ZKBS
Zentrale Kommission für die
Biologische Sicherheit

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Position statement of the ZKBS on classifying genetic engineering operations with primary cells from vertebrates

Definition of terms

Primary cells are defined as cells isolated directly from body fluids or body tissues of multi-cellular organisms. Primary cell cultures are primary cells grown in culture up to the first passage. Regarding the aspect of risk assessment, further passages of primary cell cultures and structures derived from them, such as organoids, are to be treated as primary cells until they are sufficiently characterized. Cultivation of primary cells for the purpose of virus enrichment requires an individual risk assessment.

Hazard potential

Infection or contamination of cell cultures with disease-causing agents represents a potential hazard when working with these cell cultures. However, there are only very few reported cases of illness associated with handling cell cultures. Due to unknown contaminations, the risk of transmitting pathogens to employees is greater when handling primary cells from higher organisms than when handling established cell lines, which are usually well characterized.

Based on the list of donor and recipient organisms for genetic engineering operations published according to § 6 of the Genetic Engineering Safety Regulations (GenTSV), cells and cell lines are to be assigned to **risk group 1** as donor and recipient organisms, if they do not release organisms of a higher risk group. If they release organisms of a higher risk group, classification is based on the risk group of these organisms.

Generally, cells and cell lines from higher organisms can only be cultivated using complex media and specific cell culture vessels. Since they are not viable without these conditions, they do not represent a hazard to the environment in principle. Based on this, it can thus be assumed that the requirements of the GenTSV for classifying primary cells in **risk group 1** are generally met.

However, if a reasonable suspicion exists that primary cells or primary cell cultures contain and release transmissible pathogens, it must be assumed that there is a risk for the employees. Previous experience in handling such cell materials has shown that a hazard emanates from possible infections with human pathogenic viruses. There is currently no evidence for risks associated with contaminations with non-viral pathogens.

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The possibility that viruses are present in primary cells largely depends on the species and the state of health of the donor organism as well as on the tissue or body fluid from which the material was taken. In order for primary cells to be classified in **risk group 1**, it must therefore be verified that the donor is free of disease symptoms and/or serologically negative for certain viruses.

The following section provides several examples serving as a guideline for the differentiated risk assessment of primary vertebrate cells and primary vertebrate cell cultures. Other cells – particularly primary plant and insect cells – are not considered within the framework of this position statement.

A. Risk assessment of primary vertebrate cells

For estimation of the hazard potential of primary cells it is useful to distinguish between cells established from humans, other primates, chiroptera or other vertebrates.

1. Human cells

Risk assessment of primary human cells can be based on experiences gained in transfusion and transplantation medicine. For transfusions/transplantations it is known that there is a high risk of infection for the recipient if the material used is contaminated with pathogens. For this reason, donated blood is screened for human pathogenic viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) using serological and molecular biology methods. Even if the primary cells are contaminated with HIV, HBV or HCV, the risk of infection associated with handling primary cells for the purpose of carrying out genetic engineering operations is much lower compared to the risk of infection following transfusion or transplantation. For donors not showing clinical symptoms it is thus sufficient to exclude the possibility of contaminations with the help of serological tests. This stands in accordance with the procedure of occupational health examinations, where serological tests are regarded as sufficient despite the diagnostic gap, provided that there is no actual suspicion of an exposure to or infection with HIV, HBV or HCV.

Risk assessment of primary human cells

- Primary cells from donors not showing clinical symptoms are to be assigned to **risk group 1**, if seronegativity of the donor for HIV, HBV and HCV has been demonstrated by immunological tests, or if it has been shown by other procedures that the cells are free from these viruses. In individual cases, if there is reason to suspect the presence of a certain virus of a higher risk group in the cells to be used, the primary tissue is to be checked for the absence of this virus.
- If the donor or cells are not tested for the absence of the viruses mentioned above, the primary cells – following the experience in handling diagnostic samples in medicine – are generally to be assigned to **risk group 2**.

