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Guideline on Quality of biological active substances produced by transgene expression in animals

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This guideline replaces the guideline on 'Use of transgenic animals in the manufacture of biological medicinal products for human use' (3AB7A).

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7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

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Executive summary

Transgenic animal technology has emerged as a complement to the longer-established range of prokaryotic, yeast and mammalian cell-based recombinant protein expression systems for the production of therapeutic proteins. In this document guidance is provided on the approaches that should be employed in order to achieve satisfactory quality for biological active substances proposed to be produced using this technology.

1. Introduction (background)

The principal aim of this guideline is to adapt some specific aspects of the quality guidance already in place for other recombinant production systems to the special case of transgenic animal systems. As is the case with other biotechnologically produced active substances, both the production process and its control strategy play important roles in defining the quality profile of active substances produced by transgenic animals. An additional consideration for transgenic animal-based production is that, since experience with the technology is relatively limited when compared to the longer established methods, applicants are advised to be appropriately vigilant towards transgenic system-specific aspects such as development genetics, generation and maintenance of the transgenic animal line and their impact on product reproducibility.

2. Scope

The guideline addresses the use of transgenic animals as a manufacturing technology for recombinant therapeutic proteins. It concentrates on the quality issues affecting active substances produced by the expression of one or more transgenes stably located in the nuclear genomes of animals, including the selection, generation and control of the production animals and evaluation of freedom from adventitious agents. Since it is expected that a protein that is produced by a transgenic animal, and its quality attributes, would follow the same standards as a protein produced in mammalian cells in a fermentation system, the general guidance which is already available for downstream processing systems will be applicable. Similarly, the active substances will have to comply with all of the relevant guidance already available for biotherapeutic medicinal products.

Many of the principles discussed in the Guideline on Xenogeneic Cell-based Medicinal Products (EMEA/CHMP/CPWP/83508/2009)[1] are also applicable to the generation, testing and maintenance of transgenic animals for the purposes discussed here. In particular, the applicability of the sections on animal husbandry, animal facilities, transportation, testing for infectious agents in source or founder animals and procurement steps should be considered.

Production using transiently transfected animals or cloned animals (unless the latter method was used for the generation of founder transgenic animal) falls outside the scope.

3. Legal basis

This guideline should be read in conjunction with the introduction and general principles and part I, module 3 of the Annex I to Directive 2001/83/EC[2] as amended, and with all relevant EMA Committee for Medicinal Products for Human Use (CHMP) and ICH guidelines.

Medicinal products containing biological active substances manufactured using transgenic animals fall within the scope of the Annex to Regulation (EC) No 726/2004[3] and may only be placed on the market

within the European Union if a marketing authorisation is granted in accordance with the Centralised Procedure as defined in this Regulation.

Manufacturers responsible for handling transgene-bearing animals in the European Union need to comply with relevant EU animal and environmental protection legislation, including GMO legislation, as relevant.

4. Development genetics

4.1. The host animal

The rationale for the choice of host animal and strain for the genetic manipulation should be documented.

4.2. The transgene and expression construct

Reference is made to ICH Q5B[4] and to the Note for Guidance on Production and Quality Control of Medicinal Products Derived by Recombinant DNA Technology (3AB1A)[5] regarding the description and assembly of the vector, the gene of interest (encoding the intended therapeutic protein) that will be used for transgenesis, any other sequences that will be incorporated in the vector and the final construct. The homology of the transgene sequence to the natural human sequence should be discussed if relevant.

4.3. Generation of the founder animal

Besides classical methods of transgenesis, such as zygote injection or transfection/electroporation of embryonic stem (ES) cells, alternative methods may be applied to generate transgenic animals (e.g. retroviral/lentiviral transduction of ES cells or embryos, co-injection of oocytes with sperm and DNA constructs, receptor-mediated gene transfer and cloning (nuclear transfer) from genetically manipulated cells). Whichever method is used, it should be described in detail, including the isolation of gametes, ES cells, zygotes or embryos, the *in vitro* fertilisation (if applicable), the introduction of the transgene, the re-implantation and the system to ensure traceability throughout the generation of the founder animal. Whenever retroviral/lentiviral vectors are used, only replication-deficient retroviral/lentiviral vectors should be used as described in the CHMP Note for Guidance on the Quality, Preclinical and Clinical Aspects of Gene Transfer Medicinal Products (CPMP/BWP/3088/99)[6], to minimize the risk of recombination events that may lead to the formation of replication-competent viruses.

