

**Recommendation of the ZKBS on the risk assessment of
influenza A virus strains SC35 and SC35M (A/Seal/Massachusetts/1/80)
as donor or recipient organisms according to Article 5 paragraph 1 GenTSV**

The influenza virus strains SC35 and SC35M are derived from the influenza A virus A/Seal/Massachusetts/1/80 (H7N7), which was isolated from a seal during an epidemic among these animals. During the epidemic, which took place between December 1979 and October 1980, over 400 seals died on the New England coast. The animals had symptoms of severe pneumonia with necrotic bronchitis or bronchiolitis and haemorrhagic alveolitis [1]. The isolated influenza virus, however, caused only mild respiratory disease in experimentally infected seals, suggesting the involvement of other factors in the epidemic [1]. A/Seal/Massachusetts/1/80 has a broad host range and was able to replicate in the respiratory tract of experimentally infected pigs, cats, guinea pigs, and ferrets, as well as in the brain of mice. However, the virus did not cause any serious disease in these animals [2]. In several of the investigated individuals who had been shown to be in contact with infected seals, neither respiratory disease was observed nor neutralizing antibodies to the virus were detected. Only isolated cases of partially severe conjunctivitis have been reported, which, however, completely healed [2]. The virus was thought to be of avian origin, but it replicated poorly in experimentally infected chickens, ducks and turkeys [2].

By serial passage of the original isolate on chicken embryo cells (CEC), a virus variant was created with the name SC35 [3]. SC35 proved to be highly pathogenic for chickens and can cause lethal systemic infections. The analysis of hemagglutination (HA) revealed, inter alia, an insertion of three arginines at the cleavage site of the HA precursor protein. This creates a cleavage site that can be cleaved by a large number of cellular proteases. This allows the systemic spread of the virus in the body and is a feature of highly pathogenic avian influenza A viruses (HPAIV). For mice, the variant SC35 is only slightly pathogenic after intranasal administration [4]. In ferrets, the intranasal administration of 10⁶ plaque forming units (pfu) of SC35 resulted in a respiratory illness with fever, with all infected animals surviving the disease [4].

By serial passage of SC35 in mice, a highly virulent strain for mice called SC35M was generated. Infections of mice with SC35M are lethal even at low doses (lethal dose₅₀: 250 - 700 pfu) [4, 8]. After intranasal infection, the virus causes severe, sometimes hemorrhagic, pneumonia. Furthermore, the virus is able to spread systemically in the mouse [5]. At the molecular level, SC35 and SC35M differ essentially in a few point mutations in the genes for the trimeric polymerase complex (PB2, PB1 and PA) and for the nucleoprotein NP [4, 6]. The virus thus still has an HA gene with multiple basic amino acids in the cleavage region. Several mutations seem to be responsible for the high pathogenicity in the mouse. In summary, these lead to increased viral polymerase activity, which is regarded as the cause of the virus's high virulence

in the mouse. [5, 6]. While SC35M and SC35 viruses multiply equally efficiently on chicken embryo cells, SC35M viruses replicate much more efficiently on mammalian cells [6, 7].

SC35M was originally also described as highly pathogenic for chickens. However, this phenotype could not be confirmed with SC35M viruses produced by reverse genetics [7]. It was thought that the original SC35M stock derived from passaging contained quasispecies or still fractions of SC35.

Studies on the pathogenicity of the virus for other mammals are only known for rats. After intracerebral application, the virus proved to be only slightly pathogenic [4]. Furthermore, recent studies suggest that the virus is highly sensitive to the antiviral MxA protein in humans, which is an important factor of the innate immune system. Transgenic mice expressing the human proteins MxA and MxB have a significantly increased resistance to SC35M infections compared to animals without Mx proteins [8].

Assessment

1. According to Article 5 paragraph 1 GenTSV in conjunction with the criteria in Annex I of GenTSV the variant SC35 is assigned to **risk group 3** as donor and recipient organism for genetic engineering operations.

Reasoning: The variant SC35 causes generalised influenza A in birds. It is thus a highly pathogenic avian influenza A virus (HPAIV). These are to be assigned to **risk group 3**.

2. According to Article 5 paragraph 1 GenTSV in conjunction with the criteria in Annexe I of GenTSV the recombinant variant SC35M produced using reverse genetics is assigned to **risk group 2**.

Reasoning: The variant SC35M adapted to the mouse is derived from the HPAIV SC35. However, SC35M viruses produced by reverse genetics proved to be only lowly pathogenic for chickens in several experiments. In mice, the virus causes a severe systemic infection. However, it is very unlikely that the hazard potential for humans is higher than that of an organism of risk group 2. Serious human infections with viruses of subtype H7N7 are extremely rare. The original virus A/Seal/Massachusetts/1/80 also only caused conjunctivitis in some individuals who had contact with infected seals. The high pathogenicity of the virus for the mouse, which was caused by serial passage, seems to be a not uncommon phenomenon for this animal model and has also been described in other, for humans low pathogenic influenza A viruses like A/Puerto Rico/8/34 (PR8) [9]. Furthermore, SC35M is a widely used laboratory strain which has been used in genetic engineering laboratories for several years. Reports of accidental infections with signs of diseases are not known. In addition, recent data indicate that compared to human-adapted viruses, the virus is more sensitive to the antiviral MxA protein, which is part of the human innate immune response. For these reasons, only a low hazard potential for humans is assumed. The virus produced by reverse genetics is therefore assigned to **risk group 2**.

3. According to Article 5 paragraph 1 GenTSV in conjunction with the criteria in Annex I GenTSV SC35M viruses derived from the original passage, not produced by reverse genetics are assigned to **risk group 3** as donor and recipient organisms for genetic engineering operations.

Reasoning: The virus variant SC35M was originally described as highly pathogenic for chickens. However, this phenotype could not be confirmed with SC35M produced by reverse genetics. It cannot be ruled out that virus stocks derived directly from mouse passage may still contain fractions of SC35 or other chicken-pathogenic quasispecies. Therefore, as a precautionary measure, the virus is to be classified as SC35 and therefore assigned to **risk group 3**.

Note:

Genetic engineering operations in which SC35M viruses are produced with alterations to the viral genome must be evaluated by the ZKBS on a case-by-case basis.

References

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