Board for Gene Technology

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GUIDELINES ON THE CLASSIFICATION AND CONTAINMENT MEASURES OF GENETICALLY MODIFIED VIRUSES IN CONTAINED USE

Contents

CONSIDERATIONS

These guidelines apply only to the use of genetically modified (GM) animal viruses and viral vectors that fall under the scope of the Gene Technology Act (377/1995). The Government Decree on Gene Technology (928/2004) describes the techniques and methods that produce genetically modified organisms referred to in the Gene Technology Act. Therefore, the guidelines do not apply to the use of natural viruses or the testing or classification of primary cells.

The guidance compiles general recommendations regarding the most commonly used genetically modified viruses. Under Section 9 of the Gene Technology Act, the operator has the duty to obtain information on the properties of the genetically modified organisms they use and conduct a case-specific risk assessment on the use. As a result, although these guidelines provide basic information about the safe use of specific viruses, and partly also other viruses, each operator must use their risk assessment as the basis for selecting the best suited risk management measures for their specific activities based on the characteristics of the used GM viruses.

Familiarise yourself with the general section of the guidelines before moving on to the virus-specific instructions.

CLASSIFICATION OF USE IS BASED ON RISK ASSESSMENT

The classification of the use of genetically modified viruses is based on the risk assessment performed by the operator. Decree 1053/2005 of the Ministry of Social Affairs and Health lays down provisions on the principles of risk assessment, classification and containment measures. The risk assessment is based on the biosafety level (BSL) in accordance with the pathogenicity of the natural virus behind the genetically modified virus or viral vector. Decree 748/2020 of the Ministry of Social Affairs and Health Decree on the Classification of Biological Agents lays down provisions on the biosafety level. The risk assessment also pays attention to genetic modifications and the way in which the modified virus is used. The risk assessment carried out under the valid gene technology regulation may therefore result in a classification of use that differs from the biosafety level.

Genetically modified viruses may be classified as follows based on their pathogenicity:

- Class 1 includes GM viruses that are unlikely to cause illness in humans, animals, or plants.
- Class 2 includes GM viruses that may cause illness in humans, animals, or plants but are not likely to spread among the general population or other populations. Effective prevention or mitigation measure or treatment is usually available.
- Class 3 includes GM viruses that may cause serious illness in humans, animals, or plants and which may spread among the general population or other populations but for which effective prevention or mitigation measures or treatment is usually available.
- Class 4 includes GM viruses that may cause serious illness in humans, animals, or plants and which may cause serious hazards when spreading

among the general population or other populations. No effective prevention or mitigation measures or treatment is available.

In the risk assessment, the genetic modification or the intended type of use of the virus may result in a classification that differs from the general guidelines. In the risk assessment, it is important to identify cases where the characteristics of the used insert may increase the risk. It is particularly crucial to pay attention to changes in the following factors:

- gene activity in the infected cell (transcription)
- oncogenicity of the virus
- toxicity
- development of immune response in the host (interleukins, cytokines)
- host specificity of the virus
- pathogenicity of the virus
- sensitivity of the virus to drugs

The intended type of use of a genetically modified virus influences its risk assessment and classification of use, including factors such as storage, cell culture cultivation, the amount of infectious and replication-competent virus produced, and the infection of laboratory animals. The classification of use is also influenced by the availability of effective vaccines or antiviral drugs and the vaccination coverage of the population.

The infectivity and replicative capacity of the virus, i.e. its capacity to spread and cause adverse reactions in individuals and the population also have an essential impact on the classification of use. In this context, an infective virus refers to a virus capable of penetrating a cell. A virus with replicative capacity refers to a virus exhibiting replicative activity (i.e. whose genome is replicating) in the cell, producing viral particles with infective and replicative capacity. Modified viruses or viral vectors producing only nucleic acid or recombinant protein without replicating itself are not considered replicative viruses. Virus-like particles (VLPs) are not genetically modified organisms as they do not contain viral genomes and are therefore not replicative.

When using genetically modified organisms, particularly viral vectors, there is often a need to consider whether they may revert or recombine into a natural virus. As a result, it is necessary to have knowledge of the replicative capacity and infectivity of the virus stock.

The risk assessment must pay attention to the risk of recombination between the genetically modified virus or viral vector and natural viruses that may be present in stem cells. When working with stem cells, it is recommended to ensure that the cells do not contain the same virus species as the one used in genetic modification. For example, it must be ensured with testing that the cells of human origin are HIV-negative if the intention is to infect them with a lentiviral vector. Whenever using human or primate stem cells, there is also a need to pay attention to occupational safety and health issues related to endogenous pathogens.

Viral behaviour can be observed, for example, by examining the changes caused by the virus, i.e. its cytopathic effects (CPE) in the cell using the immunofluorescence technique or polymerase chain reaction (PCR). When performing PCR testing, it is important to remember that a positive result does not stand as evidence of the

replicative capacity or deficiency of the virus. In the risk assessment, the operator must justify the method used to propose the class of use.

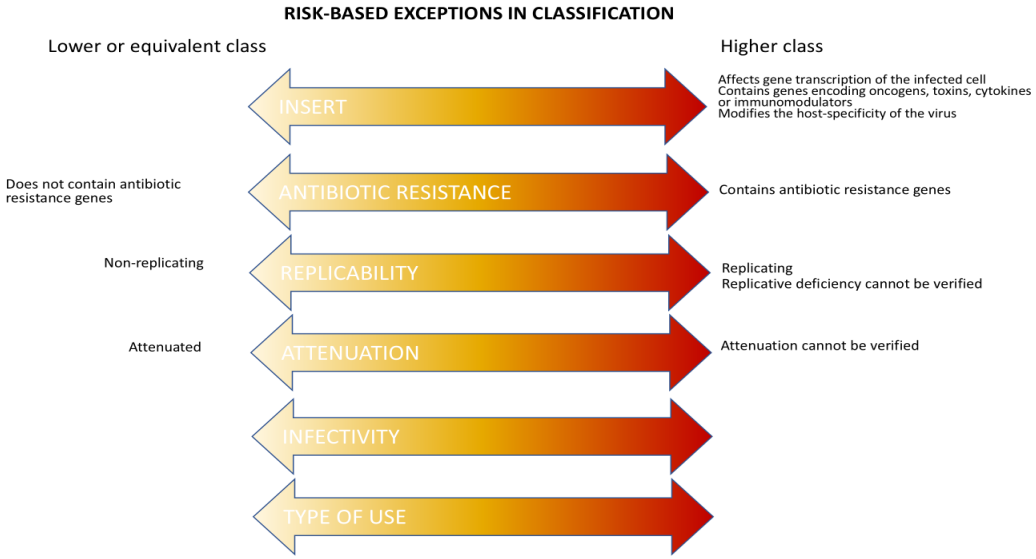

Figure 1: Examples of the factors processed in the risk assessment which may change the primary classification of use for the virus.

