Influenza A viruses possess a single-stranded, negative-sense, segmented RNA genome. They belong to the family of Orthomyxoviridae and are classified into subtypes based on the antigenic properties of their glycoproteins, haemagglutinin (HA) and neuraminidase (NA). To date, 18 different HA (H1-18) and 11 different NA (N1-11) subtypes are known, which can occur in various combinations (nomenclature HxNy, e.g. H5N1). With the exception of the H17N10 and H18N11 subtypes, all influenza A viruses can be found in birds, which is why these animals are considered to be natural reservoirs [1, 2]. Typically, infections in birds do not produce any severe symptoms. However, individual virus subtypes can cause a generalised infection with high mortality, especially in poultry. Viruses with such properties are referred to as highly pathogenic avian influenza A viruses (HPAIV). HPAIV have so far exclusively been viruses of the subtypes H5 or H7 that exhibit multiple basic amino acids at the cleavage site of the HA. Basic amino acids in the cleavage region make it possible to cleave the HA precursor protein by ubiquitous cellular proteases, thus permitting the systemic spread of the viruses in the body [3, 4]. HPAIV spread rapidly among poultry and cause great economic losses. Transmission occurs via virus-containing aerosols as well as through direct contact between mucous membranes and infectious secretions. Infection of humans by highly pathogenic avian influenza A viruses occurs only sporadically, with some subtypes, in particular some H5N1 strains, potentially causing severe diseases.


a. Avian influenza viruses of the subtypes H5 or H7 with genome sequences coding for multiple basic amino acids at the cleavage site of the haemagglutinin molecule similar to that observed for other HPAI viruses, indicating that the haemagglutinin molecule can be cleaved by a ubiquitous host protease, or

b. Avian influenza viruses with an intravenous pathogenicity index in six-week-old chickens greater than 1.2.

This definition is also used by the World Organisation of Animal Health (OIE, Office International des Epizooties). By contrast, avian viruses that do not meet the above-mentioned criteria are referred to as low pathogenic influenza A viruses (LPAIV). The terms “low pathogenic” and “highly pathogenic” exclusively refer to the pathogenicity of the viruses in birds. For example, although the novel avian virus H7N9 is highly pathogenic in humans, it is clearly classified as a low pathogenic avian virus, because it does not cause any apparent disease in poultry and also does not exhibit multiple basic amino acids in the cleavage region of the HA, as is typical of HPAIV [5].
Position statement of the ZKBS

Safety measures corresponding to containment level 3 are recommended for handling highly pathogenic avian influenza A viruses (HPAIV). The ZKBS deems the criteria listed in Appendix I No. 2 of the Directive 2005/94/EC as being relevant for the classification of an influenza virus strain as HPAIV.

Risk assessment of HPAIV-derived laboratory strains

Influenza viruses show exceptional genetic variability, which is due to their high mutation rate and the ability to undergo genetic re-assortment. Mutations in the genome can arise over a high number of passages, changes in the host organism or altered cell culture conditions and are accompanied by changes in pathogenicity [6, 7]. Re-assortment can also lead to altered pathogenicity compared to the parental strain [8, 9]. The insertion of multiple basic amino acids at the cleavage site of the HA is of exceptional significance for the high pathogenicity of avian influenza viruses in birds. The pathogenicity of HPAIV was demonstrated to be significantly reduced by deleting the basic amino acids in the cleavage region to form a cleavage site that is characteristic of low pathogenic avian influenza A viruses [10]. However, it was also demonstrated that, besides the cleavage site, there are other factors contributing to the high pathogenicity of HPAIV [11]. Recombinant or re-assorted viruses with multiple basic amino acids at the cleavage site were produced in a number of studies, of which only some were shown to be highly pathogenic in poultry [8, 12, 13]. Therefore, both the presence of multiple basic amino acids at the cleavage site of the haemagglutinin and the pathogenicity are decisive for the classification of an avian influenza virus as HPAIV. On the other hand, the classification of an avian influenza virus as LPAIV is not automatically equivalent to the allocation of the virus to risk group 2. For example, since the emergence of the novel avian influenza A virus H7N9, a virus of the subtype H7 has been known which is classified as LPAIV, but is capable of causing a severe disease in humans and has therefore been allocated to risk group 3 [14]. Therefore, avian viruses must be assessed not only with respect to their risk potential for poultry, but also with respect to their risk potential for humans and other mammals. The latter particularly applies if laboratory strains (see comment I) derived from a highly pathogenic avian virus are used.

The assessment of HPAIV and laboratory strains derived from them by the ZKBS is therefore made on the basis of the criteria listed below:

1. Isolates of a generalised avian influenza or untested HPAIV strains according to the definition in Appendix I No. 2 of the Directive 2005/94/EC of 20 December 2005, as donor and recipient organisms for genetic engineering operations, are allocated to risk group 3 in accordance with Sec. 5 (1) of the Genetic Engineering Safety Regulations (GenTSV) in conjunction with the criteria in Appendix I GenTSV.