- If the donor or cells are not tested for the absence of the viruses mentioned above and if the cells are not permissive for the viruses mentioned above, the primary cells can be assigned to **risk group 1**, if it is ensured that they are not contaminated with blood or secretions or with cells that are permissive for the viruses mentioned above.
- If primary cells are isolated from tissues or body fluids which are expected to release viral pathogens due to an illness of the donor or due to the nature of the diseased tissue, the material is to be assigned to the risk group of the respective virus.

2. Non-human primate cells

Primate cells can be carriers of viral pathogens that can be transmitted between various monkey species and also to humans (inter-species transmission). Several of these transmissible viruses persist in the animals without detectable symptoms, and symptoms only appear after inter-species transmission.

For example, retroviruses like spumaretroviruses (Simian foamy virus, SFV), lentiviruses (*Lentivirus simimdef*, formerly Simian immunodeficiency virus, SIV), *Betaretrovirus maspfimon* (formerly Simian retrovirus, SRV) or *Deltaretrovirus priTlym1*, *Deltaretrovirus priTlym2* and *Deltaretrovirus priTlym3* (formerly Primate T-lymphotropic virus 1 – 3, Simian T-lymphotropic virus, STLV) are widespread in numerous monkey species. Infection of the animals with these viruses, however, usually remains unrecognized. Infection of macaques with SIV or SRV occasionally causes AIDS-like symptoms, and infection with STLV can sometimes be associated with lymphoproliferation. Infection with SFV is widespread and usually remains asymptomatic. Numerous transmissions from monkeys to humans are reported for SFV. Infected humans remained asymptomatic as well, and the virus was not transmitted to contact persons. Thus, it can be assumed that handling of primary simian cells contaminated with SFV does not present a hazard for humans.

Simplexvirus macacinealpha1 (formerly also called (Herpes) B virus, BV) naturally infects macaques and possibly other Old World monkeys. However, infection of monkeys is largely asymptomatic whereas transmission to humans can result in a fatal encephalomyelitis. The majority of free-ranging (rhesus) macaques as well as animals kept in breeding facilities are seropositive for BV. However, the animals are usually not viraemic, and release of BV from tissues or cells obtained from them is not to be expected. Virus release is only to be expected when using cell material from viraemic animals. However, BV can persist in monkeys without being recognized by the immune system, so that seronegativity does not reliably rule out an infection.

The common marmoset (*Callithrix jacchus*) is a special case. There are no reports of infections of these New World monkeys with STLV, SIV or BV. Infections of common marmosets with inter-species transmissible pathogens cause severe disease associated with high lethality and are thus easily recognized. Since the 1960s common marmosets are used for animal experiments or are kept as pets. Diseases that can be transmitted from these animals to humans are not known. Besides rhesus macaques (*Macaca mulatta*) and cynomolgus monkeys (*Macaca fascicularis*), common marmosets are one of the primates most frequently used as laboratory animals. However, only specially bred and not wild caught common marmosets are used for this purpose.

Risk assessment of primary non-human primate cells

- Unless a special risk assessment is performed, primary cells from non-human primates from veterinary-controlled breeds not showing clinical symptoms are to be assigned to **risk group 2** due to the widespread prevalence of inter-species transmissible viruses.
- Primary cells from rhesus macaques (*Macaca mulatta*) or cynomolgus monkeys (*Macaca fascicularis*) from veterinary-controlled breeds not showing clinical symptoms are to be assigned to **risk group 1**, if they are tested negative for SIV, SRV, STLV (*Deltaretrovirus priTlym1*, -*2* und -*3*) and BV.
- If the donor or donor cells are not tested for the absence of the viruses mentioned above and if the cells are not permissive for the viruses mentioned above, the primary cells can be assigned to **risk group 1**, if it is ensured that they are not contaminated with blood or secretions or with cells that are permissive for the viruses mentioned above.
- Embryonic stem cells from rhesus macaques (*Macaca mulatta*) or cynomolgus monkeys (*Macaca fascicularis*) isolated from *in vitro* fertilized embryos of macaques from veterinary-controlled breeds that have been tested negative for SIV, SRV, STLV and BV, are to be assigned to **risk group 1**.

