Appendix H—Working with Human, Non-Human Primate (NHP), and Other Mammalian Cells and Tissues

As with any other type of laboratory activity, a risk assessment should preface work with eukaryotic cell cultures. Such work is generally considered low-risk, but risk increases when working with human and other primate cell lines and with primary cells from other mammalian species in the laboratory. This standard recognizes that employees in both research and clinical work settings face inherent risks working with human materials. Microbiological and biomedical researchers can minimize or eliminate these risks using a combination of engineering and work practice controls, personal protective clothing, safety equipment, training, medical surveillance, vaccination, signs and labels, and other provisions.

Bloodborne pathogens and risk assessment related to material source and type

Bloodborne pathogens are pathogenic microorganisms present in human blood and other potentially infectious materials (OPIM), which can infect and cause disease in persons who are exposed to blood containing these pathogens. Hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) are the most common examples of such microorganisms. Work with blood and OPIM involves risk of exposure not only to these agents, but also other opportunistic pathogens transmitted primarily by other routes (e.g., contact, droplet, and airborne) that may be present in blood or the sample material at the time it is being handled. For example, *Mycobacterium tuberculosis* may be transmitted via the airborne route and primarily present in human lung tissues, while bacterial species such as Staphylococci may be contact transmitted but present in localized tissues or blood during acute infections. Prions, responsible for spongiform encephalopathies and other diseases, may be more concentrated in neural tissues rather than blood, whereas viral hemorrhagic fever-causing viruses can be considered bloodborne pathogens but are often present in other body fluids, such as urine.[1] Numerous pathogens can be present in human materials and each agent may have a number of different characteristics to consider pertaining to the process of infection. For this reason, a risk assessment must be performed that takes into account material source, type, characteristics, and the procedures being performed with the material.

Working with human, NHP, and other mammalian cell lines may present a risk of exposure to bloodborne pathogens, as widely recognized and documented in research and healthcare settings; guidance on how to respond to potential exposures is available.[2–4] For institutions in the United States, the Occupational Safety and Health Administration (OSHA) has developed a bloodborne

pathogens standard that must be applied to all work with human blood and OPIM, including body fluids, tissues, and primary cell lines.[5]

Tissue Source Each institution should conduct a risk assessment, which can begin by appreciating the tissue source (species origin). The closer the relationship of the material is to humans, the higher the risk since pathogens usually have evolved species-specific requirements. Old World non-human primate (NHP) specimens (i.e., macaques) may contain Macacine herpesvirus (Herpes B) and Simian Immunodeficiency Virus (SIV). This material should always be considered potentially infected and should be handled with strict barrier precautions and with swift occupational responses for potential exposures. Herpes B virus infection in macaques is usually symptom-free, or causes only mild oral lesions, but in humans, the infection can be fatal.[6] Also, consider that some pathogens can cross between species (e.g., influenzas, SARS Co-V, West Nile virus). Working with other (non-human and non-NHP) mammalian, avian, and invertebrate cell lines generally presents lower risks.

Cell or Tissue Type Another important consideration is cell or tissue type and whether there is a hazard associated with the capability of the cell to form tumors (e.g., oncogene expressing). Hematopoietic cells and lymphoid tissues can have tumorigenic potential and therefore have an increased risk for handling. Neural tissues and endothelial cells may be considered to have less risk, but an assessment must determine the probability of whether such cells contain other adventitious agents and take into account the tissue or cell source(s) and parameters related to the history of that source. Epithelial cells and fibroblasts present the lowest risk in terms of cell type and tumorigenic potential.[7]

Culture Type When working with cell lines, the culture type is another important consideration. Primary cell lines are derived by sampling directly from *in vivo* organ and tissue samples and have a higher risk of containing undetected pathogens. Therefore, these culture types have shorter lifespans of unknown characterization and present a higher potential risk while culturing. Continuous cell lines (i.e., cells immortalized with viral agents such as EBV, SV-40, or other viral agents) have been modified to grow for extended passages, perhaps even indefinitely. Continuous cultures can usually be more characterized with PCR and cytometric analyses; however, cells carrying viral genomic material still can pose increased risks in the event of inadvertent exposures, particularly for immune-compromised individuals.[8] There has been a report of tumor development from an accidental needlestick injury.[9] Permissive cell lines that support viral replication may have a heightened risk of contamination with viral pathogens. Well-established, and possibly even tested, cell lines are generally considered safer, but the possibility of adventitious contamination by an

unspecified pathogen during use must be considered during the risk assessment process, and measures must be taken to lower the risk of contamination.[10]

Additional Considerations When conducting a risk assessment, consider if endogenous pathogens are present in the specimen or if the pathogens have been added intentionally. Another key consideration is if agents may have been added as a result of passaging of the line in animal model systems. Experimentally infected cell lines should be handled following safety recommendations for both potential endogenous pathogens and known pathogens added in the course of research. Any cell line with known endogenous pathogens should be handled following the safety recommendations for those pathogens. Risk assessment should also consider if any recombinant materials are expressed by the cell line and whether the cell line is a type that supports viral replication. Consult with the Institutional Biosafety Committee, or equivalent resource, when working with recombinant or synthetic nucleic acids in cell lines.[11] Helpful guidelines exist to increase awareness of the problems encountered when working with cells in biomedical research and how to address them effectively.[12]

Risk Mitigation

At a minimum, human and other primate cells should be treated as potentially infectious and handled using BSL-2 practices, engineering controls, and facilities.[13] The use of a biological safety cabinet (BSC) for culturing activities is the universally accepted best practice. Higher containment must be considered for cell lines harboring Risk Group 3 and 4 pathogens as indicated by the risk assessment; higher containment must be considered if the agents present become airborne when energy is imparted on the biological sample. Personal protective equipment (PPE) such as laboratory coats, gloves, and eye protection should be worn in tissue culture laboratories and additional PPE should be added as indicated by risk assessment. All waste culture material must be decontaminated before disposal. All laboratory staff working with human and NHP cells and tissues should be enrolled in an occupational medical program specific for bloodborne pathogens, and staff should work under the policies and guidelines established by their institution's Exposure Control Plan (ECP).

