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Swiss Expert Committee for Biosafety SECB

SECB Recommendation on

the safe handling of human and animal cells and cell cultures

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1 Purpose and principle

This SECB Recommendation is intended to support users in the assessment of risks for the safe handling of human and animal cells and cell cultures in contained systems. It is based on the provisions of the Ordinance on the Contained Use of Organisms (ContainO)[1] and the Ordinance on Occupational Safety in Biotechnology (OOSB)[2].

Cells as such are generally not infectious. They do not survive if they are injected into a body, nor can they survive in the environment.

The greatest risk when handling cells is their ability to carry and to multiply pathogenic microorganisms (viruses, mycoplasma, bacteria, fungi and parasites) unnoticed. Contamination with pathogenic microorganisms or cross-contamination with other cells can happen relatively easily while working with cells. Therefore, in addition to assessing the original risk of the cells, safe handling while working is also essential for preventing contamination.

2 Criteria for classifying cells into Groups

- The most important viral contaminants are human retroviruses and herpes viruses of humans and other primates. Mycoplasma contaminants originate from cross-contamination with infected cells (up to 35% of continuous cell lines and 1 to 5% of primary cells may be infected).
- The closer the cells are phylogenetically related to humans, the greater the potential risk of contamination (highest to lowest risk: human cells > cells of non-human primates > other mammalian cells, birds > invertebrates[3]). It is therefore inadvisable to use autologous cells (cells taken from oneself)[4],
- The tissue from which the cells originate affects the sensitivity to possible infection (highest to lowest risk: cells from peripheral blood > lymphoid cells > neuronal tissue > endothelium > gut epithelium > epithelium > fibroblasts[3]).
- Characterised cell lines may not always be free of contamination; the trustworthiness of the source should be taken into account.
- A study by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ – German Collection of Microorganisms and Cell Cultures) showed that 14.9% of 550 cells analysed had been incorrectly identified[5]. In many cases, the cells had been misidentified when they were first isolated.
- Continuous cell lines may arise through spontaneous development (CHO, Vero), uncontrolled mutation, be obtained from tumours (Hela), or have been immortalised through transformation with oncogenic viruses[3]. Cells may become neoplastic if accidentally injected.
- Inserts that are transforming or that code for pathogenicity factors or toxic proteins (e.g. oncogenic sequences from immortalisation by oncogenic viruses), or for biologically active molecules that affect the cell cycle (cytokines, hormones, growth factors, antigens), may pose a risk if they are expressed.

A precise risk assessment can only be made once the cells have been identified, well characterised, and tested for possible contamination. The absence of this information should be treated as an uncertainty leading to potential risk (WHO, Laboratory Biosafety Manual)[6].

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1 Ordinance on the contained use of organisms (ContainO), SR 814.912, https://www.fedlex.admin.ch/eli/cc/2012/329/en

2 Ordinance on occupational safety in biotechnology (OOSB), SR 832.321 (in German), https://www.fedlex.admin.ch/eli/cc/1999/445/de.

3 Pauwels K., Herman Ph., Van Vaerenbergh B., Dai Do Thi C., Berghmans L., Waeterloos G., Van Bockstaele D., Dorsch-Häsler K. & Sneyers M., 2007, Animal cell cultures: Risk assessment and biosafety recommendations. Applied Biosafety 12(1): 26–38.

4 Stacey G, Hawkins J. 2017. Cell Lines: Applications and Biosafety, p 299-325. *In* Wooley D, Byers K (ed), *Biological Safety: Principles and Practices, Fifth Edition*. ASM Press, Washington, DC. https://doi.org/10.1128/9781555819637.ch14

5 DSMZ 2003, False leukemia-lymphoma cell lines, https://www.ncbi.nlm.nih.gov/pubmed/12592342

6 WHO, 2004, Laboratory Biosafety Manual. 3rd edition, https://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Instead of obtaining additional information, such as through documentation, testing and characterisation of cells, lack of information may be compensated for by assigning a higher risk category.