GENERAL WORKING PRINCIPLES

In the contained use of a genetically modified virus or virus vector, protective measures of the containment level corresponding to the risk class must be used. The requirements for each containment level are described in the Annex of Decree 1053/2005. The principles of good laboratory practise must also be followed in all classes of use.

To ensure the safety of workers, attention must also be paid to the Government Decree (933/2017) on Protection of Workers from the Dangers of Biological Agents. The Decree covers both natural and genetically modified biological agents, and when working with GM viruses, must therefore be complied with in addition to gene technology regulations. More information about occupational safety and health issues is available on the Occupational Safety and Health Administration and Finnish Institute of Occupational Health websites.

When selecting containment measures, special attention must be paid to whether the genetically modified virus is capable of aerosol transmission. If transmission is possible, there is a need to minimise work phases where aerosol formation may occur. For instance, in tissue homogenization, centrifugation and microscopy, efforts must be taken to prevent the spread of aerosols and droplets, the workers and their environment must be adequately protected, and the decontamination measures used in the work environment should be efficient for the virus in question. Laminar HEPA filters must be replaced at regular intervals.

In the work attention must also be paid to the risk that the genetically modified virus spreads outside the contained use facilities to livestock, recreational animals, pets or wildlife. The workers should avoid regular close contact with animals particularly susceptible to an infection caused by the used viruses. If this is not possible,

containment measures must be implemented to prevent the GM virus from spreading from the contained use premises via workers.

If an effective vaccine is available for the disease caused by the virus, it is worth considering offering it to workers even, if it is not included in the national vaccination programme. More information about the available vaccines can be found in the following sources, with the last-mentioned list being the most comprehensive:

1) WHO/Immunization, Vaccines and Biologicals
   https://www.who.int/teams/immunization-vaccines-and-biologicals
2) WHO/Strategic Advisory Group of Experts on Immunization (SAGE).
   https://www.who.int/groups/strategic-advisory-group-of-experts-on-immunization
3) CDC/Vaccines & Immunizations
   https://www.cdc.gov/vaccines/index.html
4) EMA/Availability of veterinary vaccines
   https://www.ema.europa.eu/en/veterinary-regulatory-overview/research-development-veterinary-medicines/availability-veterinary-vaccines
5) FDA/Vaccines Licensed for Use in the United States
   https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states

Note! If a vaccine licensed by the FDA does not have marketing authorization from the EMA, a special permit should be applied for from FIMEA in Finland.

For more information about viruses and diseases caused by viruses: ”Mikrobiologia, immunologia ja infektiosairaudet 1”. Heikkinen, T., Järvinen, A., Meri, S., Vapalahti, O., Vuopio, J. (eds.). 4th revised edition. Kustannus Oy Duodecim. ISBN 978-951-656-548-7. Printon, Tallinn 2020.

THE USE OF GENETICALLY MODIFIED VIRUSES AND VIRAL VECTORS IN ANIMAL FACILITIES

A contained use notification of a laboratory animal unit or centre does not typically cover genetically modified viruses and viral vectors. As a result, each operator using their facilities is individually responsible for their duty to notify the GM virus work, and they should also discuss the GM activities in advance with the animal research laboratory. Users of GM viruses have the duty to assess the risks of their activities and determine the necessary risk management procedures and possible virus testing. This is typically done in collaboration with the laboratory animal unit. In practice, a good operational model has been that the operator notifies the laboratory animal unit after fulfilling their obligations under the Gene Technology Act, and if necessary, submits the relevant documents and risk assessments to the laboratory animal unit before launching the use of GM viruses there.

An operator using GM viruses must make sure that all workers are aware of the potential risks to humans and animals as well as the required risk management measures. Attention should be paid to the training of animal technicians, and training arrangements should be agreed upon between the operator and the persons in charge at the laboratory animal unit.

THE USE OF GENETICALLY MODIFIED VIRUSES AND VIRAL VECTORS IN A CORE UNIT

The notification or application of contained use must clearly indicate which genetically modified organisms are used in the core unit’s and operator’s premises and which activities will be carried out in each of them. For the use occurring in the core unit premises, the parties must agree on which party is responsible for the

notifications and later notices to the Board for Gene Technology. To avoid misunderstandings, any agreements between the core unit and the operator should be concluded in writing.

When working with genetically modified viruses in the core units, it is necessary to consider that equivalent natural (non-modified) viruses may also be used in the same facilities. Before beginning the GM work, there is a need to discuss the division of duties between the core unit's in-house staff and the operator's workers. It is also necessary to discuss how others working in the facilities will be taken into consideration and how to avoid possible recombination risk if work on natural and genetically modified viruses is simultaneously taking place in the facilities.

VIRUS GROUP SPECIFIC GUIDANCE

Genetically modified viruses were originally developed for studies investigating the gene functions and life cycles of viruses. The obtained information has been used to develop viral vectors, in which case the virus is used as a helper virus (e.g. adenovirus AAV), a part of a vaccine (e.g. adenoviral vector vaccine) or in cancer treatment (e.g. oncolytic virus therapy utilising herpes viruses). The principles for defining the classes of use for genetically modified viruses or viral vectors based on the attributes of natural viruses and the modifications performed on them have previously been described in this guideline.

The virus-specific classifications presented here apply to modifications for which some practical experience has already been accumulated. The guidelines must be applied as necessary, as they do not cover all known virus groups or viruses that have been modified or used as a basis for developing gene vectors, nor virus subtypes or virus variants. In unclear cases, you should contact the Board for Gene Technology and request further instructions.

Adeno-associated viruses (AAV) and other parvoviruses

General

Parvoviruses are small, nonenveloped ssDNA viruses. In the subfamily Parvovirinae of the family Parvoviridae, there are three genera that include viruses capable of infecting humans. The most significant of these include parvovirus (B19) in the *Erythrovirus* genus, adeno-associated viruses (AAV) in the *Dependo* genus and the human bocaviruses 1–4 in the *Bocavirus* genus. Parvoviruses also included major pathogens affecting animals, such as cattle, pigs, dogs, cats, minks and laboratory rodents.

The human parvovirus may cause various diseases such as erythema infectiosum, joint symptoms or haematological complications. Parvovirus infection in pregnant women may lead to stillbirth. A mutation of a few amino acids in the capsid protein may affect the preference of parvoviruses for binding to host cells and tissue tropism. For instance, the canine parvovirus (CPV) that appeared in the 1970s seems to be a mutant of a feline or mink parvovirus.

While AAV is not considered pathogenic to humans, AAV2 was associated with hepatitis in children in 2023. Nevertheless, the cause and effect between the pathogen and the disease remains unclear. AAV may be transmitted via aerosol, the faecal–oral route or directly via the ocular conjunctiva. Currently, there are 13 known AAV serotypes capable of the transfection of divided and differentiated cells of

humans and animal species. AAV vectors are the most common in the clinical use of gene transfer and expression vectors.