For example, primary cells can be tested for the viruses mentioned above using polymerase chain reaction (PCR) and appropriate controls. However, PCR testing of blood or serum is required for primary cells from rhesus macaques or cynomolgus monkeys infected with BV, if viremia cannot be ruled out at the time of collection of the primary cells.

- Primary cells from common marmosets (*Callithrix jacchus*) from veterinary-controlled breeds not showing clinical symptoms are to be assigned to **risk group 1**.
- Cell material taken from wild-caught primates requires an individual risk evaluation, but these cells are at least to be assigned to **risk group 2**.

3. Vertebrate cells (excluding primates)

In most cases, genetic engineering operations use rodents (mouse, rat, hamster and guinea pig) as donor organisms for primary cells. In addition, primary cells are often taken from domestic animals and pets such as cattle, pigs, goats, sheep, dogs, or rabbits, as well as from amphibians and birds. These animals can carry pathogens, in rare cases also human pathogens. For example, bats that do not show any symptoms of disease can be infected with the human pathogenic viruses *Lyssavirus hamburg* (formerly European bat lyssavirus 1, EBLV-1), *Lyssavirus helsinki* (formerly European bat lyssavirus 2, EBLV-2) and *Lyssavirus bokeloh* (formerly Bokeloh bat lyssavirus, BBLV).

Risk assessment of primary cells from vertebrates (excluding primates)

- Primary cells from vertebrates (excluding primates and chiroptera) are to be assigned to **risk group 1**, if the animals do not show any symptoms of disease. This assignment applies to animals from veterinary-controlled populations.
- Primary cells from chiroptera that have been shown to be free from the lyssaviruses EBLV-1, -2 and BBLV are to be assigned to **risk group 1**, if the animals do not show any symptoms of disease.

- Primary cells from chiroptera that have not been tested for the absence of the lyssaviruses mentioned above are to be assigned to **risk group 2**.
- If primary cells are isolated from tissues or body fluids which are expected to contain viral zoonotic pathogens, the material is to be assigned to the risk group of the respective pathogen.

B. Classification of genetic engineering operations using primary cells from vertebrates

1. Primary cells as recipient organisms

If primary cells are used as recipient organisms, the genetic engineering operations are to be assigned to the containment level corresponding to the risk group of the primary cells, provided that the transferred nucleic acids are characterized to such a degree that the hazard potential of the genetically modified organisms, after a preliminary safety assessment according to § 5 of the GenTSV, does not exceed the hazard potential of the primary cells.

2. Primary cells as donor organisms

In the following, genetic engineering operations involving establishment of genomic or cDNA gene banks in *E. coli* K12 from primary cells are assessed.

2.1. If reasonable suspicion exists that the primary cells are infected with a virus, the cells are to be assigned to the risk group of the expected virus. Genetic engineering operations using these cells as donor organisms are to be assessed as genetic engineering operations using the corresponding virus.

2.2. Primary cells from persons not showing clinical symptoms are to be assigned to **risk group 1**, if seronegativity of the donor for HIV, HBV and HCV has been demonstrated by immunological tests, or if it has been shown by other procedures that the cells are free from these viruses. If the donor or the cells are not tested for the absence of the viruses mentioned above and if the cells are not permissive for the viruses mentioned above, the primary cells can be assigned to **risk group 1**, provided that contamination with blood or secretions or with cells permissive for the viruses mentioned above can be excluded.

