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Questions and Answers on allogenic stem cell-based products for veterinary use: Specific questions on extraneous agents

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Background

Cell-based medicinal products, including stem cell-based products, are heterogeneous with regard to the origin and type of cells and to the complexity of the product.

The use of allogenic stem cell-based products in the veterinary sector, mainly for horses and dogs, is increasing and raising questions for manufacturers, authorities and users.

One of the questions under discussion concerns the freedom from extraneous agents of stem cell-based products. Freedom from extraneous agents is a high priority for any veterinary medicinal product, including stem cell-based products to be administered parenterally. The requirement to test veterinary medicinal products for potential infectious contaminants is specified in Directive 2001/82/EC and in the European Pharmacopoeia (Ph. Eur.).
Contamination could originate from the starting or raw materials or be adventitiously introduced during the manufacturing process. Differentiation between the cell sourcing steps, which include donor/tissue screening for extraneous agents (viruses, bacteria, protozoa), and the process thereafter during manufacture, where typical microbiological contamination (not related to donor/tissue) might occur (viruses, bacteria, mycoplasma), is reasonable.

Freedom from extraneous agents is crucial for donor animals used as source of tissues/fluids/cells.

Defining freedom from extraneous agents poses a real challenge given that the ideal absolute freedom from extraneous agents or residual pathogenicity is neither possible nor realistic. The detection of extraneous agents depends on the amount of agents present in the raw material as well as the methods used for sampling and detection.

Usually, the manufacture of stem cell-based products does not include terminal sterilisation of the product or removal of or inactivation steps for viruses and parasites. Therefore, it is crucial to define acceptance criteria for starting and raw materials derived from humans or animals taking into consideration the intended use.

Animal stem cells must be sourced from donor animals which are appropriately screened and tested for the absence of extraneous agents. Risk control for extraneous agents includes control of sourcing, testing of starting materials of animal origin and/or subjecting them to validated inactivation procedures, validation of the capacity of the product's manufacturing process to remove and/or inactivate viruses, and, if deemed necessary, testing of the final product.

Currently, no specific guidance is available for stem cell products for veterinary use. Guidance documents have been established for human cell-based products describing the general procedure to ensure quality during collection of source material and manufacturing process.

The EU Guide to good manufacturing practice-GMP (provided in EudraLex Volume 4) covers in Part I basic GMP principles for the manufacture of human and veterinary medicinal products [1]. Annex 2 to this guide contains the manufacture of human biological products including advanced therapy medicinal products (ATMP). The principle provisions laid down in that annex are considered to be applicable to stem cell products for veterinary use as well.

Safety aspects of extraneous agents with regard to veterinary medicinal products are included for example in the following documents:

- The CVMP Guideline on requirements for the production and control of immunological veterinary medicinal products (Annex 2 - The approach to demonstrate freedom from extraneous agents as

part of the production and control of immunological veterinary medicinal products for mammalian species and fish) (EMA/CVMP/IWP/206555/2010-Rev.1) [2].

- Note for Guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3) [3].
- The position paper of the Coordination Group for Mutual Recognition and Decentralised Procedures - Veterinary (CMDv/POS/001) on requirements for starting material of animal origin [4].

Principles on viral safety and TSE risk are also laid down in the European Pharmacopoeia (e.g. 5.2.5 'Substances of animal origin for the production of veterinary vaccines'; 5.1.7 'Viral safety'; 5.2.8 'Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products') [5].

The European Pharmacopoeia has recently adopted guidance with regard to raw materials of biological origin (5.2.12' Raw materials of biological origin for the production of cell-based and gene therapy medicinal products') [5].

The United States Pharmacopeia (USP) has established a specific chapter (1046) on cellular and tissue-based products, which gives information on several aspects of cell-based medicinal products, including freedom from extraneous agents [6].

Freedom from extraneous agents is a crucial aspect of quality evaluation of stem cell-based preparations. Therefore, appropriate acceptance criteria for starting and raw materials of human or animal origin need to be established.

Following a review of the scientific information relating to stem cells, a number of areas have been identified that would benefit from further consideration by relevant experts and, where appropriate, the elaboration of specific guidance in the form of question and answer document (Q&A).

Three specific questions for further consideration have been identified relating to freedom from extraneous agents aspects. These questions, together with an answer to each question, are presented below.

1. **Is the currently available guidance on demonstration of freedom from viruses and bacteria (list of viruses and bacteria which must be taken into account) appropriate and sufficient for allogenic stem cell-based products intended for use in horses and dogs?**

   If not, would it be beneficial to elaborate further specific guidance and appropriate requirements for stem cell-based products intended for use in horses and dogs?

   The CVMP Guideline on the requirements for the production and control of immunological veterinary medicinal products (Annex 2 - The approach to demonstrate freedom from extraneous agents as part of the production and control of immunological veterinary medicinal products for mammalian species and finfish) (EMA/CVMP/IWP/206555/2010-Rev.1) contains a reference list of viruses and bacteria which must be taken into account when demonstrating freedom from viruses and bacteria.

