# IBC
# Appendix Q
# GUIDANCE ON THE USE OF HUMAN, PRIMATE, AND MAMMALIAN CELL LINES

**Objective**
To ensure the safety of those working with human, primate, or mammalian cell lines. Even in the absence of overt contamination, these materials may contain adventitious viruses and/or other opportunistic pathogens or zoonotic agents that pose a risk to lab personnel.

**Rationale**
Human or primate cell lines may harbor viruses, bacteria, or parasites characterized as human bloodborne pathogens. For example, these pathogens can include human immunodeficiency virus (HIV), hepatitis B or C viruses, Neisseria, Treponema, or Plasmodium.

Please note that some bio-resource organizations, such as American Type Culture Collection, do not typically screen their material for bloodborne pathogens. Consequently, the handling of human and primate cell lines must conform to the OSHA Bloodborne Pathogens (BBP) Standard. Generally, this includes annual training with information on hazard communication, engineering controls, work practices, PPE, housekeeping, regulated medical waste, a written exposure control plan, and access to the hepatitis B vaccine. See the OSHA letter of interpretation for applicability of the BBP Standard with human cell lines.

Since it is extremely difficult to screen for every pathogen, all human, primate, and mammalian cell lines must be handled with Standard (Universal) Precautions - treat them as though they are contaminated with infectious agents and utilize Biosafety Level 2 (BSL-2) practices and procedures (defined below). These cell lines include, but are not restricted to those:

- Obtained from an outside source (e.g., repositories such as the American Type Culture Collection, other institutions, and investigators)
- Established within a laboratory without a complete history (passages, attempts to infect, sources of nutrient media, are all unknown)
- Contaminated with an infectious agent
- Derived from genetically manipulated cells capable of supporting the replication of infectious agents
- Previously exposed to viruses containing recombinant DNA or RNA

BSL-2 Practices and Procedures
Containment practices and procedures at BSL-2:

- Restricted access to the laboratory
- Activities within the laboratory are supervised by a competent and knowledgeable scientist
- Hand washing after contact with materials, after removing gloves, before eating, etc.
- Routine decontamination of surfaces and equipment with a disinfectant known to decontaminate agents likely to be present in the cell line - ensure adequate contact

time. Refer to the IBC Manual Appendix I - Disinfectants for Biohazardous Materials.

- Handle all waste as regulated medical waste (see Disposal section below)
- Minimize the creation of aerosols, droplets, and splashes - if this is unavoidable, use an engineering control such as a certified biosafety cabinet to conduct manipulations. Also utilize a biosafety cabinet when handling concentrated or large volumes (e.g., >500 mL)
- Use needles only when absolutely necessary. Consider the use of Safety Engineered Sharps systems or blunt tip needles (e.g., Harvard Apparatus)
- Suitable personal protective equipment –
  - Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. It is recommended that laboratory clothing not be taken home.
  - Eye and face protection (goggles, mask, face shield or other splatter guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials (e.g., chemicals) when the biological materials are handled outside the BSC or containment device.
- Safe Operating Procedures for higher risk procedures

Additional detail about BSL-2 practices and containment can be found in Section IV of *Biosafety in Microbiological and Biomedical Laboratories*, or in Appendix G-II-B of the NIH Guidelines for Research Involving Recombinant DNA Molecules.

**Authorization for Use**

You must utilize BSL-2 practices and procedures when working with cell lines regardless of how cell lines are regulated under the NIH Guidelines for Research Involving Recombinant DNA Molecules (i.e., Exempt, Non- exempt). The use of human and primate cell lines requires that the principal investigator submit a protocol to the Institutional Biosafety Committee (IBC) for review and approval. The IBC may require additional practices, controls, and containment depending on the nature of the cell lines or laboratory activities.

The IBC will evaluate each IBC protocol for the risks an individual well-established cell line may pose, the procedures and activities that will be used with the cell lines, and the skill level and experience of the research staff. The IBC may recommend that personnel fully observe the BBP Standard (e.g., annual BBP training, exposure control plan, access to hepatitis B vaccine). Alternatively, the IBC will require that researchers utilize BSL-2 practices and procedures at all times, satisfy the training points outlined above, and treat any human cell line as potentially contaminated with an infectious agent (even if ATCC, for example, recommends handling at BSL1).

