome for unknown reasons.
- History of use of the product, such as total number of doses sold and evidence of safe use of the vaccine in the target species without an unacceptable level of adverse events.
- Vaccination and requirement for booster doses (number of vaccine doses leading to continued exposure).
- Type of vaccine. Inactivated vaccines are likely to be lower risk due to the sensitivity of retroviruses to typical chemical inactivants used in the production of veterinary vaccines or the application of heat as an alternative inactivant.
- Consideration on the availability of alternative comparable products that may be free of replication competent RD114 due to the choice of starting materials of non-feline origin or the steps taken to remove or inactivate the virus during the production process.
- User safety profile of the product.

Responsibility: Manufacturers to perform the risk assessment and to evaluate the benefit-risk profile for the starting material and final vaccine.

Competent authorities to assess the level of risk and implement any necessary regulatory measures.

- Consideration should be given to on-going monitoring of feline cell lines or seed materials used for the production of vaccine antigens to identify the risk of potential emergence of replication competent RD114 through *de novo* recombination events.

  Responsibility: Manufacturers.

4. Regulatory considerations

- The continued use of authorised canine and feline vaccines containing replication competent RD114 in dogs and cats may be acceptable in conjunction with the implementation of regulatory measures to ensure negligible risk for the final product. Any regulatory applications to be submitted in compliance with the relevant EMA guidelines and Ph. Eur. within an acceptable time frame agreed with the relevant competent authorities.

  Responsibility: Manufacturers to implement risk mitigation measures.

National Competent Authorities and CVMP to assess variations and/or line extensions to existing marketing authorisations.

- In exceptional circumstances, where it is not possible to implement measures to eliminate replication competent RD114 from the final product e.g. where it has been demonstrated that heat inactivation has a deleterious impact on the quality, safety or efficacy profile of a product, the risk assessment must demonstrate a positive benefit-risk for maintaining the product on the market. Availability of vaccines to protect cats and dogs from serious infectious diseases is an important consideration for the benefit-risk profile.

  Responsibility: Manufacturers to perform the risk assessment and to evaluate the benefit-risk profile.

  National Competent Authorities and CVMP to assess the level of risk and implement any necessary regulatory measures.

5. Other felidae, canidae, mustelidae, viverridae & ursidae vaccinated with feline & canine vaccines

5.1. Points to consider for RD114 risk assessment for zoo animals, wild animals and exotic pets

- The use of EU authorised feline and canine vaccines in other Felidae (e.g. lions, tigers, jaguars, leopards, cheetahs), Canidae (wolves, foxes, jackals etc.), Mustelidae (weasels, ferrets[1]), Viverridae (civets) and Ursidae (bears), Pinnipidae (seals, sea lions, walruses) is off label as these vaccines are not authorised for use in these target species.
- According to current knowledge, the potential risk associated with RD114 in exotic species is considered no different to that in cats and dogs.
- There is a theoretical risk that replication competent RD114 could infect exotic species and integrate into the genome of the host. Replication competent RD114 is polytropic and can infect a range of feline and non-feline, cells *in vitro*.
- The risk associated with replication competent RD114 from primary and booster vaccinations remains uncertain given the paucity of scientific data on the virus.
- Manufacturers and competent authorities cannot make a specific risk assessment for off label use in any species. The potential risk associated with RD114 in exotic species is considered no different to that in cats and dogs. As always the benefit-risk assessment has to be made in the individual circumstances by the veterinary surgeon responsible for the animal concerned using their professional expertise and information provided by manufacturers, National Competent Authorities and EMA where applicable.

  Responsibility: the veterinary surgeon.

[1] some EU vaccines may be authorised for use in ferrets

Annex 1

Species to which canine and feline vaccines are used off-label

Wild animals, animals held in zoological collections and exotic pets that may be administered feline and canine vaccines off-label.

Felidae family e.g. lion (*Panthera leo*), tiger (*Panthera tigris*), jaguars (*Panthera onca*), leopards (*Panthera pardus*), cheetahs (*Acinonyx jubatus*) etc., may receive vaccines against: feline panleukopenia, feline rhinotracheitis/herpesvirus, feline calicivirus, feline leukemia, canine parvovirus, canine distemper, feline chlamydiosis, rabies and canine leptospirosis.

Canidae family e.g. wolves (*Canis lupus*), foxes (red fox, *Vulpes vulpes*), jackals, coyotes etc., may receive vaccines against: canine distemper, canine parvovirus, feline panleukopenia, canine infectious hepatitis and canine infectious tracheobronchitis, canine adenovirus, (CAV), rabies and canine leptospirosis.

Hyaenidae e.g. hyena, aardwolf, may receive vaccines against: canine distemper, feline panleukopenia and rabies.

Mustelidae e.g. raccoon, skunk, ferret, coati, genet, otter, weasel, mink, kinkajou, may receive vaccines against: canine distemper, canine parvovirus, rabies and canine leptospirosis.

Viverridae (civet family), may receive vaccines against: canine distemper, feline panleukopenia and rabies.

Ursidae (bears) may receive vaccines against: canine distemper, vaccines containing CAV, vaccines against feline panleukopenia and rabies.

Pinnipedia (seals, sea lion, walrus), may receive vaccines against: canine distemper and rabies.

