جامعة الملك عبدالله للعلوم والتقنية
King Abdullah University of Science and Technology

Institutional Biosafety and Bioethics Committee

Cell and Tissue Culture Risk Assessment

Guidance Document

IBEC requires a documented risk assessment (RA) be conducted in order to support proposed laboratory work. The RA must be appended to the registration/application paperwork, together with Pathogen Safety Data Sheet (if applicable).

This document provides guidance to Principal Investigators and research personnel on performing a RA when work involves human and animal cell and tissue culture and on mitigating risks that may be identified through the RA process.

RA Considerations

Determination of the appropriate containment level required for the laboratory work is made by reviewing the following considerations:

- ✓ Tumorigenicity/ Oncogenicity (from cells or introduced through laboratory practices; thought to be minimal – only one documented case of tumor development after an accidental needle stick)

- ✓ Presence of Infectious agents (from cells, media or introduced through laboratory practices)

- **Source Material (Biological Origin)**

  The closer the genetic relationship of the cell line to humans, the higher the risk to humans (based on host range and human immunologic response factors following exposures).

Order of risk (descending):

- human autologous & heterologous cells (e.g. exposure to foreign donor cells, cells isolated from the investigator or to pathogens grown in cells from the investigator)
- non-human primate cells
- other mammalian sources
- avian cells
- invertebrate cells

Note: Exception: lymphocytic choriomeningitis virus in rodent cells.

- **Tissue/ Cell type of origin**

Order of risk (descending):

- hematopoietic cells and tissue (blood, lymphoid tissue)
- neural tissue
- endothelium
- gut mucosa
- epithelial and fibroblasts cell

- **Culture type**

Order of risk (descending):

- primary cell cultures
- continuous cell lines (immortalized cells), intensively characterized cells (including human diploid fibroblasts).

Important Notes:

(1): RA in the case of primary human cells should also include information on:

- number of specimens from different individuals
- quantity of cells per specimen
- level of risk represented by the population from which specimens are obtained (*e.g. screened individuals? Hepatitis B - infected individuals?, etc.)*

(2): Cell cultures are shipped by commercial suppliers with paperwork describing containment recommendations (addresses mainly shipping requirements). This information should be used as a starting point for the RA and might need adjustment based on additional information and considerations listed in this document. In addition, information should be sought regarding the Blood Borne Pathogens for which the cells were tested for.

(3): Specify all species from which cells and tissues are obtained. If animals are from specific purpose-bred pathogen free animals and are not expected to contain a large number of pathogens, specify this information in the application and state that good lab practices will be used. If cells and tissues are obtained from wild species, specify common zoonoses (list risk group of pathogens for humans) that might be transmitted and what precautions are going to be taken to minimize risk.

- **Vectors**. The hazards of vectors used with the cell line must also be considered when selecting an appropriate containment level.

  - Cells immortalized with viral agents such as SV-40, Epstein-Barr Virus, Adenovirus or Human Papilloma Virus? Hazards associated with those viruses?
  - Vector is replication incompetent?, competent? or self-inactivating?
  - Tropism? (*e.g. limited tropism; incapable of infecting humans*)
  - Insert gene is: Toxic/non-toxic? Oncogenic? Immune modulatory? Increases viral tropism or pathogenicity?
  - Volumes used? (1-10ml= easy to contain and transport; OR large volumes= higher likelihood of spillage)
  - Procedures: limited to cell culture in a BSC, centrifugation with sealed tubes and safety caps or sealed rotors; OR aerosol producing, injection/administration into animals?
  - Special hazards? (e.g. *in vivo* recombination possibility between existing cell culture virus and latent virus present in the laboratory worker/host)?

- **Media**. Media (animal-derived) used when working with cell lines may have contaminants that may grow when introduced into the cell culture. The most common contaminant is bovine viral diarrhea virus (BVDV).

- **Procedures**. Generation of aerosols and use of needles or other sharps should be evaluated. Alternate, less hazardous methods to conduct research should be sought and encouraged (*e.g. use of plastic "Plasteur" pipettes, use of safety-engineered sharps*). Are procedures employed that might generate splash, sprays?

- **Growing conditions**. Changes in temperature, supplements, or growth surfaces can induce changes in oncogene expression or induce expression of endogenous or adventitious viruses.

- **Bloodborne Pathogens**. Human tissues, human cell lines, including established human cell lines, cannot possibly be tested for all adventitious agents and bloodborne pathogens. BSL-2 practices, procedures and containment must be followed for all activities. Bloodborne pathogens training is a requirement when this type of biological

material is used. Vaccination offering, training, and special additional work practices must be included in the SOPs (as applicable).

Note: Research involving human-derived materials, including established human cell lines require IBEC review and approval BEFORE the work starts.