Table 1: Classifying cells into groups

Type of cells	Group
Primary cells and cell lines where there is suspicion of contamination with a specific pathogenic microorganism. Human: e.g. cytomegalovirus, Epstein-Barr virus, human herpes 8 virus, human T-cell-leukaemia viruses I and II, hepatitis A virus, Parvovirus B19, West Nile virus or other depending on origin[7]. Primates: Yellow fever virus, Kyasanu Forest virus, Marburg, Ebola, simian haemorrhagic fever virus, rabies virus, hepatitis A virus, polio virus, herpes B virus, SV40, simian immunodeficiency virus, monkey pox virus, simian foamy virus[8] or prions[9]. Rodents: e.g. lymphocytic choriomeningitis virus, Hanta virus.	**Group of the pathogenic microorganism**
Primary cells and cell lines where contamination cannot be ruled out. The risk that primary cells from asymptomatic donors could be contaminated with pathogenic microorganisms is generally presumed to be low[10, 11].	**Group 2**
Primary cells and cell lines where contamination can be ruled out with a high probability. Depending on the origin of the cells the following criteria apply: - Human cells: primary cells derived from clinically healthy individuals if they have been tested for the absence of at least HIV, HCV, and HBV. Additional testing may be necessary, depending on the cell type used and possible risk factors of the donor (e.g. exposure to tropical agents). - Animal cells from SPF (specific pathogen free) suppliers or from animals that can be presumed to be free of epizootic diseases[12] and zoonoses. - Cells from animals that are symptom-free and are being kept under conditions according to the Guidelines of the Federation of European Laboratory Animal Science Associations (FELASA)[13]. - Cells from animals for slaughter and domestic poultry authorised for food production, if the animals' medical history is known and an official veterinarian has checked their health prior to slaughter. The same applies to cells from game (e.g. deer).[14] The DSMZ quality control can also be used as a reference for the absence of pathogenic organisms[15].	**Group 1**

[7] Sicherheit von Blutprodukten bezüglich viraler Infektionen, Niederhauser, Ch., Blutspendedienst SRK Bern (Viral safety of blood products), in pipette 3/2005, https://www.sulm.ch/pipette_magazin/files/pipette/2005-03/2005-03-019.PDF (in German).

[8] Pauwels K., Herman Ph., Van Vaerenbergh B., Dai Do Thi C., Berghmans L., Waeterloos G., Van Bockstaele D., Dorsch-Häsler K. & Sneyers M., 2007, Animal cell cultures: Risk assessment and biosafety recommendations. Applied Biosafety 12(1): 26–38.

[9] Recommendation of the Swiss Expert Committee for Biosafety (EFBS) on the classification of work using prion genes and prion proteins, https://www.efbs.admin.ch/en/recommendations/recommendations-of-the-secb/

[10] Position statement of the ZKBS on classifying genetic engineering operations with primary cells from vertebrates, http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/ZKBS/01_Allgemeine_Stellungnahmen_deutsch/10_Zellbiologie/Primaerzellstellungnahme.pdf?__blob=publicationFile&v=2 (in German)

[11] Belgian Biosafety Server, Animal cell cultures: Risk assessment and biosafety recommendations, https://www.biosafety.be/content/contained-use-animal-cell-cultures-risk-assessment-and-biosafety-recommendations

[12] Ordinance on epizootic diseases, TSV, SR 916.401, https://www.fedlex.admin.ch/eli/cc/1995/3716_3716_3716/de (in German, French or Italian).

[13] Federation of European Laboratory Animal Science Associations (FELASA), Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units, https://doi.org/10.1177/0023677213516312

[14] Verordnung über das Schlachten und die Fleischkontrolle, VFSK, SR 817.190, https://www.fedlex.admin.ch/eli/cc/2017/66/de

[15] DMSZ, Human and Animal Cell Lines, Quality control, https://www.dsmz.de/research/human-and-animal-cell-lines

Type of cells	Group
Cell lines[16, 17, 18] (including primary cells) that are identified and well characterised, and known not to be infected with specific pathogenic microorganisms (certified by the cell bank (DSMZ, ECACC, ATCC etc.) or supplier).	**Group 1**
Identified and well characterised cell lines or primary cells infected with a specific pathogenic microorganism (including deliberate infection).	**Group of the pathogenic microorganism**
Human cells containing gene sequences that could be harmful if expressed (including under extraordinary culture conditions). For example: - genes from tumour viruses responsible for the oncogenic potential of the viruses - genes that are involved to a significant extent in the development of human tumours - genes that transform mammal cells in vitro - genes that produce tumours in animal experiments[19, 20, 21].	**Group 2**
Cells that contain potentially harmful gene sequences but are unable to express them.	**Group 1, if pathogen-free**