The diseases for which vaccines may be administered are listed with respect to published vaccination schedules for the each family. The risk of RD114 in final vaccines is related to the use of feline derived starting materials and for many of the listed viruses and bacteria the risk is negligible unless the antigen is used in a combination with an antigen which may contain replication competent RD114 e.g. for canine leptospirosis the RD114 risk would only arise through a combination product containing CPV grown on CRFK cells with replication competent RD114.

Annex 2

Scientific Summary on RD114

1. Background to RD114 in canine and feline vaccines

In 2010, a scientific publication reported the detection of feline replication competent endogenous retrovirus RD114 in a number of commercially available live attenuated feline and canine vaccines in the UK and Japan. The data revealed the presence of replication competent RD114 in vaccines produced by three different manufacturers which, in the opinion of the researchers, warranted further investigation in to the potential risks of RD114 in vaccines used routinely to control serious infectious diseases in dogs and cats.

This published finding had been preceded by a report to the EMA of a putatively replication competent feline retrovirus RD114 detected in one manufacturer's live attenuated feline vaccine in 2007. To address the potential risk related to the presence of a specific retrovirus, CVMP created a Scientific Advisory Group (SAG) to perform a risk assessment and to work with manufacturers to develop a standard protocol to investigate further the presence of replication competent RD114 in cell lines used for the production of cat vaccines. An industry supported study in 2008 investigating potential RD114 contamination of feline cells used in the production of dog and cat vaccines was compromised by the use of two different methods for which the sensitivities were not directly comparable. However, it was evident that some feline cell lines were potentially contaminated with replication competent RD114 warranting further investigation.

CVMP published a scientific opinion (EMA/CVMP/433418/2010) in September 2010 on RD114 in live attenuated vaccines for use in animals and concluded that taking account of the information available at the time pertaining to replication competent RD114 in veterinary cell lines and vaccines, a hazard could not be clearly defined and consequently the risks were unquantifiable. The risks associated with the presence of replication competent RD114 in feline and canine vaccines were considered low for animal health and extremely low for human health and consequently the benefit-risk balance for vaccination against highly infectious cat and dog diseases remained strongly positive. No immediate regulatory action was warranted but corrective actions were recommended to address regulatory compliance issues on the quality of veterinary vaccines.

An ad hoc risk management meeting of the SAG involving industry participants was convened in 2013 to review the current state of knowledge on RD114 and the challenges for clearance of the endogenous retrovirus from cell cultures and finished products. The presence of replication competent RD114 in starting materials was considered principally a quality issue given the European Pharmacopoeia (Ph. Eur.) requirement for cell seeds to be free of extraneous agents.

A holistic approach on the sourcing and testing of starting materials, manufacturing the product to standards of EU GMP and application of appropriate in-process and final product controls has ensured products of high quality with an excellent safety profile, such that contamination events of veterinary vaccines have been extremely rare. However, the requirement to demonstrate absence of extraneous agents is an increasingly challenging environment for manufacturers and regulators due to advances in molecular techniques that enable the identification of nucleic acids derived from virus particles that may indicate the presence of infectious agents in starting materials used in the production of IVMPs.

From a quality perspective, CVMP considered that is was not acceptable to have vaccine batches on the market containing unwanted live virus particles, without trying to investigate and correct this issue (EMA/CVMP/433418/2010). Further consideration for improvement of affected vaccines was needed that encompassed the replacement of cell lines and introduction of manufacturing steps to allow clearance of the virus and/or inactivation of replication competent retrovirus RD114 in cell cultures or final vaccine.

CVMP and the European Pharmacopoeia (Ph. Eur.) subsequently developed RD114 specific and general additional guidance and requirements on various measures to address the risk of replicative retroviruses contaminating cell seeds and final products:

- A CVMP Reflection paper on the use of heat treatment to inactivate endogenous retroviruses in live immunological veterinary medicinal products (EMA/CVMP/IWP/37924/2014) was published in September 2015. This Reflection paper 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 immunological veterinary medicinal products (IVMPs) and to show the absence of negative impact of this treatment on the IVMP.
- A CVMP Reflection paper for the replacement of cell lines used for the production of immunological veterinary medicinal products (IVMPs) (EMA/CVMP/IWP/37620/2014). Published September 2015. This RP outlines the data requirements to be submitted by the MAH to replace the cell line as host system for production of IVMPs without significant changes to the production process or finished product specifications.
- A revision of the guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010-Rev.1) was published on 19 December 2016 and will come into force on 1 May 2017. The guideline was revised to include a new Annex 2 entitled “The approach to demonstrate freedom from extraneous agents as part of the production and control of immunological veterinary medicinal products for mammalian species and fin fish”, and replace the table of extraneous agents to be tested for in relation to the general and species-specific guidelines on production and control of mammalian veterinary vaccines (Eudralex 7BIm10a). The annex includes new requirements to test for the absence of endogenous retroviruses (replication competent) in starting materials of animal origin.
- The Ph. Eur. revision of the general chapter 5.2.4 Cell cultures for the production of veterinary vaccines that came into force in January 2016 requires cell lines to be examined for the presence of retroviruses. If the presence of retrovirus is known or established by testing such as an endpoint product-enhanced reverse transcriptase (PERT) assay, then infectivity assays should be carried out to investigate the presence of replication competent virus. Cell seeds that show the presence of infectious retroviruses are not acceptable for the production of vaccines. Only in exceptional cases of positive or equivocal results in the infectivity assay, may it be justified to use cells containing a replication competent retrovirus. Such a justification is based on a risk assessment that must demonstrate that the risk associated with the presence of infectious retroviruses is negligible in the final product.