The donor animals, from which the gametes, zygotes, ES cells or embryos are derived, should be fit for purpose and information on their origin and characteristics should be provided, including breed, strain and sub-strain. Microbiological and viral safety of the donor animals should be ensured, e.g. donor animals should be of Specified Pathogen Free (SPF) status or the transgenic animal line should be sanitized/re-derived (e.g. by embryo transfer). It is recommended that a transgenic animal line will be derived from a single genetic founder animal (unless the generation of the intended product necessitates multiple founders). Ideally the transgene should be located at a single locus. If more than one locus is present the role of each locus in the production of the recombinant protein should be ascertained. A justification should be provided for the selection of the transgenic animal line: relevant criteria to be taken into account include (but are not limited to) copy number, expression level, protein characterisation and animal health.

The transgenic animal line should be sufficiently genetically tested:

- Evidence of the incorporation of the correct transgenic sequence should be obtained. The methodology chosen shall be evaluated for the ability to detect a single deviant transgenic sequence (coding for a deviant transgenic product), among all of the inserted copies of the transgene.
- Estimates of the copy number and their orientation (head to head or head to tail) should be made, if relevant.
- In case of homologous recombination, the targeted gene deletion/replacement/mutation should be adequately demonstrated.
- The expression level of the incorporated gene and the identity of the product should be ensured.
- The tissue distribution of expression should wherever possible be shown to be consistent with the chosen strategy of expression.

4.4. Transgenic banking system

Annex 2 of the European Good Manufacturing Practices (GMP) Guidelines (EudraLex Volume 4)[7] defines the Master Cell Bank (MCB) as a homogeneous pool of microorganisms or cells that are distributed uniformly into a number of containers that are stored in such a way to ensure stability and is normally used to produce Working Cell Banks (WCB). The Master Transgenic Bank (MTB) is defined as above but for transgenic animals (or plants).

Depending on the composition of the MTB (ES cells, fertilized eggs/oocytes, embryos, sperm, etc.) the MTB may contain only haploid or heterozygote material, in which case only a fraction of the offspring will carry the transgene. When this is the case, it leads to a fundamental difference between the cells that comprise an MCB and MTB. In the case of an MCB, a pool of cells expanded from each vial is immediately capable of the manufacture of the recombinant product. In the case of an MTB, if the bank is comprised of gametes which contain the genetic construct, and these cells fuse with gametes from a breeding partner to create offspring, only a proportion will contain the transgene from the MTB. Thus the cells of an MTB are not capable of directly manufacturing the desired recombinant protein and must, through a series of reproductive phases and selection of animals, give rise to the animal which can express the transgenic product in the appropriate cells.

During these phases, a number of opportunities arise for variability between the production animals resulting in possible variability of the recombinant product. These include genetic variability due to selection of alleles in the offspring that may have an influence on the product as well as the possibility of genetic alterations (deletions, homologous recombination, point mutations) during breeding.

Consequently measures should be taken to stabilize the genetic make up, both with respect to the transgene as well as the genetic makeup of the rest of the genome, particularly so for the MTB. However, it is important to note that increased variability of production animals in a group of transgenic animals when compared to a closed cloned cell fermentation system is sometimes inevitable although the use of inbred animal lines may help to stabilise the genetic makeup.