AAV is only capable of replication with a helper virus, which is typically an adenovirus or herpes simplex. AAV viruses are relatively stable and may survive for long periods in the environment. In cells, natural AAV primarily operates episomally. In the absence of a helper virus, the AAV provirus may integrate into a specific region of human chromosome 19. AAV-based viral vectors with most of their AAV genome removed have not been demonstrated to integrate into a certain region of the chromosome; instead, the integration is random. Animal studies have shown that there is an integration risk of AAV vectors in the liver of $1x10^{-4}$ copies/ diploid genome (<0.1% vector copies). Due to insertional mutagenesis, AAV vectors cause an elevated risk of cancer, but this is considered rare; hepatocellular carcinoma has been observed in young mice in connection with AAV testing. Animal studies have also revealed an association between high AAV vector levels and inflammatory reactions in the liver and in the dorsal root ganglia when administered locally to the central nervous system.

Biological classification of wild-type viruses

Risk Group 1 when used without an adenovirus or other infective helper virus.

The human parvovirus (B19) Risk Group 2. Animal parvoviruses are also primarily included in Risk Group 2 due to the risk of disease they pose to their target species.

Classification of the use of the GM virus

The use of genetically modified AAVs that do not contain natural adenovirus or other harmful helper viruses may, as a rule, be included in class 1. The risks of genetically modified AAC also depend on the characteristics of the transferred genetic material which may result in a higher class of use. If natural adenovirus is used as the helper virus, the class of use is always at least 2. In other cases, the risks must be assessed based on the used helper virus or gene transfer method.

Other genetically modified parvoviruses are, as a rule, included in class 2. The risk assessment must pay attention to possible changes in the host spectrum or tissue tropism. In their risk assessment, the operator must particularly pay attention to the risk of direct or indirect risk of spread to animals via workers or other pathways.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. If the AAV vector is used in clinical trials, the risk assessment must pay attention to the spread of the vector and possible transmission to nursing staff or other people around the patient after the end of the course of therapy as well as the associated risk of AAV vector integration to the genome of the affected person.

Aerosol formation must be avoided when working with parvoviruses. Pregnant women must not work with human parvovirus B19. The simple capsid structure of parvoviruses is highly resistant to both lipid solvents and pH 3–9 and heat. AAVs are highly stable in various pHs and temperatures and they are also rather resistant to various regular agents used for chemical inactivation. They may be inactivated with, for example, 5% phenol, 1–10% sodium hypochlorite, 2 % glutaraldehyde, heating 10 min for 75 ºC in 0.1% sodium dodecyl sulphate (SDS) and in alkaline solutions with a pH of over 9.

Optimal inactivation agents for CPV include halogen compounds, aldehydes and sodium hydroxide. Higher protein concentrations reduce the efficacy of sodium

hypochlorite and formaldehyde. Temperatures over 100 ºC quickly render CPV inactive.

Adenoviruses (Adenoviridae)

General

Adenoviruses are nonenveloped dsDNA viruses that primarily cause respiratory infections in humans and can sometimes also lead to more severe illnesses. They may be transmitted via aerosol, the faecal–oral route, directly via the ocular conjunctiva or via blood. Adenoviruses can infect both proliferating and dormant cells. Their tissue tropism is dependent on serotype. Adenoviruses rarely integrate into the host cell genome, and as a result, they usually only cause a temporary transgene expression with a duration of 1–2 weeks. Adenoviruses are relatively stable and may survive for long periods of up to 3–8 weeks on surfaces and in aerosols and water.

Adenovirus is one of the most commonly used gene transfer vectors and is highly common in viral vector vaccines. Adenovirus has been used as a vector in vaccine candidates for ebola, rabies, HIV, malaria and tuberculosis, for instance. Adenoviral vectors are also used in oncolytic cancer therapy. Adenoviral vector-based COVID-19 vaccines have been associated with a very rare risk of a coagulation disorder.

Biological classification of wild-type viruses

Risk Group 2

Classification of the use of the GM virus

Adenoviral vectors with significantly reduced pathogenicity compared to natural viruses can be included in the class of use 1. The prerequisite for this is that the virus has been demonstrated to have been sufficiently attenuated and unable to replicate in infected humans or animals. The likelihood of reversion must be low. If the use of genetically modified virus is to be classified into class 1, you must be able to demonstrate for each virus stock that it does not contain replicative viruses, unless this is ruled out by the used production method, such as the PerC6 cell line.

The use of replicative adenoviral vectors, including CRVs (Conditionally Replicating Viruses), is included in the class of use 2.

The risk assessment of GM adenoviruses must pay attention to the possibility of recombination resulting in transfer of gene sequences to related viruses or the genetically modified virus becoming replicative. Recombination may result in infective viruses with replicative capacity, if there are complementing sequences in the cell line used for packaging viruses, in the infected cell line, in the helper virus or in the animal infected with a natural virus.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. Even in class 1 attention should be paid to preventing the formation of aerosols and airborne transmission.

If replication-incompetent viruses are used, a method for measuring viruses with replicative capacity must be applied. Validated methods, such as testing in susceptible, non-complementing cell lines, are suitable for this purpose.

If laboratory animals have been infected with GM viruses, the infective viruses possibly contained in animal secretions must be destroyed using a validated method.

Adenoviruses can be inactivated with, for example 1% hypochlorite, 2% glutaraldehyde and 0.25% SDS. Adenoviruses are also inactivated by disinfectants containing a combination of several active ingredients, such as Barrydin®, which contains quaternary ammonium compounds, guanine derivatives and alkyl polyamine, and Virkon®[1], which contains oxone, sodium dodecylbenzene sulfonate and sulfamic acid. Alcohol (ethanol) is not recommended for inactivating adenovirus, as it requires a relatively long duration of action and loses its efficacy in the presence of organic matter. Isopropanol does not inactivate adenovirus. Adenoviruses may also remain infective in ether and/or chloroform extractions.

Baculoviruses

General

Baculoviruses are dsDNA viruses infecting arthropods used in biotechnical applications, for example, to produce proteins in insect and mammal cells and for insect control. The most commonly used baculoviral vector is Autographa Californica nucleopolyhedrovirus (AcNPV), which infects at least 30 species of butterflies and moths in the *Lepidoptera* order as well as some beetle species with a fatal disease. Some species of the *Autographa* genus (Plusiinae (*Autographa gamma*)) are also found in Finland. Baculoviruses are not harmful to humans.

The rod-shaped viral particle capsid is covered by an envelope originating from the host cell membrane. The viral gp64 envelope protein contributes to the attachment of the virus to the host cell and entry into the cell. Two types of viruses appear in the infected host cell. First, virus particles bud and infect the neighbouring cells. In the late stage of infection, large amounts of polyhedrin protein are produced and combined with viral particles to form occlusion bodies, which are released as the host cells disintegrate. The polyhedrin matrix enables the infection of multiple insects as it improves the resilience of viral particles in the environment and is necessary for a viral invasion in the digestive system of insects.