2. Laboratory strains (see comment I) derived from avian influenza viruses described in no. 1 can be allocated to risk group 2 by the ZKBS if low pathogenicity in poultry has been demonstrated (if one of the criteria listed in a. – d. is met) and the risk potential for humans is assessed as low (see criteria e. – f.):
   a. They are viruses of avian influenza with an intravenous pathogenicity index lower than 1.2 in six-week-old chickens, at an infection dose of $10^6$ plaque forming units (pfu).
   b. They are viruses of avian influenza with an intravenous pathogenicity index greater than 1.2 in six-week-old chickens, infection dose $10^6$ pfu, for which no transmission has been detected between 10 experimentally infected chickens and 6 sentinel chickens. The infected chickens are brought in contact with the sentinel chickens 24 hours
after infection. They are kept in an enclosure with a maximum area of 15 m² and communal food and drink containers. Following the appearance of disease symptoms in the infected animals, the sentinel chickens are monitored for 10 days. Symptomatic infections of the sentinel chickens are measured.

c. They are viruses of avian influenza that, after nasal or intra-tracheal infection of six-week-old chickens, infection dose 10⁶ pfu, show no systemic infection.

d. They are viruses of avian influenza that, after nasal or intra-tracheal infection of six-week-old chickens, infection dose 10⁶ pfu, cause systemic infection, but no transmission has been detected between 10 experimentally infected chickens and 6 sentinel chickens. The infected chickens are brought in contact with the sentinel chickens 24 hours after infection. They are kept in an enclosure with maximum area of 15 m² and communal food and drink containers. Following the appearance of disease symptoms in the infected animals, the sentinel chickens are monitored for 10 days. Symptomatic infections of the sentinel chickens are measured.

If an HPAIV-derived laboratory strain is a virus type which is demonstrated to be capable of causing severe diseases in humans or a virus that has been adapted to a mammal, the following additional criteria must be met for allocation to risk group 2:

e. If the laboratory strain is derived from a virus type which is demonstrated to have already caused severe diseases in humans, clear evidence of attenuation in humans must be provided for the derived laboratory strain. This can be done by testing in a suitable mammalian model. In individual cases, in particular if sequence data makes a strong attenuation seem most likely (e.g. deletion of the NS1 gene), the provision of evidence of attenuation in the animal model can be dispensed with. For laboratory strains that are derived from human pathogenic H5N1 viruses, modification of the HA-encoding sequence is recommended in addition (see comment II).

f. If the laboratory strain is derived from a virus type which has so far not been associated with any severe diseases in humans but has been adapted to a mammal through passaging, it must be checked whether the adaptation leads to an increase in risk potential for humans. Pathogenicity and transmissibility in mammals are important criteria for the assessment of the risk potential; the differences between the employed animal model and the human physiology are to be taken into account. Furthermore, the results of relevant in-vitro tests (e.g. binding affinity test for α-2,6-linked sialic acids, sensitivity to antiviral cellular effectors) and the history of the virus (origin, passaging and any previous use) can be taken into account for the assessment.

Use of HPAIV-derived laboratory strains

Once an HPAIV-derived laboratory strain has been allocated to risk group 2, safety measures corresponding to containment level 2 are recommended for their handling. However, if downgraded strains are used as donor organisms for further genetic engineering operations, it should be considered that a derived laboratory strain may carry virulence markers of an HPAIV (e.g. an HA with multiple basic amino acids in the cleavage region). Therefore, when transferring nucleic acid segments from an HPAIV-derived laboratory strain to other influenza A viruses, particular attention must be paid to check whether the sequence of the segments to be transferred are identical to those of the original HPAIV. If identical nucleic acid segments are transferred, the risk potential of the original HPAIV must be included in the risk assessment.
Therefore, it is recommended that HPAIV-derived laboratory strains be named in the registration or notification of genetic engineering operations. In this case, the mere naming of the subtype is not sufficient for the assessment of the genetic engineering operation.

Comments
I. A laboratory strain is defined as a virus strain that has been adapted to virus replication in chicken eggs, in common cell lines or in a model organism through serial passaging, in exceptional cases also through genetic modifications. The term adaptation is not considered to mean a brief change of host organism for one experiment.

II. Due to their special relevance for both poultry and humans, laboratory strains derived from human pathogenic viruses of the subtype H5N1 cannot be allocated to risk group 2 as long as they carry an HA identical to the wild type.

As a rule, it is recommended that genetic engineering operations with influenza virus A strains of the subtype H5 or H7 be assessed by the ZKBS on a case-by-case basis.

References