In July 2015 CVMP adopted a mandate for an Ad Hoc Expert Group (AHEG) on RD114 to develop a risk management strategy taking into account the scientific developments in the field since the original CVMP Opinion in 2010 and the introduction of new regulatory guidance and requirements for the

presence of retroviruses in starting materials (cell substrates, virus seeds, substances of animal origin).

1.1. Retroviruses classification and characteristics

Retroviruses are a very diverse group of enveloped single stranded RNA +ve sense viruses. The viruses replicate via reverse transcriptase creating a DNA intermediate (the provirus) that may integrate into the host cell genome (note the RD114 proviruses are referred to as RD114 related sequences [RDRS] in the core strategy document). As a consequence of reverse transcription, the viral DNA contains long terminal repeats (LTRs) that contain the promoter sequences necessary for initiation of transcription of new viral RNA elements. The DNA is inserted semi-randomly into the host genome and, for some retroviruses, can be inserted into oncogenes with the potential of converting normal cells into tumour cells.

Retrovirus genomes commonly contain three open reading frames that encode proteins that are potentially found in the mature virus: group-specific antigen (gag) codes for core and structural proteins of the virus; polymerase (pol) codes for reverse transcriptase, protease and integrase; envelope (env) codes for the retroviral coat proteins.

The characteristics of retroviruses have been extensively reviewed and are probably the most studied group of viruses given the range of diseases they produce in animals and man, their ability to integrate into their host's genome and, in some instances, precipitate tumours or immunosuppression. Examples of retroviruses of veterinary importance include feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV), avian leukosis virus (ALV), caprine arthritis and encephalitis virus (CAEV), maedi-visna virus and bovine leukosis virus (BLV). However, retroviruses may also be relatively benign, having no obvious impact on an infected cell (e.g. feline and human foamy viruses).

Retroviruses were originally classified by the morphology of the virion core visualised by electron microscopy, but are now categorised into seven genera. Retroviruses can be broadly classified into two groups: simple (alpha, beta, gamma and epsilon retroviruses) and complex (lenti-, delta- and spuma-viruses).

RD114, a feline retrovirus, falls within the gammaretrovirus group. They encode only the Gag (group specific antigen, the viral core proteins), Pol (polymerase enzymes reverse transcriptase, protease and integrase) and Env (envelope glycoprotein) gene products. Defective viruses do not harbour accessory genes although some encode oncogenes (1). Representative viruses include FeLV, murine leukaemia virus (MLV) and koala retrovirus (KoRV). Sequence data of RD114 suggest the virus is a recombinant retrovirus comprising a *gag-pol* gene from a gammaretrovirus and an *env* gene from a betaretrovirus (2).

1.2. Endogenous and Exogenous retroviruses

Retroviruses are also classified into exogenous and endogenous. This definition can depend on the route of transmission. Exogenous retroviruses generally infect somatic cells and are transmitted horizontally by exposure to infectious viral particles. Endogenous retroviruses (ERVs) are genetic endogenous viral elements (loci) in the germ line genome that are derived from integrated retroviruses. They are a subclass of transposable elements (transposons) present in the genome of most vertebrates. As much as 10% of mammalian genomes (2) may contain ERV loci but only a few have been shown to be capable of expressing viral proteins or replication competent retroviruses. Infectious viruses that originated from ERV elements by either expression of intact full-length elements

or recombination of multiple ERV elements are also conventionally called ERVs. In most cases, ERVs no longer maintain their activity, but ERV insertions may produce diverse effects on their host genome including altered gene expression.

The distinction between exogenous and replication competent endogenous retroviruses is important in the context of RD114. While it is classified as an ERV, it has been shown to replicate *in vitro* in a range of cell lines including feline, canine, human cell lines (refer to section 1.4 below for details) and as such has the properties of an exogenous retrovirus in cell culture. The provirus of an exogenous retrovirus may insert semi-randomly into the chromosomes of the host genome and then be passed on to progeny cells through cell division. Most retroviruses infect somatic cells but if germline cells are infected the provirus or inserted retroviral loci may become part of the host genome.

Replication competent ERVs are derived from the germ line RDRS. The biological properties and characteristics of replication competent RD114 may vary between isolates (3, personal communication) and therefore without further characterisation of the replication competent form of the virus, caution must be applied when attempting to extrapolate the results of individual studies.

Domestic cats are known to harbour at least two exogenous retroviruses: feline immunodeficiency virus (FIV), which belongs to the same genus of retroviruses (Lentivirus) as the Human Immunodeficiency Virus (HIV) and FeLV (a gammaretrovirus). FeLV can cause significant morality in young cats resulting from development of anaemia, leukaemias, lymphomas and immunodeficiency.

1.3. Some specific examples of replication competent ERVs from recent scientific literature

RD114 is one example of a replication competent ERV, other infectious ERVs include murine leukaemia virus X [xenotropic] (MLV-X or XMRV), pig endogenous retroviruses (PERVs) and koala retrovirus (KoRV).