Primary cells from chiroptera that have been shown not to contain the lyssaviruses EBLV-1, -2 and BBLV as well as other primary vertebrate cells (excluding non-human primates) are generally to be assigned to **risk group 1**. Transfer of nucleic acid fragments from these donor organisms to *E. coli* K12 using vectors that meet the requirements of § 8 para. 2 GenTSV is to be assigned to **containment level 1**. The resulting genetically modified organisms are to be assigned to **risk group 1**.

Reasons:

The primary cells and the nucleic acid fragments transferred from them do not hold a hazard potential. The vector-recipient system corresponds to a biological safety measure according to § 8 para. 2 GenTSV.

2.3. Primary cells from persons not showing clinical symptoms that are not tested for the absence of HIV, HBV and HCV and that are permissive for these viruses or may be contaminated with blood or secretions or with cells permissive for these viruses are to be assigned to **risk group 2**, if they are used as donor organisms.

a. Transfer of nucleic acid fragments from these donor organisms to *E. coli* K12 using vectors that meet the requirements of § 8 para. 2 GenTSV is to be assigned to **containment level 1**, if according to the experimental design it is expected that either no viral nucleic acid fragments or only sub-genomic nucleic acid fragments of the respective viruses will be transferred. The resulting gene bank is to be assigned to **risk group 1**.

b. Transfer of nucleic acid fragments from these donor organisms to *E. coli* K12 using vectors that meet the requirements of § 8 para. 2 GenTSV is to be assigned to **containment level 2**, if according to the experimental design it is expected that complete genomes of the respective viruses will be transferred. The resulting gene bank is to be assigned to **risk group 2** in accordance with the General position statement of the ZKBS on the risk assessment of *E. coli* K12 derivatives with a plasmid containing the (c)DNA of the genome of a replication-competent virus (Ref. 6790-10-89, November 2019).

Reasons:

Primary cells usually do not hold a hazard potential. There could be a low risk due to infection of the cells with the viruses mentioned above. However, only certain human cells including for example hepatocytes or hematopoietic cells are permissive for HIV, HBV or HCV. Since the donors or the cells isolated from them are not tested for these viruses and since the cells are permissive for these viruses or contaminations with blood or secretions or with permissive cells cannot be excluded, these primary cells are to be assigned to **risk group 2** as a precautionary measure.

Nucleic acids fragments isolated from these primary cells are either exclusively of cellular origin or of both cellular and viral origin.

to a. Either exclusively cellular nucleic acid fragments or cellular and sub-genomic viral nucleic acid fragments are transferred. The vector-recipient system corresponds to a biological safety measure according to § 8 para. 1 and 2 GenTSV.

to b. Either exclusively cellular nucleic acid fragments or cellular nucleic acid fragments and viral genomes are transferred. The vector-recipient system corresponds to a biological safety measure according to § 8 para. 1 and 2 GenTSV.

2.4. Primary cells from non-human primates (for exceptions, see Section 2.5) from veterinary-controlled breeds not showing clinical symptoms or primary cells from chiroptera that are not tested for the absence of the lyssaviruses EBLV-1, -2 and BBLV are to be assigned to **risk group 2**.

a. Transfer of nucleic acid fragments from these donor organisms to *E. coli* K12 using vectors that meet the requirements of § 8 para. 2 GenTSV is to be assigned to **containment level 1**, if according to the experimental design it is expected that either no viral nucleic acid fragments or only sub-genomic nucleic acid fragments of the respective viruses will be transferred. The resulting gene bank is to be assigned to **risk group 1**.

b. Transfer of nucleic acid fragments from these donor organisms to *E. coli* K12 using vectors that meet the requirements of § 8 para. 2 GenTSV is to be assigned to the respective containment level in accordance with the General position statement of the ZKBS on the risk assessment of *E. coli* K12 derivatives with a plasmid containing the (c)DNA of the genome of a replication-competent virus (Ref. 6790-10-89, November 2019), if according to the experimental design it is expected that complete genomes of the respective viruses can be transferred.