Consequently, to maximise genetic stability a transgenic banking system should be established and inbred lines used, if appropriate. To extend the life span of the cell banks, a MTB and Working Transgenic Bank (WTB) should be established from the MTB and appropriately characterised, (e.g. according to the concepts outlined in ICH Q5D[8]). If feasible, the MTB should be collected from a single animal containing a haploid or diploid genomic locus of the relevant transgene(s). This material constitutes the MTB, although it is acknowledged that it may not be completely homogeneous (for example if it is composed of sperm). Progeny used to generate the WTB and production animals should

be traceable back to the MTB. If relevant, material from more than one animal may comprise the WTB to ensure that sufficient material is available to establish the production colony. However, at a minimum, the presence of the transgene, copy number and the expression level for the desired protein as well as the size and point of insertion of the transgene should be established in each animal prior to its acceptance as a contributor to the WTB.

5. Manufacturing issues

5.1. Quality system

The first production phase is specific to transgenic animal technology and includes the breeding and maintenance of animals, production of the matrix which contains the recombinant protein (e.g. milk), harvesting and primary processing.

The Annex 2 of European GMP Guidelines (EudraLex Volume 4)[7] applies to the generation and maintenance of Master and Working Transgenic Banks, collection, cutting, mixing, and/or initial processing, and all subsequent downstream processing operations during manufacture. Consequently Part A General Guidance and Part B Specific Guidance on selected product types will apply.

Where classical GMP principles prove impractical to apply to elements of the first production phase that are not covered in GMP Annex 2 (i.e. breeding and maintenance of production animals), a suitable quality system should be developed and put in place.

Ultimately, whether performed in accordance with GMP or with a defined quality system such as Good Agricultural and Collection Practice (EMEA/HMPC/246816/2005)[9], the early steps of the manufacturing process should be well controlled by the application of suitable in-process controls, provide a well-defined starting material suitable for subsequent processing under GMP, and be well documented. The operations and the documentation should be available for inspection.

Production operations for the active substance that are downstream of the first production phase of manufacture, encompassing product isolation, purification and formulation, should be conducted according to GMP. These procedures are common to all biotechnology-derived products and the general requirements are documented in the relevant CHMP and GMP guidelines.

5.2. Generation and control strategy for the production animals

The breeding strategy to obtain production animals should be clearly defined and ideally the maximum number of generations between founder and production animals should be defined. In any case, the genetic stability of the transgenic animal line should be validated over the maximum number of generations between the MTB and animals constituting the production group. In case where a maximum generational limit is not pre-established an on-going monitoring of genotypic, phenotypic and process parameters should be performed. The criteria outlined above for the genetic characterisation of the founder animal should be considered in the design of this validation. The methods used should be capable of identifying if critical genetic elements have undergone changes leading to an alteration in the intended product.

The genetic makeup of the non-transgenic line used to inter-breed with the transgenic animals should also be controlled. Herd records should be kept pertaining to the source animals and facilities.

Specific criteria should be defined by which the inclusion of transgenic animals into the production group is controlled. At a minimum this will include the presence of the transgene and the expression level for the desired protein. The impact of the genetic variability, as derived by natural allelic

differences or mutations, in those elements that are responsible for the matrix into which the recombinant product is generated, should be considered. Basic testing of the matrix itself should be performed in a representative number of animals to assure consistency between transgenic animals and over generations. In addition, any variability of the host proteins and other matrix constituents (such as lipids or glycans) that constitute the matrix should be investigated during process development, and the ability of the purification process to remove host matrix constituents should be validated in view of this variability.

The impact of the transgene on the health profile and longevity of the transgenic animals should be monitored. Additionally, impact on animal health due to expression of the recombinant protein should be considered since expressed recombinant proteins may have potent effects on the production animals.

The production group should be maintained in a well-controlled environment with restricted movement of animals, personnel, feed stuff and materials. The general principals of a Specified Pathogen Free flock are appropriate (e.g. Ph. Eur. 5.2.2 Chicken Flocks Free From Specified Pathogens for the Production and Quality Control of Vaccines)[10] with adaptation under proper justification.