XMRV was a novel gammaretrovirus which arose during passage of a human prostate tumour xenograft in immunocompromised nude mice, by activation and recombination between two endogenous murine leukemia viruses from cells of the mouse (4).

PERVs were shown to infect human cells in an *in vitro* co-culture assay and raised public health concerns for recipients of organ xenotransplants delaying the use of pig tissues in human transplant surgery. There is currently no preclinical or clinical evidence of PERV transmission through xenotransplantation (5).

Koala retrovirus was originally identified as an ERV based on the presence of the virus in the genome of almost all koalas tested (6). It possessed a full-length replication competent genome and the virus could be produced from *in vitro* culturing of peripheral blood mononuclear cells (PBMCs). It appears to share ancestry with the gibbon ape leukaemia virus (GALV), and probably arose from an ERV found in the Southeast Asian feral mice (Mus caroli). It exists as both an ERV in the germ line of koalas and as an exogenous virus that infects animals by horizontal transmission and has been linked with secondary diseases including chlamydia infection and leukaemias (6).

1.4. RD114

RD114 is now considered a polytropic retrovirus having been shown to have a broad species tropism *in vitro* in dog, mink, human and feline cells. It has been shown to be capable of replication in various cell lines including human cell lines: RD/TE671 (H, Human), HEK293 (H) and MXC (H) (7), feline cell lines

CRFK (F) (8), a lymphoma cell line MCC (F) (9), FER (F) (10) and A262 cells (a mixture of thymus, bone marrow and lymph node from a foetal kitten (11)), canine cell lines including the Madin-Darby Canine Kidney line, MDCK (12) and the mink cell line, Mv 1 Lu (13). In a recent study investigating the sensitivity of feline cell lines to RD114, six out of eight feline cell lines were shown susceptible to the RD114 pseudotype virus where resistance to RD114 virus in certain cell lines was due to receptor interference rather than receptor polymorphism.

An RD114 viral receptor, a sodium-dependent neutral amino acid transporter termed ASCT2 (14), is ubiquitous in tissues of the domestic cat and suggests that the replication competent form of the virus could infect many types of feline derived tissues. The possibility of new RD114 integrations in a range of feline tissues through iatrogenic administration of the replication competent form of the virus cannot be excluded nor the theoretical potential for inducing disease in the infected animal. ASCT2 has also been shown to be present in a wide range of mammalian species with RD114, therefore, having the potential to infect cell lines of many species including dog, cattle, sheep, horse, pig, dog, cat, ferret, mink, rabbit, and quail but not rat or mouse (14).

In a recent study investigating the sensitivity of feline cell lines to RD114, six out of eight feline cell lines were shown susceptible to the RD114 where resistance to RD114 virus in certain cell lines was due to receptor interference rather than receptor polymorphism (15).

RD114 virus is capable of retaining its replication competence for long periods at –80^{o}C (16).

1.5. Discovery of RD114 and distribution in domestic (Felinae) and large cat (Panthinae) species

RD114 was first identified in a human cell line following inoculation of cultured human rhabdomyosarcoma cells (RD cell line) into the foetuses of pregnant cats (17). Surviving kittens developed tumours from which a new C-type retrovirus (now classified as a gammaretrovirus) was identified in the bone marrow of a kitten (XC-114B). As the virus was identified in a human rhabdomyosarcoma cell line, RD114 was initially thought to be the first human RNA tumour virus, but later RD114 virus was shown to be an endogenous cat virus, originating in the brain of a foetal kitten (18).

An identical replication competent endogenous virus to RD114 was also induced from a continuous line of feline kidney fibroblast (Crandell line) cells by iododeoxyuridine treatment (19). These and other *in vitro* studies suggest that replication competent RD114 virus can emerge *de novo* through recombination events in feline cell culture. The exact mechanism of this *de novo* conversion to the replicative form remains unknown. Such an observation would also suggest that any feline cell line has the potential to express and release RD114 in its replication competent form.

RD114 shows a high level of homology with the baboon endogenous retrovirus (BaEV). This has been attributed to cross-species transmission before the evolution of the domestic cat lineage 7- 9 million years ago but after the separation of the larger cats (Pantherinae). This is due to the presence of RD114 like sequences in the genomes of all Felinae but notably absent from the genome of any of the large cats (20). BaEV did not appear to have infected an ancestor of the cat lineage, but rather it was a *de novo* recombinant between BaEV and feline endogenous gammaretrovirus that infected the germ line of the Felinae ancestor.

There is no published evidence that replication competent RD114 productively infects or replicates in cats or dogs, but as outlined above cell lines from these and other species (e.g. human, mink) will

support replication of RD114. RD114 defective particles have, however, been isolated from peripheral blood mononuclear cells (PBMCs) of domestic cats. In addition, a proportion of these samples had reverse transcriptase activity suggesting that RD114 may continue to replicate in the native animal (3). Translation and transcriptional elements of RD114 have also been observed in embryonic tissues of domestic cats but no replication competent virus has been reported from primary tissues of cats.

Through DNA studies on feline tissues it was demonstrated that multiple copies of approximately 20 RD114 retroviral-like sequences (RDRS) could be found throughout the cat genome (21) and that the characteristics of the virus were distinct from feline leukaemia virus (FeLV) that was exogenously transmitted causing serious clinical disease (22, 23). The RD114 endogenous sequences were randomly inserted in the cat DNA and whereas for the *gag* and *pol* genes there was considerable homology, the *env* gene showed divergence through either genetic drift or possible recombination with the *env* gene of another retrovirus.