   This list includes the following viruses and bacteria for horses and dogs:

Horses (equine)

Viral agents	*Bacterial agents*
African horse sickness virus Borna disease virus Endogenous retrovirus (replication competent) Equine adenovirus Equine arteritis virus Equine encephalomyelitis alphavirus Equine encephalosis virus Equine herpesvirus (EHV-1, EHV-4) Equine infectious anaemia virus Equine influenza virus Equine rotavirus Hendra virus Japanese encephalitis virus Rabies virus Vesicular stomatitis virus West Nile virus	*Burkholderia mallei* *Burkholderia pseudomallei*

Equine rhinitis viruses A and B, hepaciviruses and pegiviruses could be also relevant for horses. *Borrelia* spp*., Anaplasma phagocytophilum* and *Neorickettsia risticii* may represent relevant extraneous agents for horses, too.

Dogs (canine)

Viral agents	*Bacterial agents*
Canid herpesvirus Canine adenovirus Canine coronavirus Canine distemper virus Canine oral papilloma virus Canine parainfluenza 2 virus Canine parvovirus Rabies virus Swine herpesvirus 1	*Brucella canis* *Leptospira* spp*.*

Other viruses that could be relevant for dogs are canine influenza viruses. *Borrelia* spp*., Ehrlichia* spp*., Bartonella vinsonii, Anaplasma* spp*., Neorickettsia* spp., and other *Rickettsia* spp*.* may represent relevant extraneous agents for dogs, too.

The presence of an agent on the list or mentioned above does not mean that a test for this agent must be carried out in each case. The list is intended as guidance covering relevant agents that need to be considered and should not be seen as exhaustive. However, when not carrying out a test for a specific agent, a justification should be provided based on a risk assessment. Some agents may be ruled out, e.g. based on the animals' geographic location or after clinical examination and observation of the animals over a defined period of time before donation. The use of dedicated herds is also an option. Justification is needed for the chosen testing regime (e.g. testing of the donor or testing on the product), the testing program, and the specific timing and frequency of testing for each product depending on the type of donors used. In general, donors or the donated cells/tissues/product should be tested for all relevant extraneous agents when

exposure to or presence of the agent cannot be ruled out by screening information. Further examples for types of justification for not performing a test for a specific agent are given in Annex 2 of the CVMP guideline.

The extraneous agents to be tested are those that cannot be excluded by justification.

If donors originate from outside the EU, animals should be held under quarantine conditions for a defined period of time prior to donation and, depending on the country/region of origin, additional extraneous agents may need to be considered.

For the detection of extraneous agents highly sensitive methods should be used. *In vitro* methods have to be used, if available. The suitability of test methods used to detect extraneous agents is an essential prerequisite. The following aspects are identified as key criteria for test suitability: defined method, sensitivity, specificity, repeatability of the method and need for positive and negative controls.

For the detection of viruses, appropriate methods for virus isolation and identification should be used (cell cultures, embryonated eggs, animal inoculation) and criteria established, e.g. cytopathic effect, haemadsorption, immunostaining, etc. The sensitivity of the test for specified agents should be known not only for laboratory-adapted strains but also for field (wild) strains. Antigen and genome detection methods (e.g. PCR) can also be used.

Detection of an agent may also be based on the detection of corresponding antibodies using appropriate serological methods.

In summary, the currently available guidance on demonstration of freedom from viruses and bacteria (list of viruses and bacteria which must be taken into account) will form the basis also for allogenic stem cell-based products intended for use in horses and dogs. Additionally viruses and bacteria are identified which may represent relevant extraneous agents for horses and dogs. At present, preparing further specific guidance for stem cell-based products intended for use in horses and dogs appears not to be required.

2. As no EU guidance is currently available on the demonstration of freedom from parasites, especially protozoa, which protozoa should be specifically taken into account for allogenic stem cell-based products intended for horses and dogs?

Protozoan parasites, including vector-borne infections and intestinal protozoa, are responsible for a number of different diseases in dogs and horses. Although many infections are acquired by direct ingestion of infective stages, others may be transmitted by arthropods. These parasites are distributed worldwide or regionally. Therefore, investigations for protozoa may be relevant for dogs and horses depending on the animals' region of origin and the prevalent epidemiological situation in this region as well as the travel history of the animals. Well-documented clinical information on the dogs and horses used as donors should be available. The following parasites, especially protozoa, should be taken into consideration:

Horses: *Babesia caballi, Theileria equi, Trypanosoma equiperdum*.

Dogs: *Babesia* spp*., Leishmania* spp*., Trypanosoma cruzi, Neospora caninum, Dirofilaria immitis*.

3. Are there any recommendations regarding other aspects or approaches to be taken into account (risk control, risk analysis, risk mitigation, risk management) concerning the freedom from extraneous agents of allogenic stem cell-based products for horses and dogs?

Stem cell-based products for veterinary use should only pose a minimal risk of transmitting extraneous agents to the recipient.