[16] ATCC 2007: Cell line authentication test recommendations. ATCC Technical Bulletin 8, https://www.lgcstandards-atcc.org/Services/Testing_Services/Cell_Authentication_Testing_Service/Cell_Line_Authentication_Test_Recommendations.aspx?geo_country=ch

[17] DSMZ, Catalogue of Human and Animal Cell Lines, https://www.dsmz.de/collection/catalogue/human-and-animal-cell-lines/catalogue

[18] ZKBS List of well-characterised cell lines, classified into groups: https://zag.bvl.bund.de/zelllinien/index.jsf;jsessionid=N3t3ygIYN9QJ-pJn6ULRVQs3_XlgUuu16OWB3DdP.subs208?dswid=8943&dsrid=411 (in German)

[19] Recommendation of the ZKBS (2016): Precautionary measures in handling nucleic acids with neoplastic transformation potential: https://www.zkbs-online.de/ZKBS/SharedDocs/Downloads/02_Allgemeine_Stellungnahmen_englisch/10_cell_biology/Nucleic%20acids%20oncogenes%20(updated%202016).pdf?__blob=publicationFile&v=3

[20] SECB Recommendation: Risk assessment of activities with oncogenic and cytokine-encoding sequences, https://www.efbs.admin.ch/en/recommendations/recommendations-of-the-secb/

[21] General position statement of the ZKBS on the risk assessment of the expression of Tat-fusion proteins: https://www.zkbs-online.de/ZKBS/SharedDocs/Downloads/01_Allgemeine%20Stellungnahmen/11_Zellbiologie/Tat-Fusionsprotein_2006.pdf?__blob=publicationFile&v=4

3 Allocation to a class of an activity using cells

General comment: an activity using human or animal cells should only be allocated to Class 1 if there is sufficient information available on the properties of the cells to classify them with certainty as Group 1, and if they are being handled under safe, sterile working practice (Section 4).

Table 2: Allocating activities using cells to classes

Activity	**Class**
Cultures of characterised cells belonging to Groups 1–3 ***Examples:***	**Class allocated according to Group of the cells or the pathogenic microorganism**
- Well characterised primary human tissue cells, which have tested negative for pathogenic microorganisms, cultured with growth factors to produce bone grafts.	Class 1
- Cultivation of cells for purposes of (e.g. biochemical) analysis that (may) contain a Group 3 organism where no attempt is made at explicit multiplication of these organisms	Class 2
- Cultivation of CESS, an established human lymphoblast cell line that contains the entire genome of the Epstein Barr virus (EBV, Group 2) and can produce EBV.	Class 2
- Co-culture of human primary cells (Group 2) that contain cytomegalovirus (Group 2) with SPF pig cells (Group 1).	Class 2
- Culture of primary canine cells (Group 2) infected with canine distemper virus (Group 2).	Class 2
- Culture of ScGT-1, a murine neuroblastoma cell line infected with scrapie (Group 2).	Class 2
- Culture of HuT 102, a human T-lymphocyte cell line able to produce human T-cell lymphotropic virus (HTLV-1, Group 3).	Class 3
Production of viruses or viral vectors in cell cultures[22] ***Examples:***	**Class allocated according to Group of the vectors or virus**
- Production of replication deficient, ecotropic retroviral vectors (Group 1) in established murine cell lines (Group 1).	Class 1
- Production of infectious, replication deficient lentiviral vectors (Group 2) in Group 1 cell lines, if this does not produce native viruses or replication-competent vectors.	Class 2
- Production of VSV-G pseudotyped, replication-incompetent viral vectors containing sequences of the human endogenous retrovirus HERV (Group 3) in established cell lines (Group 1).	Class 3
- Production of HIV in Group 1 cell lines.	Class 3
Culture of cells that are inadequately characterised or where there is a suspicion of infection with pathogenic microorganisms ***Examples:***	**Class 2** **Class 3 (if Group 3 microorganisms are suspected)**
- Culture of various primary human tumour cells (RG2) for analysis of mutations in specific sequences responsible for tumorigenic properties, using DNA hybridisation tests.	Class 2
- Culture of liver carcinoma cells from HBV-seropositive persons.	Class 3

[22] SECB Recommendation: Classification of work with genetically modified viral vectors, https://www.efbs.admin.ch/en/recommendations/recommendations-of-the-secb/