An early publication reported approximately 20 related RD114 loci (21) in the genome of the domestic cat used renal tissue from a very limited number of animals. More recent evidence suggests the number of RDRSs may vary considerably depending on the breed of cat examined or its geographical location (24). Six RDRS were identified in domestic cats which showed considerable diversity between breeds. Most of the loci have deleted-*env* regions and no complete infectious provirus loci has been identified. More recent searches attempting to identify the provirus using the genome database of domestic cats (24) has again failed to identify an infectious provirus sequence.

Domestic cats have an RD114 virus-related sequence (RDRS) on chromosome C2, termed RDRS C2a (24), but other RDRSs vary depending on the regions where cats live or breed. This finding supports the view that RDRS C2a is the oldest RD114 related provirus that entered the host genome before domestic cats diverged whilst more recent RDRSs reflect later introductions of RD114-like retroviruses into evolutionary isolated lineages. RD114 viral genomes have not been detected in large felids, such as lions and pumas (25).

All domestic cats, therefore, have RD114 retroviral-like sequences (RDRSs) elements within their genomes, and several feline cell lines produce replication competent RD114-like viruses. Infectious RD114 virus can be resurrected by the recombination between two non-infectious RDRSs (24). By co-transfection of two defective RDRS in HEK293 cells, infectious RD114-like viral particles were generated through recombination. The researchers expressed concern that new integrations through recombination events had the potential to induce diseases in cats, particularly if the integration occurred in the vicinity of proto-oncogenes. The same theoretical pathogenesis could equally apply to iatrogenic infection of a replication competent virus. A role of RD114 in feline lymphomas has been postulated given the sustained prevalence of such tumours despite the decline in FeLV infections through successful vaccination programmes (24) and the report of RD114 expression in a natural lymphoma (22).

De novo recombination events may result in virus negative feline cell lines becoming positive for replication-competent RD114. Routine monitoring of feline cell lines for the presence of replication competent RD114 could be an effective strategy to mitigate the risk of recombination events transforming a previously compliant cell line to being a producer of a replication competent retrovirus.

1.6. RD114 replication in vitro and in vivo

RD114 replication-competent viruses are now classified as polytropic, as they are able to replicate in both feline and non-feline cell lines. Whether or not domestic cats express replication competent

RD114 remains unknown. A recent study examined peripheral blood mononuclear cells (PBMCs) from 296 domestic cats for the presence of RD114. No replication competent virus was identified but 25 PBMC samples expressed PERT activity suggesting ERV activity *in vivo* in a representative population of cats (3). However, sera from healthy cats and cats with various diseases do not contain antibodies to retrovirus RD114 viral proteins which indicate that RD114-like antigens may not be expressed or that cats are immunologically tolerant to the virus in its replication competent form (26).

The existence of RDRSs in the genome of domestic cats does not provide conclusive evidence for tolerance to iatrogenic infection with replication competent RD114. RD114-like retroviruses may have integrated into the genome of the domestic cat through the course of evolution but such an observation does not exclude the possibility of a disease risk associated with iatrogenic infection of replication competent RD114 in domestic cats through vaccination. If domestic cats do not express RD114 in the natural state, iatrogenic exposure could result in an infectious event that may not have occurred in domestic cats or their ancestors for many hundreds of thousands of years.

The presence of replication competent RD114 in some commercial batches of certain feline and canine vaccines on the EU market originates from the use of feline origin starting materials during the establishment of seed viruses and/or the use of feline cell cultures during the manufacture of the vaccines which contained replication competent RD114. One of the challenges for manufacturers using starting materials of feline origin is the potential for *de novo* recombination. Initial investigations may indicate that a feline starting material is negative for reverse transcriptase (RT), but this does not address the risk for the potential emergence of replication competent RD114 through *de novo* recombination events from passaging of feline derived cell lines.

The absence of RD114 retroviral sequences in the genome of large cats reflects the evolutionary lineage of the Felinae and Pantherinae families that separated from a common ancestor before RD114 infected the genome of the domestic cat lineage. The risks associated with the administration of RD114 contaminated vaccines to large cats held in wildlife parks and zoos are considered no different to the risks associated with iatrogenic infection of domestic cats; iatrogenic exposure could result in an infectious event that has not occurred in large cats through the course of evolution and the risks associated with such an infection are unknown.

1.7. Canine retroviruses and susceptibility to RD114

There are no published reports of exogenous retroviruses in the family of Canidae despite many disease causing retroviruses having been identified in many domestic animals including cows, horses, poultry, sheep, goats and cats.

Recent analysis of the canine genome identified canine endogenous retroviruses (CfERV)-like proviruses representing only 0.15% of the dog genome but with no elements containing complete open reading frames (27, 28). In comparison, the human genome has been estimated to contain up to 10% retroviral DNA which is more typical of mammalian species (1, 29). The most recent retroviral integration event in the dog genome is thought to have occurred within the last 200,000 years (30).

In vitro canine cells are permissive to infection by replication competent RD114. It has been reported that RD114 virus infects a variety of canine cell lines including Madin Darby canine kidney line (MDCK).