Reasons:

Primary cells usually do not hold a hazard potential. There could be a low risk due to infection of primary non-human primate cells or untested cells from chiroptera with interspecies transmissible viruses. Therefore, these primary cells are to be assigned to **risk group 2** as a precautionary measure.

Nucleic acids fragments isolated from these primary cells are either exclusively of cellular origin or of both cellular and viral origin.

to a. Either exclusively cellular nucleic acid fragments or cellular and sub-genomic viral nucleic acids fragments are transferred. The vector-recipient system corresponds to a biological safety measure according to § 8 para. 1 and 2 of the GenTSV.

to b. Either exclusively cellular nucleic acid fragments or cellular nucleic acid fragments and viral genomes are transferred. The vector-recipient system corresponds to a biological safety measure according to § 8 para. 1 and 2 GenTSV.

Annotation to the experimental design:

- When cutting the DNA of the donor cell with a restriction enzyme it should be considered that genomes of possibly present DNA viruses (e. g. HBV) can also be cleaved by the same restriction enzyme. Thus, sub-genomic nucleic acid fragments that can be inserted in the vector as well as nucleic acid fragments that cannot be inserted could be generated. If the extra-chromosomal genome of the DNA virus contains no restriction sites for the respective restriction enzyme, transfer of the complete viral genome with the help of the vector is not expected.
- Circular viral DNA genomes (e. g. of polyoma- or papillomaviruses) cut at a single site by a restriction enzyme can be transferred as a complete genome, but the genetic continuity is disrupted at the restriction site (see also General position statement of the ZKBS on the risk assessment of *E. coli* K12 derivatives with a plasmid containing the (c)DNA of the genome of a replication-competent virus (Ref. 6790-10-89, November 2019)). Viral DNA integrated in the host genome (also proviruses) can be completely transferred to the vector via restriction sites in the flanking cellular DNA, if there are no internal restriction sites for the respective restriction enzyme.

- Generation of cDNA banks involves transfer of expression products of genes. In case of a virus infection of the donor cells, it can thus be generally assumed that only sub-genomic nucleic acid fragments will be transferred.
- In case that the expected virus is an RNA virus (e. g. HCV or HIV), the transfer of the cDNA of a complete viral genome cannot be excluded, if the primer used for synthesis of the complementary strand matches sequences at the 3' terminus of the viral genome and allows reverse transcription to proceed over the full length of the viral genome. Fragment lengths of 6 kb or more are only seldomly achieved. In case of cDNA generation from retroviral genomes, production of infectious nucleic acids is not expected, since there are no complete *long terminal repeat* regions.

2.5. Primary cells from rhesus macaques (*Macaca mulatta*) or cynomolgus monkeys (*Macaca fascicularis*) from veterinary-controlled breeds not showing clinical symptoms are to be assigned to **risk group 1**, if they are tested negative for SIV, SRV, STLV and BV. Embryonic stem cells from rhesus macaques (*Macaca mulatta*) or cynomolgus monkeys (*Macaca fascicularis*) isolated from *in vitro* fertilized embryos of macaques from veterinary-controlled breeds that have been tested negative for SIV, SRV, STLV and BV are to be assigned to **risk group 1**. Primary cells from common marmosets (*Callithrix jacchus*) from veterinary-controlled breeds not showing clinical symptoms are also to be assigned to **risk group 1**. Transfer of nucleic acid fragments from these donor organisms to *E. coli* K12 using vectors that meet the requirements of § 8 para. 2 GenTSV is to be assigned to **containment level 1**. The resulting genetically modified organisms are to be assigned to **risk group 1**.

Reasons:

The primary cells and the nucleic acid fragments transferred from them do not hold a hazard potential. The vector-recipient system corresponds to a biological safety measure according to § 8 para. 1 and 2 GenTSV.

2.6. Cell material taken from wild-caught non-human primates requires an individual risk eval uation, but these cells are at least to be assigned to **risk group 2**. If such cells are used as donor organisms, classification is carried out on a case-by-case basis.