In addition to infecting insect cell lines, baculovirus also transfects the dividing and non-dividing cells of several mammal species, including humans. However, baculovirus does not replicate in mammalian cells and its expression signals only activate in insect cells. The gene sequence expressed in a baculoviral vector intended for mammalian cell expression is typically inserted downstream of a strong viral promoter that functions in mammalian cells. The baculovirus genome does not insert into the host cell genome.

Classification of the use of wild-type viruses

Risk Group 2 based on the risk to insects. Risk to humans and other animals is non-existent.

Classification of the use of the GM virus

GM baculoviruses that lack polyhedrin or p10 are capable of infecting cell cultures but cannot cause disease in arthropods. When using attenuated baculovirus that

[1] Note! Virkon must currently be collected separately and may not be disposed of down the drain.

does not generate occlusion particles, the virus does not pose a hazard to the arthropods in the environment. In this case, the use can be categorised in class 1. If using less attenuated baculoviruses that may survive and infect hosts if spread into the environment, there is a need for a case-by-case assessment of whether their use should be categorised in class 2.

The potential risks affecting human health of baculoviral vectors are related to the nature of the transgene. A potentially harmful insert, such as a bacterial toxin, oncogene or growth factor may result in a higher classification. It may be necessary to classify GM baculoviruses containing potentially harmful inserts regulated by a promoter in mammalian cells to class 2.

The complement system rapidly inactivates baculovirus, but the virus also triggers innate immune responses in mammals. As a result, exposure to baculoviruses may cause an infection response.

Baculovirus may be pseudotyped with a heterological viral glycoprotein, such as VSV. Pseudotyping increases the ability of the baculovirus to infect mammalian cells and decrease the sensitivity of the virus to the complement. VSVg pseudotyping may also increase the durability of the virus in the environment. Viral tropism can also be altered by inserting a sequence that binds the gp64 protein target cell receptor. The change in tropism must be taken into consideration in the risk assessment.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. When using baculoviruses whose expression signals only operate in insect cells and which have been attenuated by inactivating or deleting polyhedrin, the work can be carried out in containment level 1. Such GM viruses not protected by a polyhedrin matrix are more sensitive than wild-type viruses to heat and ultraviolet light and quick to inactivate in the environment. For example, ethanol can be used for inactivating them. Inactivation of waste is recommended even in this case.

As an occlusion particle will survive for several weeks in nature, polyhedrin producing baculoviruses and any waste containing them must be inactivated in accordance with class 2.

Herpesviruses

General

Herpesviruses are large, enveloped DNA viruses. There are nine human herpesviruses, and many herpesviruses also affect animal species. Infections caused by herpesviruses are rather common and there is a high seroprevalence against most herpes viruses. Herpesviruses are characterised by a lifelong, latent infection that is occasionally reactivated. Typical diseases caused by herpesviruses include labial herpes and genital herpes, varicella and herpes zoster. The infections caused by herpesviruses are easy to diagnose and they can be primarily treated with antiviral drugs. Vaccines are available against some herpesviruses and herpes zoster.

Herpesviruses are modified to investigate the lifecycle of herpesviruses as well as to serve as viral vectors. Herpesvirus-based vectors are commercially available. T-VEC, an oncolytic virus used to treat melanoma, was developed from herpes simplex virus with the removal of the neovirulence gene and immune evasion factors. HSV-

DNA does not integrate into the genome and naturally contains a drug susceptibility gene (thymidine kinase). These are safety factors when using HSV vectors.

Biological classification of wild-type viruses

Risk Group 2

Classification of the use of the GM virus

Class 2 unless it has been demonstrated that the virus is replication incompetent. A plasmid vector (*E. coli* bacterium as host) is not infective and can be included in class 1.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. Even class 1 infectious waste must be inactivated with a verified method. Special caution is required regarding pregnant workers, as an acute herpes virus infection is hazardous to foetal development and infants.

As enveloped viruses, herpesviruses can be easily inactivated with commonly used disinfectants (such as 70% ethanol used to disinfect surfaces and hands), and they are relatively sensitive to drying. However, they can survive for several days, particularly in cold rooms, for example, in a culture medium or other materials with protein content.

As herpesviruses are highly species-specific, it is unlikely that they will spread to other species. Genes related to virus pathogenesis and virulence have typically been removed from modified viruses, which reduces their infectivity and replicative capacity.

Influenza viruses

General

Influenza viruses are enveloped RNA viruses whose genome consists of eight separate RNA genome segments. They are characterised by the reassortment of RNA segments between different virus strains. The virus also undergoes mutations through point mutations. These changes are particularly significant if they affect the viral surface proteins, haemagglutinin and neuraminidase. The same influenza virus subtype may occur in various animals, which may result in the emergence of new virus variants, even a pandemic. Influenza virus occurs seasonally, and a seasonal influenza vaccine is developed against it annually. Effective drugs are available if the infection is diagnosed in the early stages of the infection.

The genetic functions of the influenza virus can be modified by altering various RNA segments that are cloned to plasmids *in vitro*. Recombinant viruses are produced by transfecting all eight RNA segments into the same cell in plasmid form, so that RNA is transcribed from them, functioning like the virus and producing an infective virus.

Classification of wild-type virus

Seasonal influenza Risk Group 2, avian influenza Risk Group 3.

Classification of the use of the GM virus

As a rule, follows the classification of the unmodified virus. A plasmid vector (*E. coli* bacterium as host) is not infective and can be included in the class of use 1.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. Even class 1 GM viral waste must be inactivated with a verified method.

As enveloped viruses, influenza viruses can be easily inactivated with commonly used disinfectants (such as 70% ethanol used to disinfect surfaces and hands), and they are relatively sensitive to drying. However, the viruses can survive for several days, particularly in cold rooms, for example, in a culture medium or other materials with protein content.

When modifying viruses, special attention should be paid to the virus subtypes and their natural capacity to infect different species. The impact of genetic modification on viral pathogenicity must be examined, paying attention to the pandemic potential of the subtype. Due to the high mutability of influenza viruses, special attention must be paid to preventing the GM virus from spreading into the environment.

As an effective vaccine is available against the virus, it must be ensured that workersreceive up-to-date instructions on vaccine availability and that new workers are familiarised with vaccination instructions.

Coronaviruses

General

Coronaviruses are enveloped RNA viruses. Coronaviruses include pathogens affecting both humans and animals. Coronaviruses are rather species-specific, but cross-species transmission has been observed and human coronaviruses are presumed to have originally been transmitted to humans from other animal species. In humans, different coronavirus species cause both mild and severe disease, which affects their classification. Different human coronaviruses also spread in various ways between individuals. Coronaviruses are common and their typical clinical picture involves the common cold. Coronaviruses are prone to mutate. A good example of this is SARS-CoV-2 which caused the COVID-19 pandemic and has dozens of different viral variants. Vaccines and antiviral treatments are available for SARS-CoV-2.