The identification of RD114 or RD114-like viruses in commercial canine vaccines (16, 30, 31, 32) led to investigations into the source of contamination. Canine parvovirus (CPV), a core-antigen included routinely in puppy and dog vaccination schedules, is widely grown on feline-derived cells such as CRFK

cell lines. RD114 isolated from commercial canine vaccines was shown to be identical to the virus isolated from CRFK cells (31).

The origin of RD114 in canine vaccines is not limited to feline starting materials used during production of the vaccine. RD114 contamination of canine vaccines has been reported in vaccines produced on non-feline cell lines. The use of RD114 containing feline cell lines in the initial stages of establishing the CPV viral seed stocks was the most likely source of RD114 in final vaccines with many CPV viral seed stocks shown to contain replication competent RD114 virus (31).

In order to examine the potential of RD114 to cause disease in dogs, researchers infected small groups of SPF dogs with live or inactivated RD114 and recorded clinical signs and immunological and biochemical markers over a period of several weeks for evidence of pathogenesis. No differences were observed between RD114 challenged dogs and control animals. RD114 provirus was not detected in blood, lymph node, spleen or bone marrow samples of the RD114 group infected with 10^5 infectious units. Furthermore, neutralising antibodies were not elicited in any of the dogs. These results suggest that RD114 did not replicate and disseminate in the dogs, and that the virus is unlikely to be pathogenic in dogs (33, 34).

However, to fully assess the potential risks for viral integration into the genome and replication in challenged animals would require much larger groups of dogs, studied for much longer periods of time with a greater understanding of the minimum infectious dose and mechanisms of potential pathogenesis. The potential susceptibility of different categories of dogs or breeds is also unknown. In the recent study (33) SPF dogs of 10 months of age were challenged with RD114 but in veterinary practice, vaccination occurs in puppies as young as 5-6 weeks.

Considering the limitations of the investigations to date and as canine cell lines are permissive to infection by replication competent RD114, the implications of iatrogenic infection of dogs with replication competent RD114 due to the use of contaminated vaccines is unknown due to the paucity of scientific data on the subject. As a consequence, the Risk Management Strategies for feline and canine vaccines are treated similarly.

1.8. Assays for identification of retroviruses in starting materials

1.8.1. Introduction

Viruses can be introduced into the Master Cell Seeds (MCSs) by several routes such as:

- Derivation of cell lines from infected animals;
- Use of virus to establish the cell line;
- Use of contaminated biological reagents such as animal serum components;
- Contamination during cell handling.

MCSs containing endogenous retrovirus sequences have the potential to transmit viral sequences vertically from one cell generation to the next, since the viral genome persists within the cell. Such sequences may be constitutively expressed or may unexpectedly become expressed as an infectious virus (*de novo* recombination).

Molecular assays such as massive parallel sequencing, degenerative PCR and panmicrobial microarrays have resulted in detection of an array of viral sequences in live human vaccines including endogenous

retroviral sequences, Avian leukosis virus (ALV), simian retrovirus (SRV) and porcine circovirus-1 (PCV1) (35). Swine torque teno virus (TTV) has been detected in commercial pig vaccines, using PCR (36) and four mycoplasma hyopneumoniae vaccines were positive for TTV1 and two by PCR.

The ICH Topic Q5A (R1) (37) provides detailed guidance on the testing approach for the presence of retroviruses for products derived from cell lines of human or animal origin and a similar approach can be used to investigate cell lines and viral seed materials, used in the production of IVMPs, for the presence of ERV RD114.

In the human arena a variety of tests are used to detect retroviruses in MCSs including infectivity assays in sensitive cell cultures and electron microscopy (EM) studies. If infectivity is not detected and no retrovirus or retrovirus-like particles have been observed by EM, reverse transcriptase (RT) or other appropriate assays should be performed to detect retroviruses which may be non-infectious. Induction studies have not been found to be useful.

In the veterinary field Ph.Eur 5.2.4 requires a validated *in vitro* test to be carried out to detect the presence of retroviruses in cell lines. If the presence of retrovirus is known or established by testing such as an endpoint product-enhanced reverse transcriptase (PERT) assay, then infectivity assays should be carried out. A PERT assay may be suitable to detect infective retrovirus after passage on permissive cells.

1.8.2. New extraneous test requirements for cell lines and other starting materials

Prior to 2016 there was no requirement in the EU to test starting materials (substances of animal origin, cell substrates and virus seeds) of any species for the presence of ERVs for the development and production of IVMPs. For starting materials of feline origin, tests for the absence of extraneous agents were listed in EudraLex Volume 7B (now withdrawn) and required materials to be tested for exogenous retroviruses feline leukaemia virus and feline immunodeficiency virus. There was no requirement to test for RT activity or examine starting materials for replication competent ERVs. However, there was a general requirement that all starting materials should be free of contamination:

- *Ph. Eur. 0062 Veterinary Vaccines*

  2.1.3 Seed lots. If the master seed lot is shown to contain living organisms of any kind, other than the virus of the species and strain stated, or foreign viral antigens, then it is unsuitable for vaccine production.

  2.1. PREPARATION OF THE VACCINE. The methods of preparation, which vary according to the type of vaccine, are such as to maintain the identity and immunogenicity of the antigen and to ensure freedom from contamination with extraneous agents.

