

MAMMALIAN CELL AND TISSUE CULTURE BIOSAFETY

Scope

In accordance with recognized biosafety standards and practices (listed below) and the *UNL Biosafety Guidelines*, a Principal Investigator (PI) is required to conduct a risk assessment in support of any proposed work that is subject to review and approval by the Institutional Biosafety Committee (IBC). This SOP provides guidance on conducting a risk assessment when the work involves mammalian cell and tissue cultures. It also provides guidance on reducing risks of injury or exposure that may be identified through this process.

Primary References:

- **Biosafety in Microbiological and Biomedical Laboratories (BMBL)**, 5th ed. U.S. Dept. of Health and Human Services (CDC and NIH), 2009
- **Biological Safety- Principles and Practices**, 5th ed. Dawn P. Wolley and Karn B. Byers, editors. ASM Press, Washington D.C., 2017

Risk Assessment

Following is a summary of considerations during the risk assessment process, which will result in an initial determination of the appropriate containment level to be selected for cell and tissue culture work. In general, potential presence of pathogenic agents and/or tumorigenicity must be considered, whether arising from the cells themselves or introduced through laboratory practices. Potential for pathogenic agents is of utmost concern.

- **Tumorigenicity:** must be considered, but risk is thought to be minimal since there has been only one documented case of a researcher developing a tumor following an accidental needle stick [1]. Although the growth of tumor cells from a different person is unlikely in healthy individuals, as with pathogen exposure risk, anyone with a compromised immune system is at greater risk.
- **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 be kept in mind, for example: lymphocytic choriomeningitis virus in rodent cells [2] or, rabies virus infected canine cells, which would increase the risk.

- **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 will often provide biocontainment recommendations based on characterization of the cells. This recommendation is an initial reference, which may need to be modified based on other risk assessment considerations described herein, in addition to IBC review and approval. When manipulating primary human cells, risk assessments should also consider presence of recombinant or synthetic nucleic acids or 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 have 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.) or 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 used with a cell line must also be considered when selecting an appropriate containment level. These may include certain human hepatoma cell lines (contains Hepatitis-B genome), in addition to 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) standard for Bloodborne Pathogens (BBP) (29 CFR 1210.1030) may apply to PIs and laboratory workers who handle human cell lines and animal cells intentionally infected with Bloodborne pathogens.

As outlined in the UNL Biosafety Guidelines, 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 may be initiated **only after** submission of a completed IBC protocol registry form and approval by the IBC.

Established human cell lines, which are characterized to be free of contamination from human hepatitis viruses, human immunodeficiency viruses, and other recognized bloodborne pathogens, are **not** subject to the BBP standard. However, they must still be handled using BSL-2 containment conditions and universal precautions.

Compliance with the BBP standard requires the PI and employees to enroll in the UNL BBP program and complete required training. For additional information, refer to **UNL's Bloodborne Pathogen Exposure Control Plan** (available on the EHS website).



Non-human primate and other animal cell lines which are known to be or likely infected/contaminated with human microbes or agents classed as bloodborne pathogens, especially hepatitis viruses and HIV, are also subject to the BBP standard and IBC approval prior to initiation of work.

- **Administrative controls.** Procedures should be developed to minimize generation of aerosols when manipulating cultured cells. Use of sharps, such as needles, should also be evaluated, with alternatives available, if possible. Researchers are always encouraged to find alternative methods to conduct their research which allow a decreased risk of injury or exposure. Manipulation of human or animal cells is usually done under BSL-2 containment. For additional guidance on containment levels and procedures, please see the EHS SOP, **Biosafety Containment Levels**, available on the EHS website.
- **Engineering controls.** To reduce the risk of exposure or product contamination, cell cultures should be manipulated in a certified biosafety cabinet. Guidance on the proper use and maintenance of a biosafety cabinet can be found in the EHS SOP **Working in a Biosafety Cabinet**. An instructional video on proper Biosafety Cabinet use is also available on the EHS website (ehs.unl.edu) (Training>Video Resources>Biosafety Cabinets). Additionally, approved sharps containers should be employed for proper disposal of contaminated needles, pipette tips, etc.



NOTE: Use of a 2D or 3D **Bioprinter** for artificially building human tissues from individual cells requires a **separate** risk assessment, specialized training, and containment evaluation, in addition to an approved IBC protocol. Please contact UNL's Biosafety Officer for additional guidance prior to beginning such work.

Safe Practices

The work practices described below are recommended best practices and should be observed in addition to other work practices discussed in training, the referenced documents, and requirements imposed by the IBC.

- **NEVER** use autologous cells. Autologous cells, if accidentally reintroduced to a host, can evade normal immune responses.
- If you are sick, **DO NOT** work with mammalian cell cultures as you could inadvertently contaminate your culture with a human-infectious organism.[5]
- Observe good aseptic technique when working with infected or potentially infected cells and tissues.
- Use well characterized cell lines. Always consider non-human primate cells, blood, neural and lymphoid tissues as potentially hazardous.
- Read all information provided with the cells from the supplier, including any certificates of analysis.
- Use serum- 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 creation of biological aerosols:
 - Do not create aerosols by mixing fluids with a pipette.
 - Discharge pipettes against the wall of containers to avoid splashes.
 - Special attention should be given when opening rubber-stoppered vessels.
- Use of sharps should be limited. Especially when working with bloodborne pathogens.
- Wear appropriate lab attire and Personal Protective Equipment (PPE), as described in biosafety training and the approved IBC protocol. After each work session, remove PPE carefully and wash hands with soap and warm water. Disposable PPE should be handled as biohazardous waste. Reusable PPE (e.g., lab coats) should be laundered periodically or decontaminated if grossly contaminated or a spill or splash has occurred.
- 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 approved disinfectant as needed. Filters should be disposed of as biohazardous waste.

- A container of freshly prepared dilute bleach solution or other approved disinfectant can be used for immediate disinfection of pipette tips and serological pipettes used while working in the biosafety cabinet.
- Decontaminate all reusable glassware and plasticware immediately after use. Guidance on proper decontamination can be found in the EHS SOP, ***Chemical Disinfectants for Biohazardous Materials***, found on the EHS website. Approved disinfectants should always be used according to manufacturer's instructions.
- Notify the PI and Biosafety Officer/EHS immediately of any injuries or possible exposures.
- Familiarize yourself with applicable emergency response procedures, including use and location of the biohazard spill kit.

Guidance to address culture contamination

Although there is not necessarily a risk to workers when cell and tissue cultures become contaminated, it can ruin research and compromise results. The guidance below provides some simple steps to determine the source of contamination and how to eliminate it.

1. Autoclave, or disinfect, the contaminated culture and associated media and dispose of them.
2. Thoroughly clean and disinfect all BSCs, incubators, centrifuges, refrigerators, microscope stages and any other equipment, including pipettors, that may have been in contact with the contaminated cultures. Use of a broad spectrum disinfectant that has bactericidal, virucidal and fungicidal properties is recommended.
3. If these steps do not eliminate the contamination, it is recommended to conduct a gaseous decontamination of the BSCs and incubators associated used for cell culture. Surface decontamination alone may not be sufficient as it is difficult to reach all internal surfaces of these equipment with liquid disinfectant.
4. All media, media components and other reagents used for the contaminated cell lines must be discarded and all other cell lines in use within the laboratory should be quarantined and tested for mycoplasma to detect any spread of contamination.

Other References

1. Gugel EA, Sanders ME. Needle-stick transmission of human colonic adenocarcinoma. *N Engl 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.