Classification of wild-type virus

The classification is based on the infectivity and pathogenicity of the virus. SARS, SARS-2 and MERS Risk Group 3. Others Risk Group 2.

Classification of the use of the GM virus

SARS, SARS-2 and MERS class 3. Other GM coronaviruses class 2. A plasmid vector-CDNA or viral vector in an *E. coli* host is not infective and can be categorised into the class of use 1.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. Diagnostic samples of the SARS-2 virus may be handled in containment level 2 facilities, but the viruses must be cultured in containment level 3 facilities. Even class 1 GM viral waste must be inactivated with a verifiable method.

As enveloped viruses, it is easy to inactivate coronaviruses with general disinfectants; for example, 70% ethanol can be used to disinfect surfaces and hands.

Coronaviruses are relatively sensitive to drying but they may survive for several days in a culture medium, for example.

When modifying viruses, special attention should be paid to the virus type and its natural infectivity and capacity to infect different species. The impact of genetic modification on viral pathogenicity must be examined, paying attention to the pandemic potential and vaccine response of the type.

As an effective vaccine is available against the SARS-2 virus, it must be ensured that employees receive up-to-date instructions on vaccine availability and that new employees are familiarised with vaccination instructions.

Lentiviruses (HIV, FIV, SIV)

General

Lentiviruses are enveloped RNA viruses that belong to the Retroviridae family. A typical modified lentivirus is HIV-1, which causes a chronic infection that leads to immunodeficiency (AIDS) and death without medication. While the clinical course can be significantly slowed down with medication, the disease is incurable and there is no vaccine for it.

Lentiviruses are commonly used as gene-transfer vectors. Lentivirus-based vectors can infect both dividing and non-dividing cells, with the transgene they carry integrating into the genome to achieve stable gene expression.

The most commonly used lentiviruses include HIV-1-based vectors, but vectors based on HIV-2, SIV (Simian Immunodeficiency Virus) and FIV (Feline Immunodeficiency Virus) are also available. Where applicable, the guidelines for HIV-1 based vectors will be used for them.

Biological classification of wild-type viruses

Risk Group 3.

Classification of the use of the GM virus

Genetically modified HIV and SIV belong to the class of use 3 unless it can be confirmed that their infection capacity and virulence are clearly reduced. For risks posed to animals, FIV is included in class 3. It has minor or non-existent health risks to humans.

Necessary measures

Work with infective HI and SI viruses is always carried out in containment level 3. If the integration of a gene linked to the lentivirus genome into the host cell genome may cause harmful effects, or if the potential effects cannot be reliably assessed, it is important to consider whether enhanced risk management measures are needed to protect against aerosol, droplets and open wounds.

When working with material containing viruses with replicative capacity, you should be prepared to start post-exposure prophylaxis as quickly as possible in case of an accident. It has been shown that timely initation of prophylactic medication reduces the likelihood of HIV infection.

As enveloped viruses, lentiviruses can be easily inactivated with commonly used disinfectants (such as 70% ethanol used to disinfect surfaces and hands), and they are relatively sensitive to drying. However, the virus can survive for several days, for example, in a culture medium or other materials with protein content, especially in a

cold room. Pseudotyping may have a stabilizing effect on the virus, which must be taken into consideration when assessing possible methods of exposure, for instance, when handling specimens containing the virus, including samples collected from infected animals.

When material infected with lentivirus has been inactivated, for example by fixation, so that it no longer contains infective viruses, containment level 1 can be used.

1. HIV-1-based vector systems

Classification of use in various situations

The primary risks in using HIV-1-based vectors involve the formation of viruses with replicative capacity and possible oncogenic effects caused by transgene expression or an expression cassette integration event. The risks are affected by modifications that increase the biosafety of the used vector system and the activity of the protein produced by the sequence inserted into the vector. Risk assessment must pay attention to the effects caused by pseudotyping, such as tissue tropism and stability. There is also a need to pay attention to the target cell or tissue that the virus affects as well as the effect of changes in the surface structures of the virus on the complement activity. If stem cells or patient material are used, it is necessary to ensure that these tissue or cell materials do not contain the HI virus.

Several generations of HIV viral vectors have been developed, and it is recommended to use at least a third-generation plasmid system. In third-generation systems, the transfer plasmid encoding the transgene and the plasmid's packaging proteins are encoded from four separate plasmids that do not contain overlapping sequences. This method improves the biological safety of the use. Such lentivectors are typically so-called SIN (self-inactivating) vectors where the replication of the transfer plasmid has been prevented by removing certain regulatory regions. The lentivirus vector particles are targeted to a specific cell using pseudotyping, in which one of the system plasmids encodes the heterological envelope protein such as the vesicular stomatitis virus glycoprotein (VSV-g).

The infective lentiviral vectors and pseudotyped viral vectors are always included in at least class 2 even if it has been confirmed that the viral vector batch does not contain viruses with replicative capacity. As a rule, material infected with a lentiviral vector, such as a cell line, is categorised under class 2 before it has been shown that the infected material is free from lentivirus particles. After this, you may move on to work in containment level 1. However, it is necessary to use containment level 2 facilities and implement risk management measures, if the integration of a gene carried by the vector into the genome may cause adverse effects, or if these effects cannot be reliably assessed. The transgenes increasing the risks include oncogenes, genes encoding siRNA that inactivates tumour suppressor genes, and toxin genes.

Lentivirus particles can be identified from the viral culture supernatant using, for example, p24- ELISA (antigen test for capsid protein) or the quantitative PCR method (Scherr et al. 2001; https://pubmed.ncbi.nlm.nih.gov/11570495/). Supernatant collected on the day of infection may be used as a positive control and supernatant of an uninfected culture as a negative control. The culture will often only be negative after the third inoculation. Once it has been reliably shown that the culture contains no infective viruses, the use can be categorised into class 1.

Any waste must be inactivated even if it has been demonstrated that the infected material belongs to class 1.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. Since exposure to HIV-based virus through blood, mucous membranes, or broken skin can lead to infection, it is essential to minimize the possibility of wounds, splashes, and aerosols. The protective measures are determined based on the nature of the activities. It is essential to avoid using sharp or easily breakable objects. If this is not possible, workers must be instructed on the safe use of these objects, and the training must be documented. The viruses must not be handled in open containers outside the laminar flow cabinet. Personal protective equipment must be worn as necessary to protect the mouth, nose and eyes from splashes and aerosols. Protective gloves must be worn at work. Workers must not work with class 2 HIV constructs when the skin on their hands is clearly non-intact (e.g. wounds and eczema).

The premises must have access control, or the users and usage times must be recorded. It is also recommended to keep records of the organisms and viral content used in testing. When infecting animals with lentiviral vectors, the measures of containment level 2 must be complied with to minimise the exposure risk of the worker. As the lentivirus does not replicate in rodents, their care can be transferred to containment level 1 facilities a few days after infecting, provided that the infection site is carefully cleaned and the animals are moved to clean cages. However, HIV replication may occur in animals implanted with human tissue or other tissue permissive of HIV. When infecting such animals with lentiviral vectors, the minimum containment level to be applied is 2.