- *Eudralex Vol 7B Rules Governing Medicinal Products in the European Union (now withdrawn)*

  1.1.2 Preparation of starting materials of animal origin. All batches of substances shall be shown to be free from contaminants as described below and/or shall be subject to a suitable validated inactivation procedure.

In January 2016, a revision of Ph.Eur 5.2.4 cell cultures for the production of veterinary vaccines came into force which requires primary cells and cell lines to be tested for the presence of retroviruses and introduced for the first time in the veterinary sector a mandatory general test for retroviruses.

Ph. Eur. 2.5.4 also states that cell seeds that show the presence of infectious retroviruses are not acceptable for the production of vaccines except in exceptional cases based on a risk assessment including all available data and any downstream processing steps until the final product stage. The results of the risk assessment must demonstrate that the risk associated with the presence of infectious retroviruses is negligible in the final product.

Furthermore, the CVMP Guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010 Rev.1), that replaces Eudralex Vol 7B, introduces the requirement for general tests for the presence of endogenous retroviruses (replication competent) to be performed on animal origin starting materials (cell lines, viral seeds and substances of animal origin) from most species (except dogs) and some specific tests for known exogenous retroviruses.

1.8.3. Fluorescent Product Enhanced Reverse Transcriptase (FPERT) and real-time reverse-transcription-PCR assays for detecting the presence of retroviral reverse transcriptase (RT)

The introduction of PERT and subsequently FPERT (qualitative and quantitative) assays has increased the sensitivity over more conventional RT assays by a significant margin (up to 10^6 fold more sensitive). Published test methods for FPERT assays report sensitivities of 10^{-7} Units of RT enzyme or 10^2 IU MLV (international units of Murine Leukaemia Virus). Such methods are therefore able to detect a few molecules of RT in a sample, although inhibitors such as anti-foaming agents or serum can affect the sensitivity of FPERT assays considerably in the absence of further sample processing. They have become the gold standard for detection of RT in samples and can be either qualitative or quantitative. The results from FPERT assays must be considered carefully due to the possibility of both false positive and false negatives. Ph. Eur. 5.2.4 states that since the sensitivity of the PERT assay is very high, interpretation of a positive signal maybe equivocal.

Samples containing high levels of polymerase activity may affect the results of analysis. DNA polymerases may originate from cell lysates, virus seeds and unpurified viral bulk harvests and can give rise to false positive signals. Prior treatment of samples, such as ultracentrifugation and addition of DNA polymerase suppressants such as calf thymus DNA can reduce the chance of false positives but any equivocal results should be treated with caution.

A real-time quantitative reverse-transcription-PCR (qRT-PCR) method has been described (38) and has advantages of being a rapid quantitative method for detecting RD114 viral RNA in starting materials and vaccines. The qRT-PCR had a LOD of less than 100 RD114 particles and is several log10 more sensitive than the number of infectious particles detected using the LacZ method (see section 1.8.4).

The existence of a standard test for RD114 with appropriate biological reference preparations (BRP) would enable manufacturers to conduct tests according to an official method and facilitate direct comparisons on the level of RT and/or RD114 in starting materials and final product used in the production of IVMPs on the EU market.

However, in the absence of suitable EU/international reference preparation manufacturers are required to develop and validate in-house tests to detect the presence of RT to meet the requirements of Ph. Eur. 5.2.4 which states: A validated in vitro test is carried out to detect the presence of retroviruses in cell lines. If the presence of retrovirus is known or established by testing such as an endpoint PERT assay, then infectivity assays should be carried out.

1.8.4. Infectivity assays for RD114 to detect replication competent virus

Cell lines transformed by infection with RD114 virus can be used as infectivity assays. There are a number of permissive cells lines for RD114 that are suitable for such an assay including TE671/RD, HEK293, HeLa, and QN10 cell lines. Only QN10 cells develop clear specific foci after incubation, whereas for most other susceptible cell lines infection is usually confirmed using supplementary methods such as the identification of RT in cell supernatant. Human TE671/RD cells are not susceptible to feline herpesvirus type 1, feline calicivirus and feline panleukopenia virus, and therefore suitable for monitoring feline vaccines containing live attenuated components that may interfere with other detection systems unless neutralised with suitable antiserum.

The feline sarcoma-positive leukemia-negative (S+L-) fibroblast cell line (QN10 cells) is highly susceptible to RD114 viruses and has been a preferred cell line for classical infectivity assays with cell seed materials or for basic virological studies with RD114 (Z, ZZ) to generate unique foci following infection of replication-competent gammaretroviruses.

An alternative approach for detecting infectious gammaretroviruses is the *lacZ* marker rescue assay (39) which detects the retroviruses by transducing *lacZ* marker gene to the target cells, which shows *lacZ*-positive foci if the infectious virus is present. The *lacZ* marker rescue assay is considered more sensitive than classical infectious assays. By inoculating RD114 samples into TE671 cells transduced with the *lacZ* marker gene, less than 10 infectious units were detected (16). The *lacZ* marker assay can equal the sensitivities of PCR to detect proviral DNA but are not as sensitive as PERT assays to detect RT or nested PCRs for specific viral sequences.

Infectivity assays are relatively insensitive in detecting extremely small quantities of virus but are useful for identifying the presence of replication competent virus. There is a possibility that feline derived QN10 cells may also produce RD114 viruses and therefore induce transformed foci or interfere with inoculated RD114 viruses and therefore appropriate controls are needed to identify such an eventuality.