For information on procedures regarding implanting human, primate, or other mammalian cells into rodents, refer to the IBC Manual Appendix R- Animal Biosafety Level Determination Matrix.

**Training**

All individuals working with any human, primate, or mammalian cell lines must receive adequate training. Individuals must complete the Bloodborne Pathogens and Biosafety training provided by URI EH&S. Training is required annually. Additionally, the principal investigator must provide or ensure that personnel receive appropriate orientation and specific training for the safe performance of the work. This training must include:

- Communication about the potential hazards of working with cell lines
- Work practices, and instruction on engineering controls used to minimize exposure
- Suitable personal protective equipment
- Familiarity with laboratory Safe Operating Procedures and the approved IBC protocol
- What to do in case of exposure or accidental release of contaminated materials (see Personnel Exposures and Accidental Spills sections below

**Disposal**

Human, primate, and mammalian cell lines and items in contact with these materials must be disposed as Regulated Medical Waste to increase the safety of individuals who must handle the waste, as well as comply with the Rhode Island Medical Waste Regulations (Regulation 250-RICR-140-15-1). Briefly:

- Segregate disposable solid waste (e.g., intact plastic ware, gloves, paper) in red biohazard bags inside biohazard waste boxes
- Dispose of sharps waste (e.g., syringes, needles, Pasteur pipettes, broken glass, microscope slides and coverslips) in sharps disposal containers
- Bags and containers must be disposed via URI EH&S Guidelines. Regulated medical waste must not enter the normal solid waste stream
- Decontaminate liquid wastes with disinfectant (e.g., household bleach to a final dilution of 1:10 for 30-60 min), or in an autoclave, and dispose in the sanitary drain followed with water.

For more detailed information, refer to the IBC Manual Appendix H - Managing Biohazardous Waste SOP.

**Accidental Spills**

- Don suitable personal protective equipment (PPE) such as gloves and lab coat. Wear PPE to prevent mucous membrane exposure (face mask and safety glasses) if the spill is large (beyond what could be handled with a few paper towels) or if you anticipate splashing
- Remove any sharps such as broken glass with a secondary device (e.g., tongs, forceps) and discard in a sharps disposal container

- Cover spill with absorbent material (e.g., paper towels, gelling substances) and add disinfectant (e.g., freshly prepared 10% bleach). Alternatively, wipe up gross contamination and add disinfectant to the "cleaned" surface. With either approach, allow adequate contact time for the disinfectant (at least 5-
- 10 min, depending on disinfectant used), and perform a second application of disinfectant
- Segregate spill cleanup materials into red biohazard bags or sharps disposal containers. Refer to section above on Disposal
- Remove PPE, discard in red biohazard bag, and wash hands with soap and water

For more detailed information, refer to the IBC Manual Appendix J - Biohazard Spill Management Plan SOP.

**Personnel Exposures**

The consequences of exposure and appropriate post-exposure treatments are not well defined. Thus, the emphasis should be placed on prevention. Follow safe work practices as described in the IBC Manual Appendix B - Standard and Special Microbiological Practices; use engineering controls and practice Standard (Universal) Precautions to prevent lab acquired infections.

- Personnel who sustain an exposure to non-intact skin or mucous membranes from any cell culture fluids should wash the affected areas with soap and water. Use an eye wash to rinse exposures in the mucous membranes
- If the exposure results in a puncture wound, encourage bleeding under running water and perform necessary first aid
- Notify a supervisor and seek medical evaluation.
- Complete the URI Incident/Injury Report Form.

**References**

1. American Type Culture Collection, I have just received a human tumor cell line. What are the biohazards associated with this line? How should I work with it? https://www.atcc.org/support/product-technical-support/faqs/biohazards-associated-with-human-tumor-cells.
2. CDC/NIH. 2020. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. Working with Human, NHP and Other Mammalian Cells and Tissues.
3. College of Veterinary Medicine, Medical Waste Disposal, accessed 7 June 2019.
4. New York State Department of Environmental Conservation, Guidance for Regulated Medical Waste Treatment, Storage, Containment, Transport and Disposal.
5. New York State Department of Health, Managing Regulated Medical Waste.
6. Pauweis, K., et al., 2007. Animal cell culture: Risk assessment and biosafety recommendations. Applied Biosafety, 12, 26-38
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