As enveloped viruses, it is easy to inactivate lentiviruses with general disinfectants; for example, 70% ethanol is suitable for disinfecting surfaces and hands. Lentiviruses are relatively sensitive to drying. However, they can survive for several days, particularly in cold rooms, for example in a culture medium or in other protein containing material. Pseudotyping may impact the stability of the virus. This must be taken into consideration when assessing possible exposure routes, for example, when handling samples extracted from infected animals or other samples.

2. **Feline Immunodeficiency Virus (FIV) based vector systems**

Classification of the use of the GM virus

In classifying genetically modified FIV vectors, attention must be paid to both animal disease risks as well as the above guidelines concerning the effect of the pseudotyping of lentiviral vectors and inserts on the classification.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. The safety measures used for pseudotyped FIV vectors that can infect human cells are similar to those for HIV-based vectors. FIV vectors must be produced with three-plasmid co-transfection. It is necessary to demonstrate that the finished viral preparation does not contain a replicative virus also when using FIV vectors.

When using FIV vectors capable of infecting and replicating in felines, the risk assessment must pay special attention to the possibility that the virus would come into contact with a host animal. If you identify in your risk assessment a hazardous

situation that could facilitate the spread of the GM virus, the approaches used and the waste inactivation measures must eliminate the animal disease risk.

Papillomaviridae

General

Papillomaviridae are non-enveloped dsDNA viruses that only infect the epithelial cells of the skin or mucous membranes. In humans, they cause, for instance, skin warts, laryngeal papilloma and genital condyloma. Papillomaviridae also contribute to the onset of several tumours and cancers such as cervical cancer. The human papillomaviridae are not known to infect other animal species.

Papillomaviridae may integrate into the host cell genome, particularly in severe epithelial dysplasia and cancers. To replicate, papillomavirus requires a highly differentiated epithelial cell, which cannot be cultured in vitro. A typical experimental infection is not possible with papillomaviridae. 3D cell culture models enable producing relative amounts of papillomaviridae. Commonly used cell lines, such as HeLa, CaSki, and SiHa, originate from cancers caused by human papillomavirus and contain segments of the papillomavirus genome. However, the virus cannot replicate in these cell lines and they cannot produce infective viral particles.

Biological classification of wild-type viruses

Risk Group 2

Classification of the use of the GM virus

Primarily class 2.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. The replication characteristics of genetically modified papillomaviridae are comparable to wild-type papillomaviridae. Papillomaviridae are highly tissue-specific in immunocompetent individuals, and different virus types occur on the skin and mucous membranes. However, health risks cannot be completely ruled out in situations where the virus ends up in an employee’s body via an ulcer, for example. As a result, people with non-intact skin must not work with papillomavirus particles. Appropriate protective clothing must be worn when working with the viruses and the use of sharp objects must be avoided as far as possible.

Picornaviruses

General

Picornaviruses are small, globular non-enveloped RNA viruses. They are among the most common human pathogens. Well-known picornaviruses include poliovirus, rhinovirus, enterovirus and parechovirus. Picornaviruses have been found in nearly all examined animals. The different types of picornaviruses are well known, and cross-immunity is limited. A picornavirus infection is transient, and the immune system effectively removes the virus from the body. Typical illnesses caused by picornaviruses include the common cold, enteroviral vesicular stomatitis with exanthem and meningitis. There are no drugs or vaccines for picornaviruses with the exception of polioviruses and hepatitis A. Picornaviruses have been tested as therapeutic viruses in cancer treatment. The viruses used for research purposes

include non-modified cell-adapted picornaviruses as well as genetically modified viruses.

Classification of wild-type virus

Risk Group 2

Classification of the use of the GM virus

A picornavirus vector containing the viral genome in the form of cDNA, i.e., as a plasmid, belongs to class 1 because it is non-infectious. It behaves similarly to plasmids and is not expressed naturally in human cells without the presence of appropriate promoters. When such a vector is used for producing a virus or if the genome in the cDNA vector is modified through mutation or components of viruses are swapped, the resulting virus is included in class 2. A virus-like particle (VLP) produced using picornaviruses is not a genetically modified organism as it does not contain the viral genome and it is non-infectious.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. If the picornavirus vector is used in clinical trials, the risk assessment must consider the possible spread of the vector to medical staff or people close to the subject after the treatment period has ended. Picornaviruses have a highly stable simple capsid structure, and the virus can remain infective on surfaces for several days. Enterovirus particles can survive at a very low pH (2.5). While the virus can be inactivated with heat processing (over 56 °C, 30 min), a more reliable method involves treating the materials with Virkon[2], Oxivir or 10 % sodium hypochlorite. Ethanol does not destroy the structure of the picornavirus.

Retroviruses (not including lentiviruses)

General

Retroviruses are enveloped viruses whose genome consists of two positive-strand RNA molecules. The reverse transcriptase enzyme in the virus produces DNA from its viral RNA. The resulting DNA provirus is incorporated into the host cell genome, where it serves as a template for viral mRNA transcription. The long terminal repeats (LTR) that flank the viral genome contain the promoter and enhancer segments (U3), which regulate viral expression. All retroviruses contain the *gag* gene cluster, which encodes structural proteins, the *pol* region, which encodes reverse transcriptase and integrase, and the *env* region, which encodes the envelope glycoproteins. Some retrovirus groups also contain genetic sequences enhancing replication and encoding modifying proteins, which may affect pathogenicity.

Many retroviruses are oncogenic as they may cause insertional mutagenesis or because some host cell genomic DNA has integrated into the RNA genome of the virus. The latter event usually leads to a defective but acutely transfection-capable strain that requires a helper virus for its replication. Retroviruses typically spread through exposure to bodily fluids or via a needle prick. While most of these viruses are highly immunogenic, the host immunity is usually more likely to suppress replication rather than to fully eliminate infection.

[2] Note! Virkon must currently be collected separately and may not be disposed of down the drain.

For laboratory purposes, murine (MiMLV), avian (ALV) and feline (FeLV) retroviruses are used.

Biological classification of wild-type retroviruses

Non-primate retroviruses are typically non-pathogenic to humans, so they are primarily classified in Risk Group 1. If the retrovirus may cause animal diseases, it may be classified into a higher risk group. For example, the Bovine leukaemia virus (BLV) is included in Risk Group 2 and Equine Infectious Anemia Virus (EIAV) in Risk Group 3.

Classification of the use of the GM virus

The primary risks in using retrovirus-based vectors involve the formation of viruses with replicative capacity and possible oncogenic effects caused by transgene expression or an expression cassette integration event. The risks are affected by modifications that increase the biosafety of the used vector system and the nature of the sequence inserted into the vector. The risk assessment must pay attention to the effects caused by pseudotyping (tissue tropism, stability) and the fact that changes in the surface structures of the virus may affect the susceptibility of the virus to a complement. In addition to health risks affecting humans, there is a need to examine the risk of animal disease in a scenario where the genetically modified virus spreads outside the contained-use facility.