It is important to define a regime for monitoring the health status of the production group, and to pre-define procedures if animals in the group are suspected or discovered to be infected with a pathogen. The monitoring protocol should include regular observations and regular checks by appropriately qualified personnel. If the testing of transgenic animals is not suitable, the inclusion of “sentinel” animals (non-transgenic, sacrificial animals) to live alongside the production group should be considered, so that these animals could be transported off site at defined intervals for in-depth analysis for specific pathogens. This is, however, useful only if the production animals live in close proximity to the sentinels, such that transmission of infection is likely to occur. Direct testing for specific pathogens should be conducted at regular, defined intervals. The pathogens to be tested for will depend on the individual species and the location of the production facility.

If infected animals are suspected, a pre-defined procedure should be implemented regarding the specific animal(s), the whole group, and any production material that has been obtained from the group. The procedures defined should take into account the impact of the disease, and its pathogenicity for humans. If an infection is confirmed, a follow-up on the timing of the infection shall be performed to clarify which batches of production material are affected.

Unexpected deaths should be fully investigated. When production animals die unexpectedly or are euthanized (except under pre-defined circumstances) the cause of death, or the condition leading to the need for euthanisation, should be ascertained and where appropriate, archival samples should be stored.

If vaccines, antibiotics or other pharmaceuticals or additives are used on the production animals, the persistence of the substance/reagent in the animal, the matrix within which the transgenic product is expressed, and the toxicity of the substance/reagent in humans should be taken into consideration in defining a period of time before the treated animals can contribute to the pool of starting material. Until this time period has elapsed, starting material should not be derived from animals treated with these substances/reagents.

5.3. Production of starting material

The production of starting material typically includes breeding, collection of the matrix containing the recombinant product from the group of transgenic animals, initial processing and storage. Matrix may

be pooled at this stage, or immediately prior to downstream processing. The following aspects should be considered and described:

Description of the site

- Geographical location, with boundaries exactly defined
- Housing arrangements for the animals
- Supervision of the site
- Measures taken to ensure the isolation of the transgenic animals from other local animals
- Food stuff, water supply, bedding, chemicals (including fertilisers and pesticides) used on site should be defined, and specifications should be set, where appropriate
- Local fauna
- The quality and/or good practice system in operation at the site.

Housing and feeding strategy

- Description of housing strategy (indoor, covered areas and open fields)
- Strategy to maintain the SPF status of the animal group, with adaptions under proper justification
- Environmental conditions
- Measures taken to ensure suitability of food and water supply.

Harvesting and primary processing

- Criteria for initiation of collection of transgenic material (harvesting)
- Frequency of harvesting
- Harvesting procedures
- Procedures and validation of the immediate steps after harvesting, including pooling strategy (if relevant), transport and storage arrangements, and physical and chemical treatments applied
- Location, conditions and duration of storage of matrix
- Cleaning and sterilisation of equipment.

5.4. Downstream processing

Since it is expected that a recombinant protein and its quality attributes produced by a transgenic animal would follow the same standards as those produced by the same construct inserted in mammalian cells in a fermentation system, the general guidance that is already available for downstream processing systems will be applicable. Similarly, the active substances and finished products will have to comply with relevant guidance already available for biotherapeutic medicinal products.

If a batch of starting material comprises several sub-lots, then consideration should be given to a pooling strategy that minimises variability of the composition of the pool for the downstream process.

If the animal production system allows the possibility of leaching of host proteins from one particular compartment into the transgenic production system (for example from animal blood into milk), then it

should be shown, possibly through in-process controls or batch release testing, that the quantity of leachate is under strict control.

6. Freedom from contamination with adventitious agents

6.1. Non-viral

Depending on the kind of harvested material (e.g. blood or milk), it may be impractical to guarantee that the starting material is free from microbial contaminants such as mycoplasma, fungi and bacteria and these may contaminate, and replicate in, the crude harvested material. In this case, steps should be taken to minimise the bioburden associated with such contaminants and they should be removed or reduced to inconsequential levels as soon as possible after the start of downstream processing. Monitoring for them will be required.