Activity	Class
Use of genetically modified cells[23]	**Class according to assessment of the risk posed by the cells, vectors and inserts**
- Properties of the host cell lines (for hybridomas the properties of both cells should be taken into account), - Vector used for the transformation (plasmids, viral vectors), - Transfer of viral sequences, transfer of virulence factors, - Experiments to activate endogenous retroviruses, - Expression of recombinant gene products, presence of helper viruses. Note should also be taken that particular genes (e.g. tumorigenicity) are only expressed with increasing age (e.g. inserted retroviral sequences), i.e. following a number of passages of a cell culture, or only under specific culture conditions (pH, presence of nutrients or additives). ***Examples:***	
- Expression of non-functional human Interleukin in commercially available, certified insect cell lines (*Spodoptera frugiperda*) that have been transfected with non- infectious, commercially available, well characterised vectors (Baculo virus).	Class 1
- Expression of human growth factors in well characterised Group 1 insect and murine cell lines and established, well characterised Group 1 human cell lines.	Class 1
- Overexpression of DNA repair proteins in well characterised Group 1 primary cells.	Class 1
- Transduction of primary murine neuronal cells from mice that have been kept under FELASA conditions (Group 1) with attenuated pseudorabies vectors (Group 2).	Class 2
- Transfection of primary human Beta-cells from the liver (RG2) with adenoviral vector (Group 2) carrying siRNA for diabetes genes.	Class 2
Handling cells (Group 1) for biochemical studies, without culture	**Observe OOSV provisions on protection against exposure to potentially infectious material**
The handling of cells (including blood and biopsies) which does not include the multiplication of the cells or of any pathogenic micro-organisms present and that does not require the identification of pathogenic microorganisms, does not count as a handling of pathogenic microorganisms and therefore does not fall under the scope of the ContainO. Adherence to the SUVA recommendations for the prevention of infections transferable by blood in medical laboratories is recommended (1997, Order number: 2869/19.d). ***Examples:*** Extraction of proteins from primary human samples to analyse the crystal structure of the proteins.	

[23] Public Health Agency of Canada, 2015, Canadian Biosafety Standards and Guidelines, Second edition: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/second-edition.html

4 Safe, sterile working practice

Contamination of cell cultures with pathogenic microorganisms or cross-contamination with other cells can be significantly reduced or even prevented by adhering to safe, sterile working practices [24].

The contamination of cells while working is due to

- pre-existing contamination of the cells obtained;
- contaminated materials such as cell culture media (e.g. bovine viral diarrhoea virus in bovine serum albumin) or additives;
- cross-contamination with other cells used in the same laboratory and that are known or not known to be contaminated, or through other microbiological or molecular biological work in the same laboratory;
- negligence of the person who handles the cells (documented examples are: failure to wear gloves, talking, sneezing, dusty sleeves inside the biological safety cabinet);
- the air supply or via other environmental routes.

These contaminations can be avoided by applying the following measures[25]:

- observing and monitoring Good Laboratory Practice: using sterile, certified media and consumables, measures of personal hygiene (gloves, lab coats), regular decontamination of work benches and equipment, working in a Class II biological safety cabinet that is tidy and well maintained;
- using master cell banks with care and managing them strategically, dividing cells into master and working cell banks with low passage numbers;
- using as little antibiotics as necessary;
- testing the cell lines used for evidence of contamination, or to confirm the absence of contamination.

Tests to identify the contamination of cell lines can be obtained from most commercial suppliers of cell lines; or they will perform the tests themselves.

In the case of contamination it is recommended that the cell line be disposed of correctly and if possible work should continue using a fresh culture from the Master cell bank. Removing contaminant, e.g. mycoplasma or viruses, requires a great deal of effort or is almost impossible.
Instead of routinely testing cell lines, higher safety measures can also be taken. If however high quality standards and quality programmes are available, it may be possible to carry out the activity at a lower risk class.

[24] WHO 2013, Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks, Annex 3, TRS No 978, https://www.who.int/publications/m/item/animal-cell-culture-trs-no-978-annex3

[25] Corning Incorporated 2017, Understanding and managing cell culture contamination, https://www.chemie-brunschwig.ch/documents/suppliers-information/CLS-AN-020-cell-culture-contamination-guide-A4.pdf

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- WHO, 2020, Laboratory biosafety manual, 4rd Edition, https://www.who.int/publications/i/item/9789240011311.
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