The use of a three-plasmid system is recommended when oncogenic retroviral vectors, such as ALV and MoMLV, are used. The host range of originally ecotropic, rodent specific retroviral vectors can be expanded by pseudotyping, which involves replacing an Env protein with another envelope protein. For example, using the vesicular stomatitis virus (VSVg) glycoprotein in pseudotyping results in amphotropic viruses that are infectious to several mammal species, including humans. Xenotropic viruses infect, for example, human cells, but not rodent cells. The use of the SIN (self-inactivating) system reduces the risk of transactivation caused by the transfer plasmid. When producing GM retroviruses, the cell lines used must be packaging cell lines specifically designed to minimise the risk of generating replication-competent viruses through recombination.

Regardless of their replicative capacity, the use of retroviruses that are capable of infecting human cells always belongs at least to class 2. The characteristics of an introduced insert may lead to a higher classification. The genes increasing the risks include oncogenes, genes encoding siRNA that inactivates tumour suppressor genes, and toxin genes.

Working with replication-incompetent ecotropic retroviral vectors (with the exception of vectors based on primate retroviruses) is primarily included in class 1 unless the introduced insert or a risk for animal disease increases the containment level. If the replication-incompetence of the ecotropic retroviruses has not been demonstrated, their class of use is determined based on the animal disease risk, unless the insert affects the classification.

Necessary measures

The activities must comply with the containment level corresponding to the classs of use.

When using amphotropic or xenotropic retroviruses capable of infecting human cells, each recombinant virus batch must be tested using special cell lines developed for

this purpose or some other reliable testing system to exclude replication-competent viruses. Supernatant collected on the day of infection may be used as a positive control and supernatant of an uninfected culture as a negative control. The culture will often only become negative only after the third inoculation.

If the integration of the gene delivered by the retrovirus into the genome may have harmful effects or it is impossible to reliably assess the potential effects, the work must be carried out in compliance with containment level 2 requirements in accordance with the nature of the activities. When working with amphotropic or xenotropic retroviruses, it is essential to avoid using sharp or easily breakable objects. If this is not possible, workers must be instructed on the safe use of these objects and the training must be documented. The viruses may not be handled in open containers outside the laminar flow cabinet. Personal protective equipment must be worn as necessary to protect the mouth, nose and eyes from splashes and aerosols, and protective gloves must be worn when working. The premises must have access control, or the users and usage times must be recorded. It is also recommended to keep records of the organisms and viral content (titers) used in testing.

When the produced ecotropic viral batch has been tested to ensure it does not contain replication-competent viruses, the use is considered to meet the requirements of class 1 contained use. Waste must be inactivated even in this case.

Once it is confirmed that cell lines infected with ecotropic or amphotropic retroviruses cannot produce replication-competent viruses, work with them may be conducted at containment level 1, in accordance with the protective measures required for cell cultures. Waste must be inactivated even in this case.

By lysing or fixing the infected cell lines, work may be carried out at containment level 1 regardless of what the retroviruses used for the infection are like in terms of host-specificity or replication-competence.

When infecting animals with amphotropic or xenotropic retroviral vectors that may also infect human cells, the measures of containment level 2 must be followed to minimise the risk of exposure of workers. The animal may be transferred to containment level 1 premises a few days after infection, if the used retrovirus is incapable of replication in the laboratory animal. In connection with the transfer, the infection site must be carefully cleaned and the animal must be transferred to a clean cage. There is no need to check laboratory animal secretions as they do not contain infective recombinant viruses.

When infecting animals with ecotropic replication-competent retroviruses (excluding primate retroviruses), the containment level in accordance with the animal disease risk applies. If it can be reliably confirmed that replication-competent viruses do not develop in the animals infected with ecotropic retroviruses, the work conducted with them may be carried out in containment level 1.

Retroviruses are sensitive to drying and are quick to inactivate outside the host cell, but they can also survive for long periods in materials with protein content, such as a culture medium. As enveloped viruses, it is easy to inactivate them with general disinfectants (e.g. 70% ethanol for disinfecting surfaces and hands). Virus pseudotyping may impact the stability of the virus, which must be taken into

consideration when assessing possible exposure routes, for example when handling samples extracted from infected animals and other samples.

Sendai virus

General

Sendai virus is a virus of the respirovirus genus in the family *Paramyxoviridae* causing respiratory infections in rodents. Human parainfluenza viruses 1 and 3 belong to the same genus.

Sendai virus is a large, enveloped, 150–250 nm–diameter negative sense, single-stranded RNA virus. Sendai virus is currently known as murine respirovirus, but the previous name is also commonly used. The virus has also been previously known as murine parainfluenza virus type 1 and haemagglutinating virus of Japan (HVJ).

Sendai virus transmits easily via aerosols and respiratory tract secretions, and it is known to cause respiratory tract infections in at least mice, rats, hamsters and guinea pigs. There are known differences in the susceptibility of different mice and rat strains to Sendai virus infections. For example, the C57BL/6 strain of mice is highly resistant, whereas the 129/J and DBA/2J strains are highly sensitive. While Sendai virus infections have been reported to be common in pet mice and rats, the infections of immunocompetent animals usually heal naturally without treatment.

The Sendai virus genome encodes six proteins: nucleocapsid protein NP, phosphoprotein P which is a minor subunit of the RNA polymerase complex, large protein L of the RNA polymerase, hemagglutinin-neuraminidase HN, and Fusion protein F. Sendai virus infects cells via the sialic acid receptor. As the sialic acid receptor is a highly common and conserved cell surface receptor, Sendai virus can infect different animal cells.

While Sendai virus is not known to be pathogenic to humans, it can easily infect human cells in cell culture conditions. Sendai virus does not integrate into the host cell genome and replicates in the cytoplasm using its own RNA polymerase.

Biological classification of wild-type viruses

Risk Group 2

Classification of the use of the GM virus

Sendai virus is considered very safe to humans, and it is not known to have caused any diseases to humans. Sendai virus has been used in the development of vaccines for human pathogens in the family Paramyxoviridae (including RS virus, parainfluenza 1) but also in HIV vaccine development. In particular, modified replication-incompetent Sendai viral vectors are commonly used in producing induced pluripotent stem cells (iPSC).

The cells reprogrammed using Sendai viruses are not genetically modified in themselves as the viruses do not integrate into the host cell genome. Sendai viral vectors may remain in the cells depending on the cell line for up to over 10 passages. PCR or antibody test may be used to demonstrate that the cells no longer contain Sendai virus.

The use of Sendai virus must be taken into consideration when working with laboratory animals, as the virus may cause infertility in addition to pulmonary

changes in rodents and expose them to other infections. Infected animals should not be used in research.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. The formation of aerosols must be avoided when working with the Sendai virus. Sendai viruses lack stability, and no special measures are required for their inactivation. However, due to the very high potential of the virus to spread, strict measures must be imposed to prevent its spread.