1.8.5. Recommended approach for the detection of RD114 in feline derived starting materials (cell and virus seeds, intermediate products and final vaccines)

To meet the current Ph. Eur and CVMP Guidelines the following feline derived starting materials should be tested for the absence of replication competent RD114:

- Master Cell Seeds (MCSs); to comply with Ph. Eur 5.2.4.
- Cells from working cell seed at highest passage level; to comply with Ph.Eur 5.2.4.
- Working Cell Seeds (WCSs); no requirement but potentially useful for detecting *de novo* recombination events.
- Master seed viruses (MSVs); to comply with Guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010-Rev.1).
- Working Seed Viruses (WSVs); no requirement.
- Intermediate products (active ingredient); no requirement. However, recommended as part of CVMP risk management strategy if seed materials test positive for RD114.

- Final vaccines; no requirement. However, recommended as part of CVMP risk management strategy if seed materials, test positive for RD114.

There are no specified assays, only examples of suitable methods described in Ph. Eur. 5.2.4, an endpoint PERT assay for the detection of RT followed by infectivity assays if the PERT test is positive. In the absence of any suitable reference assay or standards, it is for the manufacturer to identify and validate the methods of choice with the specific product to be tested and for the regulatory authorities to assess their suitability for the intended purpose. For a quantitative assay, the following parameters are determined during validation: accuracy, precision, specificity, quantitation limit, linearity, range and robustness.

Published methods for classical infectivity assays, FPERT and qPCR (including qRT-PCR) would be useful to provide guidance to both manufacturers and regulators on the limits of quantitation that can be expected. The FPERT is described as a suitable method in Ph. Eur.5.2.4. with reference to Ph.Eur 2.6.21 for the approach required to validate nucleic acid amplification techniques such as PCR.

For detection of replication competent RD114, Ph. Eur.5.2.4. describes infectivity assays on permissive cells followed by an endpoint PERT assay. There are no recommended infectivity assays but a number of publications describe suitable permissive cell lines e.g. QN10, HEK293 or TE671 cells.

2. Conclusions

RD114 is a replication competent endogenous retrovirus belonging to the gammaretrovirus family. Multiple copies of RD114-like-sequences have been found throughout the domestic cat genome with the numbers of copies varying between breeds. Whether or not cats express replication competent RD 114 remains unknown. RD114 is absent from the genome of the large cat family. Studies have shown that peripheral blood mononuclear cells (PBMCs) of domestic cats from cats may contain reverse transcriptase activity and translations and transcriptional elements of RD114 have also been observed in embryonic tissues of domestic cats.

There are no reports of exogenous retroviruses in the canine family and the dog genome contains a relatively low percentage of retrovirus-like sequences compared to other mammalian species.

There is no published evidence that replication competent RD114 can infect or replicate in cats or dogs. However, RD114 is capable of replication in various cell lines *in vitro* including feline, canine and human cell lines and as such has a broad species tropism including canine cell lines.

Immunological investigations in dogs and cats have not identified any specific active immune responses to RD114. Clinical studies have not identified any clinical signs associated with RD114 and there has been no disease associated with RD114 to date.

The risk associated with iatrogenic infection of cats through vaccines containing replication competent RD114 remains unknown. There is a theoretical possibility that a new integration in the vicinity of an oncogene could induce disease in infected animals.

Considering the limitations of the investigations to date and as canine cell lines are permissive to infection by replication competent RD114, the implications of iatrogenic infection of dogs with replication competent RD114 due to the use of contaminated vaccines is also unknown. As a consequence, the risk management strategies for feline and canine vaccines are treated similarly.

Replication competent RD114 can be formed by *de novo* recombination between two non-competent retroviral sequences. Routine monitoring of feline cell lines for the presence of replication competent

RD114 could be an effective strategy to mitigate the risk of recombination events transforming a previously compliant cell line to being a producer of a replication competent retrovirus.

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Annex 3: Abbreviations / acronyms

AHEG	Ad hoc expert group
ALV	Avian leukosis virus
BLV	Bovine Leukosis virus
CAEV	Caprine Arthritis and Encephalitis virus
CVMP	Committee for Veterinary medicinal products
EMA	European Medicines Agency
ENV	Envelope gene, Env is a viral gene that encodes the protein forming the viral envelope
ERV	Endogenous retrovirus
FeLV	Feline leukaemia Virus
FIV	Feline Immunodeficiency Virus
GAG	Group specific antigen
KoRV	Koala retrovirus
LTRs	Long Terminal Repeats are identical sequences of DNA that repeat at either end of proviral DNA formed by reverse transcription of retroviral RNA
MLV	Murine Leukemia Virus
MLV-X (XMRV)	Murine Leukemia virus X [xenotropic]
MA	EU Marketing Authorisation
NCA	National Competent Authority
PBMCs	Peripheral Blood Mononuclear Cells
PERT assay	Product Enhanced Reverse Transcriptase assay designed to detect the presence of retroviral reverse transcriptase
PERV	Pig Endogenous Retroviruses
Ph.Eur	European Pharmacopoeia
Pol	Polymerase enzymes including reverse transcriptase, protease and integrase enzymes
RD114	An endogenous retrovirus of cats
RDRS	RD114-Related Sequences
RT	Retroviral reverse transcriptase