IBC
APPENDIX N
MAMMALIAN CELL CULTURE RISK ASSESSMENT SOP

PRIMARY REFERENCES

- **Biosafety in Microbiological and Biomedical Laboratories (BMBL)**, 6th Ed., US Dept. of Health and Human Services (CDC and NIH), 2020.
- **Biological Safety: Principles and Practices**, 5th ed. Dawn P. Wooley and Karen B. Byers, editors. ASM Press, Washington, D.C., 2017.

APPLICABILITY

Per well-established biosafety practices and requirements of the Institutional Biosafety Committee (IBC), Principal Investigators (PI's) are required to conduct a risk assessment to support proposed work that is subject to review and approval by the Committee. This SOP provides guidance on how to conduct a risk assessment if you are working with mammalian cell or tissue cultures. Guidance is also provided on how to reduce risks of injury or exposure that might be identified in this process.

RISK ASSESSMENT

There are many hazards to consider when conducting your risk assessment. Two of the highest risks are tumorigenic cell lines or pathogens that are either experimental in nature or introduced into the process from a source such as contaminated media.

Tumorigenicity

While this must be considered, the actual risk is thought to be low because there has only been one documented case of a researcher who developed a tumor due to a needle stick. However, consideration is warranted because even though the likelihood of developing a tumor is low, the consequences of a needle stick could be extreme. This would be a much higher risk proposition if an individual is immuno-compromised.

Species of the Source Cells

The closer the genetic relationship of the cell line to humans, the higher the risk to humans. This concept is based on host range and human immunologic response factors. In decreasing order of risk: human heterologous > non- human primate > other mammalian sources > avian > invertebrate. Exceptions must also be considered, for example: lymphocytic choriomeningitis virus may be present in rodent cells [2] or, rabies virus might infect canine cells, both of which would increase the risk of working with these cells.

Tissue type of origin. In decreasing order of risk: pluripotent stem cells > hematogenous cells and tissue (blood, lymphoid tissue) > neural tissue > endothelium > gut mucosa, epithelial cells > fibroblasts.

Culture type

In decreasing order of risk: whole tissue > primary cell cultures > continuous cell lines (immortalized cells) > intensively characterized cells (including human diploid fibroblasts). Commercial suppliers often provide biocontainment recommendations based solely on characterization of the cells. This recommendation is an initial reference, which may need to be modified based on other risk assessment considerations described here, in addition to IBC review and approval. When manipulating primary human cells, risk assessments should also consider the presence of recombinant or synthetic nucleic acids or vectors (see Appendix U: Biosafety Considerations for Research with Lentiviral Vectors), along with subsequent product, the quantity of cells per specimen, the number of specimens from different individuals, and the level of risk represented by the population from which specimens are obtained.

Media

Cell culture media or supplements derived from humans or animals may contain contaminants. When purchasing media or media supplements, such as animal serum, a reliable vendor is recommended to minimize the occurrence of contaminants. Consult the supplier's Certificate of Analysis to verify testing against toxins, mycoplasma, other viruses (Hepatitis-B, Rabies, etc.) and prions. Always prepare media aseptically, adhering to appropriate administrative and engineering controls [3,4].

Growing conditions

Changes in temperature, supplements, or growth surfaces can induce changes in oncogene expression, induce expression of endogenous viruses, or alter interactions between recombinant virus and endogenous genomic provirus.

Viral Contamination

The presence of viruses and/or viral genetic material in a cell line must also be considered when selecting an appropriate containment level. These contaminants may be found in certain human hepatoma cell lines (contains Hepatitis-B genome), as well as in cells immortalized with viral agents such as SV-40, EBV, Adenovirus, or HPV. Some viral vectors used in transformation studies are replication incompetent (e.g., 3rd and 4th generation Lentivirus). Some vectors, however, may be replication competent (Vaccinia virus, HIV-1, viral orthologs), which present a higher risk. The vector type, as well as any recombinant material contained within, must be considered when evaluating overall risk.

Bloodborne Pathogens

The Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens (BBP) Standard, (29 CFR 1210.1030), applies to PIs and laboratory workers who handle human cell lines. As outlined in Appendix A- Exposure Control Plan for Researchers (URI Biosafety Manual), work with all human cells and organ/tissue cultures must be regarded as containing bloodborne pathogens and are subject to the BBP standard as well as review and approval by the IBC. Cultures included in this category include those that are:

- potentially infectious or contaminated with bloodborne pathogens
- well-established cell lines
- human embryonic stem cells; and
- pluripotent cells and their derivatives.

Experiments using any of these materials may be initiated ***only after*** your IBC protocol registration document has been approved by the IBC.

Handle them using BSL-2 containment conditions, practice universal precautions and always wear gloves when working with them. Annual BBP + Biosafety training is required.

Engineering controls

To reduce the risk of exposure or contamination of your cells, always manipulate your cultures in a certified biosafety cabinet. Guidance on the proper use and maintenance of a biosafety cabinet can be found in the URI Biosafety Manual Appendix C: Safe Use of Biosafety Cabinets.

https://web.uri.edu/ehs/biosafety/

Four instructional videos on proper Biosafety Cabinet use are also available on the EH&S Biosafety web page under the "Resources" heading: (https://web.uri.edu/ehs/biosafety/).