Stem cell-based products are obtained from various anatomical structures of the organism, e. g. adult skin, adipose tissue, umbilical cord tissue, bone marrow. Not all pose the same risk of being contaminated by extraneous agents; e.g. umbilical cord tissues are considered less likely to contain communicable infectious agents compared to adult tissue or fluids.

When considering the testing of starting materials, three approaches can be differentiated:

- A single primary cell isolate used directly for the cell-based medicinal product.
- Primary cells cultured for a few passages before being used for the cell-based medicinal product.
- Cells based on a well-defined cell bank system consisting of a master cell bank and a working cell bank.

An initial risk analysis may be performed based on existing knowledge of the type of product and its intended use. The risk posed by the administration of a cell-based medicinal product is highly dependent on the origin of the cells, the manufacturing process, non-cellular components and the specific therapeutic use.

Determination of donor eligibility is very important and therefore, qualification of donor animals used as source for the stem cells is one of the most critical aspects. A donor would only be considered eligible to donate cells where it was considered free from risk factors for, and clinical evidence of, relevant disease agents and diseases. Such a donor eligibility determination should be completed for each donation of cells or tissues used in the manufacture of an allogenic stem cell product. The donor's age should be considered as the probability of contact with an extraneous agent increases with age. It is well known that neonatal tissues (umbilical cord tissue, cord blood) carry less risk than adult tissues. If starting material from newborns (e.g. umbilical cord) is used, it may be necessary to test their mothers too.

One prerequisite for determining eligibility of donors is the establishment of donor selection criteria. A risk-based, product-specific approach should be used to determine the selection criteria for eligible donors. The selection criteria should consider the characteristics of the product, the intended recipient population, the donor population, and the risk of transmission of disease agents to recipients. A combination of donor screening using historical and clinical information, donor testing for specific disease agents, and product/source material testing for specific disease agents can be used to demonstrate the absence of a disease agent.

Information to be recorded in support of donor eligibility should include:

- A list of relevant extraneous agents to be taken into account when considering which screening and testing procedures are appropriate.
- Criteria for qualification of donor animals/determination of eligibility as donors.
- Donor screening methods including historical information (e.g. source, identification, travel history, vaccinations, treatment for parasites), physical examinations, daily health observations, general health screening tests, procedures to reduce the risk of exposure to extraneous agents.
- Donor testing methods, including validation of test methods.

- Documentation with respect to donor screening, testing and donor eligibility determination and evaluation of the results.

As for allogenic stem cell therapies, donors free from extraneous agents are desirable it is necessary to clarify what will be the fate of the donor in case of positive tests or under which conditions use of cells/tissues from such donors could be accepted.

Absence of extraneous agents should be also ensured for other materials of biological origin needed for collection, selection, culture or modification of cells, such as other cells, enzymes, antibodies, cytokines, sera. As a consequence, each biological substance used in the procedure should be clearly specified and evaluated as to its freedom from extraneous agents.

Furthermore, aseptic manufacturing is necessary for reducing the presence of extraneous agents.

The following statement of Appendix I of ICH Topic Q 5 D (CPMP/ICH/294/95) should be considered too: “Appropriate testing regimens and test methods for cells used in the production of specific products will vary depending on the donor species used as a source of tissue, adventitious agents potentially present, the nature of the product, its intended clinical use, aspects of the manufacturing process, and the extent of testing performed on the final product. Applicants should explain and justify the approach taken with respect to their specific product” [7].

When mesenchymal stem cells (MSC) originate from peripheral blood, bone marrow or umbilical cord blood, the collected fluid is the starting material. When MSC are extracted from a tissue forming part of an anatomical structure, (e.g. neonatal tissue from umbilical cord tissue located around blood vessels), the extracted tissue and not the anatomical structure itself may be considered as the raw material.

References

1. EudraLex - Volume 4 of "The rules governing medicinal products in the European Union".
2. The CVMP Guideline on requirements for the production and control of immunological veterinary medicinal products (Annex 2 - The approach to demonstrate freedom from extraneous agents as part of the production and control of immunological veterinary medicinal products for mammalian species and fish), EMA/CVMP/IWP/206555/2010-Rev.1.
3. Note for Guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products, EMA/410/01 Rev.3.
4. Position paper of the Coordination Group for Mutual Recognition and Decentralised Procedures - Veterinary on requirements for starting material of animal origin, CMDv/POS/001.
5. European Pharmacopoeia, Ph. Eur. 5.2.5 ‘Substances of animal origin for the production of veterinary vaccines’, 5.1.7 ‘Viral safety’, 5.2.8 ‘Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products’, 5.2.12’ Raw materials of biological origin for the production of cell-based and gene therapy medicinal products’.
6. U.S. Pharmacopeia, chapter 1046 ‘Cell and gene therapy products’.
7. ICH Topic Q 5 D, Note for guidance on quality of biotechnological products: Derivation and characterisation of cell substrates used for production of biotechnological/biological products, CPMP/ICH/294/95.