6.2. Viral

The overall strategy for assuring the viral safety of a transgenic recombinant protein will involve the well-established approach that is used for most biological medicinal products:

- controls and tests on starting materials, raw materials, reagents and excipients,
- *in vitro* and *in vivo* tests for the absence of adventitious agents at critical production stages, such as appropriate unprocessed bulk and/or processed bulk levels,
- validated virus inactivation/removal procedures.

At the level of the transgenic animals, barriers or containment approaches applied to the agricultural steps (husbandry and harvesting) and aimed at preventing the adventitious entry of extraneous agents will play a critical role. Good husbandry and testing animals for specific agents is addressed in Section 5.2 – Generation and Control Strategy for the Production Animals. The health monitoring system as described in the EU guideline (CPMP Note for Guidance on Production and Quality Control of Animal Immunoglobins and Immunosera for Human Use - CPMP/BWP/3354/99)[11] should be applied whilst the annex to the referred guideline provides examples of viruses which the manufacturer should consider when establishing a system of health control of the animals used. Reference is also made to the OIE (World Organisation for Animal Health (OIE, Office International des Epizooties; http://www.oie.int/eng/en_index.htm)[12] for information concerning the prevalence of viruses in specific geographic regions. Many of the principles discussed in the Guideline on Xenogeneic Cell-based Medicinal Products (EMEA/CHMP/CPWP/83508/2009)[1] are also applicable to ensuring the freedom of transgenic products from pathogenic agents.

Individual donations or pooled unprocessed bulk harvested from the transgenic animals should be tested for viral contamination by a variety of *in vitro* and *in vivo* tests, unless otherwise justified. With regard to the tests applied, reference is made to the ICH guideline Q5A (Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, CPMP/ICH/295/95)[13] and the guidance presented therein should be applied as appropriate. This includes the performance of studies to investigate the capacity of the downstream purification process to inactivate or remove viruses, bearing in mind that downstream processing should incorporate a dedicated step (or steps) that is effective in inactivating or removing viral contaminants in addition to purification steps which themselves should be included in the evaluation. For the production of material to be used during clinical evaluation, the guidance provided in the Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products, EMEA/CHMP/BWP/398498/2005[14], may have value.

6.3. TSE

From a TSE point of view, the use of a TSE irrelevant species of transgenic animal is desirable; however, where a TSE relevant species is used, justification should be provided and the most current version of the EC Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products, EMA/410/01[15] should be applied. Steps should be taken to minimise TSE contamination, such as embryo washing at the level of production of the transgenic founder, and a detailed history of all animals used in establishing the transgenic animal line as well as the production animals themselves, a history of the premises where the animals are kept, the use of a closed herd and the measures taken when introducing new animals into the herd, the monitoring and testing of animals, the TSE category of the harvested material from the involved species and any ability of downstream processing steps to remove or inactivate TSE agents should be documented.

6.4. Risk assessment

Taking each of the above considerations into account, applicants should present a risk analysis of the potential for contamination of the active substance with adventitious microbial, viral or TSE agents. On the basis of this analysis, which should be quantitative insofar as is possible; the applicant should propose an integrated step-wise strategy that reliably assures the microbial, viral and TSE safety of each batch of the transgenic medicinal product.

Definitions

Matrix: The animal tissue or fluid in which the recombinant protein is expressed. It is derived from the host animal and composed of host related impurities.

Starting material: The animal tissue or fluid, plus the recombinant protein which comprises the bulk harvest from the transgenic animal.

Transgenic animal line: All animals which are derived from a single founder transgenic animal.

References

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11. Note for Guidance on Production and Quality Control of Animal Immunoglobins and Immunosera for Human Use (CPMP/BWP/3354/99)
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12. OIE - World Organisation for Animal Health (OIE, Office International des Epizooties)
http://www.oie.int/eng/en_index.htm

13. ICH Q5A - Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin (CPMP/ICH/295/95)
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002801.pdf

14. Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products (EMEA/CHMP/BWP/398498/2005)
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