Those working with laboratory animals (especially rodents) or who have rodents as pets should not work with Sendai viruses.

A mouse population that suddenly develops respiratory symptoms should always be tested for a Sendai virus infection (SV antigen test). It is worth noting that a positive SV antigen test in rodents other than mice or rats (such as guinea pigs), may be a result of a parainfluenza infection, as guinea pigs in particular are sensitive to human parainfluenza viruses.

Vaccinia viruses

General

The vaccinia virus (VACV or VV) is an enveloped dsDNA virus belonging to the poxvirus family related to cowpox and smallpox viruses. Unlike other DNA viruses, poxviruses do not replicate in the host cell nucleus but rather in the cytoplasm of the host cell. This requires a large genome for encoding various enzymes involved in replication and transcription. During its replication cycle, VV produces four infectious forms which differ in their outer membranes. There are changes in the abundance of the infectious forms and they are believed to play slightly different roles in the spread between hosts, between cells and in the host organism. The vaccinia genome also encodes several proteins that protect the virus against interferons.

A vaccinia infection is mild and typically asymptomatic in healthy humans, although mild skin rash and fever may occur. As the immune reaction caused by the vaccinia virus provides protection against smallpox (Variola), vaccinia has been used as a vaccine against smallpox. The live-virus vaccines have caused complications in people who are immunocompromised and there is varying information about related mortality. After the eradication of smallpox, smallpox vaccines have been only administered to risk groups which may come into contact with the vaccinia virus in healthcare or research, for instance. Safer vaccines have been developed / are available, because the threat of using the smallpox virus for bioterrorism exists.

In addition to the vaccine use, the vaccinia virus has been used as a gene delivery vector in animal and human tissue as well as in gene therapy. The best-characterised strains of vaccinia virus include Western Reserve, Copenhagen, Dryvax (i.e. “Wyeth”; vaccine strain), ACAM2000 (vaccine strain), and Modified Vaccinia Ankara (MVA). The last-mentioned strain is an attenuated (nonvirulent) strain, which may be safer than the others.

Biological classification of wild-type viruses

Risk Group 2

Classification of the use of GM viruses

GM vaccinia viruses derived from the wild-type vaccinia virus (e.g. WR, NYCBOH, Copenhagen, Lister), can be included in class 2.

Highly attenuated strains of the vaccinia virus (e.g. MVA, NYVAC), which are incapable of replication or replicate poorly in mammalian cells and are incapable of causing a progressive infection can be classified in class1.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. Even class 1 GM viral waste must be inactivated with a verified method.

The entry of the infective virus into the digestive tract, non-intact skin or mucous membrane and particularly the eyes via droplets or aerosols must be prevented. Similarly, care must be taken to ensure that the virus does not enter the body through needle pricks. The virus may also spread from the injection sites of animals infected with vaccinia and vaccinated people, their secretions, respiratory secretions and tissues. Contact transmissions from the vaccine site are also possible. Some poxviruses remain stable in the dry form and may spread via objects. These spreading pathways must be eliminated. The work with a virulent virus must be carried out in a laminar flow cabinet and workers must wear eye protection.

Immunocompromised individuals or those with non-intact skin or severe skin rash must not work with the virus.

Zoonotic viruses

General

Zoonoses are diseases transmitted from animals to humans. Viral zoonoses are caused by viruses in several different virus families. The most commonly found zoonotic viruses found in Finland are the Puumala virus (hantavirus), tick-borne encephalitis virus and Sindbis virus. Globally, this group also includes viruses such as dengue viruses, Zika virus and chikungunya viruses. Due to the high number of different zoonotic viruses, they are not separately discussed here. es. Instead, the operator must apply the general guidelines in this section.

Zoonotic diseases are transmitted by animals such as moles, ticks and arthropods. In some cases, this may lead to a transmission from an animal to humans. The infection may be confined to the infected individual or lead to long transmission chains in the population.

Many zoonotic viruses are significant human pathogens, posing a risk of serious illness. The mortality rate of a rabies infection is 100%, but death can be avoided by post-infection vaccination and antibody treatment. Effective vaccines are available for the prevention of yellow fever, Japanese encephalitis and tick-borne encephalitis.

Zoonotic genetically modified viruses are primarily only used in studies.

Classification of wild-type viruses

Puumala virus, tick-borne encephalitis virus, dengue viruses and chikungunya viruses are Risk Group 3 viruses.

Sindbis virus and Zika virus are included in Risk Group 2.

Classification of the use of GM viruses

The classification of replication-competent viruses is primarily based on the natural virus unless its virulence or replication competence has been reduced. This must be confirmed with an appropriate method. The cDNA clone generated from the viral genome does not produce an infective virus in bacteria and its class of use is therefore 1. When using replicon vectors containing no structural genes of the virus, the work can be included in class 1. If the replicons are packaged to virus replicon particles (VRP) by using helper RNA containing the structural proteins, recombination may produce viruses with infection capacity. This must be taken into consideration when determining the class of use. The use of VRPs can be categorised into class 1 if they are produced through methods that minimise the possibility of recombination (such as a three-plasmid system) or if it can be reliably demonstrated that the batch does not contain infectious virus (e.g. with the lack of CPE). The risk assessment must include justifications for what is done to ensure that no recombinants are formed.

When working with replicating vectors, it is essential to pay attention to the possible effect of the used insert on the host cells and organism and the replication characteristics of the virus in them. The protein produced by the virus, such as cytokine or growth factor may increase the rate of viral replication and as a result, lead to unexpected situations. When working with laboratory animals, it is important to determine whether the used genes are also functional in human cells.

Necessary measures

The activities must comply with the containment level corresponding to the class of use. If laboratory animals are infected using replication-competent viruses, facilities and measures in line with containment level 2 or 3 must be applied.

The capability of the virus to spread via aerosols must be taken into consideration and the formation of aerosols must be avoided during work. Falcon tubes with punctured caps to accommodate the homogenizer rod may be used for tissue homogenisation. Closed tubes must be used in centrifugation. Multiwell plates are better suited than culture dishes for microscopy; both should be taped with parafilm already in the viral workspace to make sure they remain sealed. Laminar HEPA filters should be replaced at regular intervals.

Due to the risk of infection, viral waste must be always inactivated regardless of the classification. Liquid waste can be processed where applicable with Virkon[3], Oxivir or hypochlorite. Surfaces and containers may be treated with 70% ethanol or Virkon. Dry waste can be autoclaved, incinerated, or both. The use of disposable materials is recommended. If waste is not inactivated on site, any solid waste must be

[3] Note! Virkon must currently be collected separately and may not be disposed of down the drain.

collected from the viral workspace and put in special containers labelled for infection waste.

The feasibility of vaccinating employees must be assessed in advance, depending on the specific virus.