



### **Position statement of the ZKBS on risk assessment of the recipient cell lines COS, 239 and Raji**

When handling the cell lines COS-1, COS-7, 239 and Raji, the catalogue of the American Type Culture Collection (ATCC) recommends "biosafety level 2". According to the regulations of the Genetic Engineering Act and the Genetic Engineering Safety Regulations for risk assessment of these cell lines, the ZKBS takes the following position:

#### **I. COS-1 and COS-7 cell lines**

The establishment of COS cell lines was published in 1981 (2). Here, a CV-1 cell line, an established kidney cell line from Green African Monkeys, which is permissive for SV40 virus replication, was transformed with an SV40 mutant that had a deletion of 6 bp in the replication origin and was thus no longer able to replicate. As a control, wild type SV40 was used, and the CV-1 cells transfected with wild type DNA lysed due to viral replication after ca. 6 weeks. Transfection with the mutants gave rise to three cell populations (COS-1, 3 and 7), of which COS-1 is the only cloned cell population and the only one that could be precisely analyzed. COS-1 carries 2 copies of the defective SV40 mutant genome integrated into the host cell's genome. One copy comprises the complete early region and part of the late region (only VP2), the second copy comprises only the late genes Vp1 and VP2.

All 3 COS cell lines express the SV40 T-Antigen and are permissive for infection and replication of SV40 virus.

Since transfection of the CV-1 cell line involved recombinant SV40 plasmids, according to § 3 of the GenTG, COS cell lines are defined as GMOs.

Normally COS-1 or COS-7 cell lines are used.

#### **Position statement of the ZKBS:**

COS-1 and COS-7 cell lines are, according to § 5 para. 2 in connection with Appendix I part A II No. 2 of the GenTSV, assigned to **risk group 1** and the handling of COS-1 and COS-7 cell lines are assigned to **containment level 1** according to § 7 para. 3 of the GenTSV.

COS-1 and COS-7 cell lines comply with the requirements of § 6 para. 4 GenTSV. In connection with vectors containing the replication origin of SV40 but no late SV40 genes, or the late SV40 genes but no functional SV40 replication origin, and which fulfill the conditions of § 6 para. 5 of the GenTSV, these are considered recognized **biological safety measures**.

#### Reason:

COS-1 and COS-7 cell lines have been used for a long time in many molecular biology laboratories. A reversion of the integrated SV40 mutant to wild type would lyse these cell lines due to lytic replication of wild type SV40. Spontaneous lysis has not been observed. It is therefore assumed that the defect in the mutant SV40 replication origin in COS cell



lines is stable. In COS1 cells the genomes of the SV40 mutants are further characterized by additional deletions. COS cells thus represent established monkey cells that contain defective viral genomes and release no infectious virus particles.

1. Tooze, J., (1981). DNA-Tumor Viruses, CSH NY
2. Gluzman, Y., (1981). Cell 23 , 175 - 182.

## II. 239 cell line

Establishment of the cell line 239 was published in 1977 (2). It involved transforming human embryonal kidney cells with randomly sheared fragments of Adenovirus type 5. The shearing process produced an average size of DNA fragments carrying about one third of the viral genome. One of the few transformed clones obtained led to the establishment of the 239 cell line. DNA analyses revealed that 4 – 5 copies from the left end (12% of the viral genome, E1a, b genes) and 1 copy of the right end (10%, E4 gene) were present in 239 cells. RNA analyses showed that the left end is transcriptionally active. The 239 cell line is permissive for infection with Adenovirus type 5 and other human pathogenic adenoviruses, where the adenoviruses go through a lytic replication cycle.

Since the human embryonal kidney cells were not transfected with recombinant DNA, 239 cells are not considered GMOs according to § 3 of the GenTG.

### Position statement of the ZKBS:

According to § 5 para. 2 in connection with Appendix part B, part All of the GenTSV the 239 cell line is assigned to **risk group 1**.

The 239 cell line fulfills the requirements of § 6 para. 4 of the GenTSV. In connection with vectors that do not contain the late adenoviral genes and fulfill the conditions of § 6 para. 5 of the GenTSV, this represents a recognized **biological safety measure**.

### Reason:

The 239 cell line has been used in many molecular biology laboratories for a long time. If the transfected adenoviral gene regions had recombined to a complete viral genome, the cells would have lysed due to replication of the adenovirus and no cell line would have been established. The DNA fragments of the adenovirus are stably integrated into the cell line's genome. No viral DNA fragments of the middle genomic region, which code for late viral proteins, have ever been identified. Transcripts have only been detected for the left adenoviral genome end, which contains the transforming region. The 239 cell line therefore represents an established human cell line that contains part of a viral genome and releases no infectious virus particles.

1. Tooze, J., (1981). DNA-Tumor Viruses, CSH NY.
2. Graham, F.L., Smiley, J., Russell, W.C. and Nairn, R. (1977). J.Gen.Virol. 36, 59 - 72.

### III. Raji cell line

The establishment of the Raji cell line was published in 1964 (1). It was derived from a Burkitt's lymphoma of an 11 year-old boy. Electron microscope analyses could not identify any virus particles. Molecular biology analyses revealed the presence of a latent Epstein-Barr virus (EBV) genome in the Raji cell line, which contained two deletions at different locations in the genome (2). One of these deletions affects a gene essential for lytic replication (3). Even treatment with TPA, which leads to release of infectious EBV in other cells containing latent EBV, did not result in either an increased replication rate of EBV DNA or the production of infectious EBV in the Raji cell line (4).

#### Position of the ZKBS:

According to § 5 para. 2 in connection with Appendix part B, part All of the GenTSV the Raji cell line is assigned to **risk group 1**.

The Raji cell line fulfills the requirements of § 6 para. 4 of the GenTSV. In connection with vectors that do not contain the EBV DNA fragments deleted in the Raji cell line and fulfill the conditions of § 6 para. 5 of the GenTSV, this represents a recognized **biological safety measure**.

#### Reason:

The Raji cell line has been used in many molecular biology laboratories for a long time. Viral particles or a complete viral genome could not be identified. The Raji cell line therefore represents an established human cell line that contains part of a viral genome and releases no infectious virus particles.

1. Pulvertaft, R.J.V. (1964). Lancet 1, 238.
2. Polack A., Delius, H., Zimber, U. and Bornkamm, G.W. (1984), Virology 133, 146 - 157.
3. Fixman, E.D., Hayward, G.S. and Hayward, S.D. (1992). Journal of Virology 66, 5030 - 5039.
4. Hudewentz, A., Bornkamm, G.W. and zur Hausen, H. (1980), Virology 100, 175 - 178.