Please refer to Section II for additional information about the risk assessment process and risk mitigation.

References

1. Kuhn JH, Clawson AN, Radoshitzky SR, Wahl-Jensen V, Bavari S, Jahrling PB. Viral Hemorrhagic Fevers: History and Definitions. In: Singh SK, Ruzek D, editors. Viral Hemorrhagic Fevers. Boca Raton (FL): CRC Press; 2013. p. 3–13.

2. Siegel JD, Rhinehart E, Jackson M, Chiarello L; Healthcare Infection Control Practices Advisory Committee. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health Care Settings. Am J Infect Control. 2007;35(10):S65–S164.
3. Kuhar DT, Henderson DK, Struble KA, Heneine W, Thomas V, Cheever LW, et al. Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to Human Immunodeficiency Virus and Recommendations for Postexposure Prophylaxis. Infect Control Hosp Epidemiol. 2013;34(9):875–92. Erratum in: Infect Control Hosp Epidemiol. 2013;34(11):1238.
4. US Public Health Service. Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis. MMWR Recomm Rep. 2001;50(RR-11):1–52.
5. Bloodborne pathogens, 29 C.F.R. Part 1910.1030 (1992).
6. NASPHV; Centers for Disease Control and Prevention; Council of State and Territorial Epidemiologists; American Veterinary Medical Association. Compendium of measures to prevent disease associated with animals in public settings, 2009: National Association of State Public Health Veterinarians, Inc. (NASPHV). MMWR Recomm Rep. 2009;58(RR-5):1–21.
7. Pauwels K, Herman P, Van Vaerenbergh B, Dai Do Thi C, Berghmans L, Waeterloos G, et al. Animal Cell Cultures: Risk Assessment and Biosafety Recommendations. Apple Biosaf. 2007;12(1):26–38.
8. Caputo JL. Safety Procedures. In: Freshney RI, Freshney MG, editors. Culture of Immortalized Cells. New York: Wiley-Liss; 1996. p. 25–51.
9. Gugel EA, Sanders ME. Needle-stick transmission of human colonic adenocarcinoma [letter]. N Engl J Med. 1986;315(23):1487.
10. McGarrity GJ. Spread and control of mycoplasmal infection of cell culture. In Vitro. 1976;12(9):643–8.
11. National Institutes of Health. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). Bethesda (MD): National Institutes of Health, Office of Science Policy; 2019.
12. Geraghty RJ, Capes-Davis A, Davis JM, Downward J, Freshney RI, Knezevic I, et al. Guidelines for the use of cell lines in biomedical research. Br J Cancer. 2014;111(6):1021–46.
13. United States Department of Labor [Internet]. Washington (DC): Occupational Safety and Health Administration; c1994 [cited 2019 April 10]. Applicability of 1910.1030 to establish human cell lines. Available from: https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=INTERPRETATIONS&p_id=21519

Appendix I—Guidelines for Work with Toxins of Biological Origin

Biological toxins encompass a vast range of peptides, small molecules, and macromolecular proteins that cause disease by interfering with biological processes. As their name suggests, biological toxins reside between traditional definitions of biological and chemical agents. They are produced by living organisms, are unable to replicate, and do not result in communicable diseases. The production of novel or existing toxins by synthetic means is becoming increasingly accessible.[1,2] Many biological toxins have been evolutionarily optimized to rapidly disrupt critical biological functions at low concentrations. Their extraordinary, highly specific toxicity is mediated through a diverse set of mechanisms, including enzymatic activity against critical cellular targets, blockade of membrane ion channels and receptors, and perturbation of essential cellular functions. The remarkable combination of specificity and potency has resulted in the widespread use of diverse biological toxins for clinical and research purposes, including botulinum neurotoxins, tetrodotoxin, conotoxins, scorpion toxins, snake venom toxins, and immunotoxins. Because laboratory workers in a wide range of medical and scientific disciplines are likely to encounter biological toxins at some point during their career, it is critically important that laboratory workers understand and are able to assess the risks associated with their use.

Laboratory workers can be exposed to biological toxins through a variety of routes, including inhalation of powders, aerosols, or volatile substances; ingestion; injection; and absorption through dermal, mucosal or ocular tissues. Many biological toxins are highly potent, and internalization of even relatively low doses may result in death or severe incapacitation. Consequently, it is critically important for those working with biological toxins to understand and implement appropriate laboratory safety principles. A number of principles for the safe use of many toxins commonly encountered in the clinical or research environment are summarized below, including for those biological toxins regulated by the Federal Select Agent Program as Select Toxins (see below).

General Considerations for Toxin Use

The primary risks during laboratory use of biological toxins result from accidental injection, absorption through skin or mucous membranes, inhalation, and ingestion. Laboratory work with most toxins in amounts routinely employed in the biomedical sciences can be performed safely with minimal risk to the worker and negligible risk to the surrounding community. Under most circumstances, toxins can be handled using established general guidelines for toxic or highly-toxic chemicals with the incorporation of additional safety and security measures