Additionally, approved sharps containers must be used for proper disposal of contaminated needles and other sharps.

Administrative controls

Develop procedures to minimize generation of aerosols when manipulating cultured cells. Evaluate the use of sharps, such as needles, and use alternatives when available. Always follow Safe Work Practices (see below) to minimize the risk of exposure.

Safe Work Practices

The following are recommended best practices and should be observed in addition to other work practices described in your training, the referenced documents, and requirements

imposed by the IBC.

- **NEVER** use autologous cells. Autologous cells can evade normal immune responses if accidentally reintroduced into a host.
- If you are sick, **DO NOT** work with mammalian cell cultures as you could inadvertently contaminate your culture with a human-infectious organism.[5]
- Practice good aseptic technique when working with cells and tissues.
- Wear a clean lab coat when working with cell cultures. Dedicated lab coats that can be left in the culture room are recommended.
- Use well characterized cell lines. Always consider non-human primate cells, blood, neural and lymphoid tissues as potentially hazardous.
- Read all documentation provided with the cells from the supplier, including the certificate of analysis.
- Use serum- free or protein-free media, if feasible, to reduce the risk of contamination.
- Work with only one cell line during a work session to avoid cross-contamination.
- Disinfect work area thoroughly before beginning work on a different cell line.
- Avoid creating bioaerosols:
  - Do not create aerosols by mixing fluids with a pipette (aspirating).
  - Discharge pipettes against the wall of containers to avoid splashes.
  - Special care should be taken when opening rubber-stoppered containers.
- Limit your use of sharps especially when working with bloodborne pathogens.
- Wear appropriate lab attire and Personal Protective Equipment (PPE), as described in your biosafety training and the approved IBC protocol. After each work session, remove PPE carefully and wash hands with soap and warm water. Discard disposable PPE as biohazardous waste. Reusable PPE (e.g., lab coats) should be laundered frequently by a laundry service or decontaminated if grossly contaminated from a spill or splash. Do **not** launder lab coats at home.
- Protect all vacuum lines with disposable in-line HEPA filters and/or liquid traps. Check the integrity of filters and fluid levels in traps before and after each work session.
- Change filters and use approved disinfectant as needed. Filters should be disposed of as biohazardous waste in a clear plastic bag.
- A flat Nalgene tray containing freshly prepared 10% bleach or other approved disinfectant can be used to disinfect pipette tips and serological pipettes in the BSC.

Note: even 10% bleach can cause severe pitting in stainless steel. Always wipe the area next to the tray with several rinses of distilled water at the end of each work session.

- Notify the PI and Biosafety Officer/EH&S immediately of any injuries or possible exposures.
- Familiarize yourself with applicable emergency response procedures, including the use and location of the biohazard spill kit.

Managing Cell Culture Contamination

While contamination itself poses no health risk to lab personnel, it can compromise your results and ruin your research. The following are some simple steps to help you manage contamination. This can be a tedious process, so it is much better to practice good aseptic technique to prevent contamination of your cells in the first place.

1. Autoclave, or disinfect, the contaminated culture and all associated media, then dispose them. If the pH is outside the pH 5-9 range after disinfecting with bleach, collect it in a container and dispose of it as chemical hazardous waste.
2. Thoroughly clean and disinfect all BSCs, incubators, centrifuges, refrigerators, microscope stages and any other equipment, including pipettors, that have been in contact with the contaminated cultures. Use a broad-spectrum disinfectant that has bactericidal, virucidal and fungicidal properties.
3. If these steps do not eliminate the contamination, conduct a gaseous decontamination of the BSCs and incubators that are used for cell culture. Surface decontamination alone may not be sufficient as it is difficult to reach all internal surfaces with liquid disinfectant.
4. All media, media components and other reagents used for the contaminated cell lines must be discarded. Quarantine all other cell lines used within the laboratory and tested for mycoplasma to detect any spread of contamination. You may have to repeat the process.

References

1. Gugel EA, Sanders ME. Needle-stick transmission of human colonic adenocarcinoma. N Eng J Med. 1986;315: 1487. doi: 10.1056/NEJM198612043152314.

2. Dykewicz CA, Dato VM, Fisher-Hoch SP, Howarth MV, Perez-Oronoz GI, Ostroff SM, et al. Lymphocytic choriomeningitis outbreak associated with nude mice in a research institute. JAMA. 1992;267: 1349-1353.

3. Uryvaev LV, Dedova AV, Dedova LV, Ionova KS, Parasjuk NA, Selivanova TK, et al. Contamination of cell cultures with bovine viral diarrhea virus (BVDV). Bull Exp Biol Med. 2012;153: 77-81. doi: 10.1007/s10517-012-1648-1.

4. Nagayama K, Oguma K, Sentsui H. Survey on vertical infection of bovine viral diarrhea virus from fetal bovine sera in the field. J Vet Med Sci. 2015;77: 1531-1534. doi: 10.1292/jvms.14- 0556.

5. Jennings A. Viral contamination of cell cultures. In: Stacey G, Doyle A, Hambleton P, editors. Safety in Cell and Tissue Culture. Dordrecht: Springer Netherlands; 1998. pp. 49-63. doi: 10.1007/978-94-011-4916-7.

6. University of Nebraska-Lincoln: Mammalian Cell and Tissue Culture Biosafety SOP.