Objective
Human, primate, or mammalian cell lines, even in the absence of overt contamination, may contain adventitious viruses and/or other opportunistic pathogens or zoonotic agents. Since it is extremely difficult to screen for every pathogen, all human, primate, and mammalian cell lines must be handled with standard precautions- treat 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 or synthetic nucleic acid molecules

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
- Handwashing 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
- Handle all waste as regulated medical waste
- 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- at a minimum gloves and lab coat are recommended
- Standard 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 and Synthetic Nucleic Acid Molecules. The Institutional Biosafety Committee (IBC) may require additional practices, controls, and containment depending on the nature of the cell lines or laboratory activities.

You must utilize BSL-2 practices and procedures regardless of how cell lines are regulated under the NIH Guidelines for Research Involving Recombinant Synthetic Nucleic Acid Molecules (i.e., exempt, non-exempt). Additionally, the use of human, primate, and mammalian cell lines requires that the principal investigator complete the biohazard section of the Memorandum of Understanding (MUA).
Training
All individuals working with any human, primate, and mammalian cell lines must receive adequate training. 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 to minimize exposure
- Suitable personal protective equipment
- Familiarity with laboratory Standard Operating Procedures, and the content of the MUA filed with the IBC
- What to do in case of personal exposure or accidental release of contaminated materials (see Personal Exposures and Accidental Spills below)

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, 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, regulate medical waste, a written exposure control plan, and access to the hepatitis B vaccine. See the following OSHA letter of interpretation for applicability of the BBP Standard with human cell lines.

The IBC will evaluate each MUA for the risks an individual well-established cell line may pose, 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 OSHA 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). Contact Environmental Health & Safety (255-8200) for information regarding OSHA training.

*** Please note that this adaptable performance option does not exist for work with primary cells. ***

Disposal
Human, primate, and mammalian cell lines and items in contact with these cell lines must be disposed as Regulated Medical Waste to increase the safety of individuals who must handle the waste, as well as comply with regulations promulgated by the New York State Departments of Environmental Conservation and Health. Briefly:

- Segregate disposable solid waste (e.g., intact plasticware, gloves, paper) in red biohazard bags
- Dispose of sharps waste (e.g., syringes, needles, Pasteur pipettes, broken glass) in sharps disposal containers
- Bags and containers must be disposed via the Veterinary College Regulated Medical Waste program. 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
Accidental Spills

- Don suitable personal protective equipment (PPE) such as gloves and lab coat. Wear mucous membrane protection (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.

Personal Exposures

The consequences of exposure and appropriate post-exposure treatments are not well defined. Thus, the emphasis should be placed on prevention.

- Personnel who sustain an exposure to 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 at Cornell Health, Occupational Medicine (255-6960).
- Complete a Cornell University Injury/Illness report.

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? [http://www.atcc.org/support/faqs](http://www.atcc.org/support/faqs)
2. American Type Culture Collection, Are ATCC human cell lines tested for viruses such as Epstein-Barr (EBV) virus, human immunodeficiency virus (HIV, AIDS virus), human T cell leukemia (HTLV), and hepatitis B virus? Are ATCC cell lines tested for bovine viral diarrhea virus (BVDV)? [http://www.atcc.org/support/faqs](http://www.atcc.org/support/faqs)
3. CDC/NIH. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. Working with Human, NHP and Other Mammalian Cells and Tissues.
4. College of Veterinary Medicine, Medical Waste Disposal