- **New Technology**. In the NIH Guidelines, that has been adopted by KAUST as its source document, it is specified that "*The NIH Guidelines cannot anticipate every possible situation. Motivation and good judgment are the key essentials to protection of health and the environment. […] The utilization of new genetic manipulation techniques may enable work previously conducted using recombinant means to be accomplished faster, more efficiently, or at larger scale. […] an appropriate risk assessment of experiments involving these techniques must be conducted taking into account the way these approaches may alter the risk assessment. As new techniques develop, the NIH Guidelines should be periodically reviewed to determine whether and how such research should be explicitly addressed*." If you plan to introduce new technology in cell and tissue culturing in your work, discuss it first with KAUST biosafety specialist and IBEC to assess risk and determine feasibility.

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Risk Assessment Sheet

RISK LEVEL

Researchers: use this sheet to complete the risk assessment for your work, based on the information provided in the previous pages.

Cell Source	**High**	**Medium**	**Low**	
Tissue/ Cell Type	High	Medium	Low	
Culture Type	High	Medium	Low	
Culture Media	High	Medium	Low	
Vector	High	Medium	Low	
Procedures	High	Medium	Low	
New Technology	High	Medium	Low	
Other *	High	Medium	Low	
OVERALL RISK				

Note*: Personnel proficiency and experience must also be considered when assessing risk.

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General Guidelines for Working Safely with Cell Lines and Tissue Cultures

Researchers can use this as basic biosafety guidelines that they can update with laboratory specific information when developing their Standard Operating Procedures (SOPs)

- All mandatory safety training must be taken and documented
- Training in aseptic technique must be taken and documented.
- Use of autologous cells is prohibited. Autologous cells, if accidentally self-inoculated, can evade normal immune responses.
- Well characterized cell lines from recognized suppliers, are preferred.
- Read certificates and Safety Data Sheets information provided by vendors with the shipped cell lines.
- Quarantine new cell lines when they are brought into the laboratory. Continue separation until the new culture does not show growth of contaminating agents (*e.g. bacteria, fungi, or mycoplasma*).
- Work with only one cell line at a time.
- Disinfect work area thoroughly before beginning work on a different cell line.
- Rely on good technique rather than on antibiotics to keep cell lines free of contamination.
- Use serum or protein-free media if feasible.
- When preparing media that will be used with more than one cell line, aliquot media into containers labeled separately for each cell line. This will reduce the chance of contamination.
- Avoid creation of aerosols:
    1. Discharge pipettes against the wall of containers to avoid splashes/aerosols.
    2. Do not create aerosols by mixing fluids with a pipette.
    3. Pay special attention when opening rubber-stoppered vessels.
- Use of sharps is strongly discouraged.
- Personal Protective Equipment:
    1. Lab coat - buttoned or tie-back
    2. Eye protection (*e.g. safety glasses; goggles or face shield - to protect against liquid splashes or sprays when work is done outside of BSC*).
    3. Surgical mask – in addition to eye protection when work is conducted outside of the Biological Safety Cabinet (BSC) and ensure protection of the mucous membranes.
    4. Gloves:
        a. lab coat's sleeves and gloves must completely overlap. Double gloving can provide additional protection and use of two contrasting colors will increase visualization of small tears or holes.
        b. Outer contaminated gloves may be removed and replaced.
        c. Remove contaminated gloves carefully and wash hands with mild soap and warm water at the end of the procedure and always before exiting the lab.

- Wash hands after removing gloves, before leaving the laboratory and at any time after handling materials known or suspected to be contaminated. Keep hands away from eyes, nose, mouth in order to avoid potential exposure of the mucous membranes.

- Biosafety Cabinet (refer to laboratory SOP on proper use of BSC):
  - Check certification label (must be current, within one year) and check airflow (verify flow monitor).
  - Use appropriate disinfectant (*e.g. 1:10 household bleach solution*) and wipe the interior, sides and sash of the BSC.
  - Plan ahead and take all needed supplies to the BSC before beginning work. Disinfect them as you place them within the BSC.
  - Delineate areas for clean and dirty materials inside the BSC. Use plastic-backed paper to control small spills and better organize work area.
  - Place a container of an appropriate disinfectant (*e.g. 1:10 bleach solution*) within the BSC for pipette tips or Pasteur pipettes (*encourage the use of "Plasteur" pipettes rather than Pasteur pipettes*). Use of a horizontal collection tray/container is highly recommended. Do not dispose of contaminated materials in a sharps container or other collection container located outside the BSC (will avoid aerosols formation and small drips and spills).
  - Reusable glassware and plasticware must be immediately decontaminated after use.
  - Vacuum lines must be protected with disposable air filters and/or liquid traps (an overflow flask should be used). Integrity of filters and fluid levels in traps must be verified before and after completion of work. Filters and disinfectant must be changed as needed. Filters must be autoclaved before disposal.
  - Label flasks with waste contents and date or use the Cell and Tissue culture label available on KAUST Biosafety website at: https://hse.kaust.edu.sa/Services/Pages/bio.aspx

- Special hazards are encountered during use of flow cytometry or cell sorting. Please refer to the standard published by the Society of Analytical Cytology and for specific recommendations and evaluation of hazards available here: http://isac-net.org/PDFS/d2/d2a43c32-5f89-44d7-adf6-c137d38f81b1.pdf