

# University of Denver Institutional Biosafety Committee (IBC)

## Guidance on the Use of Human, Primate, Mammalian, and Insect Cells

### Objective

Human, primate, mammalian, or insect cells or cell lines may contain adventitious viruses, opportunistic pathogens, or zoonotic agents. Based on their source, these cells or cell lines must be handled with standard **BSL-1** or **BSL-2** practices and procedures (defined below). Laboratories at the University of Denver are not currently equipped to work with **BSL-3** (or higher) agents. Working with BSL-3 (or higher) materials requires IBC approval of protocols and facilities prior to initiation of studies.

The principal investigator (PI) is responsible for initially evaluating the biosafety level of each individual primary cell culture or cell line using the general guidelines outlined below. Those items in BSL-1 are not known to cause disease in healthy adult humans. Materials in BSL-2 present a moderate risk and should be handled under BSL-2 practices and procedures.

### Primary Cell Culture

- Primary cultures of insect or mammalian cells from a source known to **NOT** contain infectious agents should be treated using **BSL-1** practices and procedures (see below).
- Primary cultures of insect or mammalian cells from a source where infection status is **UNKNOWN** (or known to be infected) must be treated as though they are contaminated with infectious agents and utilize **BSL-2** practices and procedures (see below).

### Cell lines

- Human, primate, mammalian, or insect cell lines from a source shown to **NOT** contain infectious agents should be treated using **BSL-1** practices and procedures.
- Human, primate, mammalian, or insect cell lines from a source from a source where infection status is **UNKNOWN** (or known to be infected) must be treated as though they are contaminated with infectious agents and utilize **BSL-2** practices and procedures.
- When in doubt, all human, primate, mammalian, or insect cell lines obtained from an outside source (e.g. repositories such as the American Type Culture Collection [1], other institutions, or investigators) must be treated using biosafety levels used by that source (for example, if ATCC recommends handling the cell line at a particular biosafety level).

### Laboratory Biosafety Level Criteria

For detailed descriptions of BSL-1 and BSL-2 practices and procedures, also see Section IV of *Biosafety in Microbiological and Biomedical Laboratories* [2] or Appendix G-II-A and G-II-B of the NIH *Guidelines for Research Involving Recombinant DNA Molecules* [3]. The IBC Committee may require additional practices, controls, and containment depending on the nature of the primary cell culture, cell lines, or laboratory activities.

### Containment practices and procedures at BSL-1:

- *Working in an annually certified BSL-1 biosafety cabinet is highly recommended.*
- The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
- The laboratory supervisor must ensure that laboratory personnel receive appropriate training in standard aseptic technique (see Training below).
- Persons must wash hands after contact with materials and before leaving the laboratory.
- Surfaces and equipment must routinely be decontaminated with a disinfectant known to decontaminate agents potentially present in primary cell cultures or cell lines.
- Procedures must be performed to minimize the generation of aerosols, droplets, and splashes.

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- Precautions must be taken with sharp objects.
- Suitable personal protective equipment (gloves at a minimum) is recommended.
- All potentially infectious material must be decontaminated using an effective method prior to disposal (see Disposal below).
- Decontaminated waste may enter the normal waste stream.

## **Containment practices and procedures at BSL-2:**

- *Working in an annually certified BSL-2 biosafety cabinet is required.*
- The laboratory supervisor must restrict access to the laboratory.
- A competent scientist must supervise all activities in the laboratory.
- The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special aseptic technique before working with BSL-2 agents (see Training below).
- Persons must wash hands after contact with materials and before leaving the laboratory.
- Surfaces and equipment must routinely be decontaminated with a disinfectant known to decontaminate agents potentially present in primary cell cultures or cell lines.
- Procedures must be performed to minimize the generation of aerosols, droplets, and splashes.
- Precautions must be taken with sharp objects. Use needles **only** when absolutely necessary.
- Suitable personal protective equipment (at a minimum gloves and a lab coat) is recommended.
- All potentially infectious material must be decontaminated using an effective method prior to disposal.
- Standard Operating Procedures (SOPs) should be developed and provided to IBC for higher risk procedures.
- Persons working with BSL-2 agents must be provided with the opportunity for medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory. When appropriate, a baseline serum sample should be stored.
- All waste must be treated as regulated medical waste (see Disposal below).

## **Training**

All persons working with human, primate, mammalian, or insect cells or cell lines must receive adequate training. The PI must provide or ensure that personnel receive appropriate laboratory orientation and specific training for the safe performance of the work. The PI must also ensure that all personnel also enroll in appropriate training sessions available through the Environmental Health and Safety Department (<http://www.du.edu/ehs/>).

At a minimum, laboratory orientation and training must include:

- Information about the potential hazards of working with primary cell culture or cell lines.
- Instruction on standard containment practices and procedures for work at BSL-1 and/or BSL-2 (as appropriate).
- Instruction in appropriate work practices, aseptic technique, and engineering controls to minimize exposure.
- Suitable personal protective equipment (at a minimum gloves, a lab coat, and eye protection).
- Familiarity with all laboratory SOPs and the content of the IBC protocol submitted to the IBC.
- Instruction in how to deal with accidental spills or personal exposure to contaminated material.
- Personnel must receive annual updates or additional training when procedural or policy changes occur.

The IBC will evaluate each protocol for the risks an individual cell or cell line may pose, procedures and activities that will be used with the cells or cell lines, and the skill level and experience of the research staff. Bearing this in mind, the IBC may require that researchers take additional precautions. For example, because human or primate cell lines may harbor viruses, bacteria, or parasites characterized as human blood borne pathogens [4], laboratory personnel may be required to fully observe the OSHA Blood

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borne Pathogens Standard (e.g. annual OSHA training, exposure control plan, access to hepatitis B vaccine). Alternatively, the IBC may require that researchers utilize BSL-2 practices and procedures at all times, satisfy the training points outlined above, and treat any cell or cell line as potentially contaminated with an infectious agent (even if ATCC, for example, recommends handling at BSL-1). Contact Environmental Health & Safety (303-871-7501) for information regarding OSHA training.

### Disposal

#### *Disposal of BSL-1 Waste*

Those cells and cell lines in BSL-1 and items that come in contact with these cells may be disposed of in the normal waste stream following appropriate treatment. Briefly:

- Decontaminate disposable solid waste (e.g. intact plastic ware, gloves, paper) in an appropriate disinfectant (e.g. a freshly prepared solution of 10% household bleach for 60 min.).
- Alternatively, segregate disposable solid waste into clear autoclavable plastic bags and decontaminate by autoclaving.
- Dispose of decontaminated solid waste in the normal solid waste stream.
- Dispose of sharps waste (e.g. syringes, needles, Pasteur pipettes, broken glass) in sharps disposal containers.
- Decontaminate liquid wastes with disinfectant (e.g. a 1/10 dilution of household bleach for 60 min.), or in an autoclave, and dispose of in a sanitary drain followed by excess water.

#### *Disposal of BSL-2 Waste*

Those cells and cell lines in BSL-2 and items that come in contact with these cells must be disposed of as Regulated Medical Waste to increase the safety of individuals who must handle the waste, as well as to comply with regulations promulgated by the Colorado Department of Public Health and Environment. Briefly:

- Segregate disposable solid waste (e.g. intact plastic ware, gloves, paper) into 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 of via an approved Regulated Medical Waste transporter and treatment facility. Contact Environmental Health & Safety (303-871-7501) for more information.
- Regulated medical waste must not enter the normal solid waste stream.
- Decontaminate liquid wastes with disinfectant (e.g. a 1/10 dilution of household bleach for 60 min.), or in an autoclave, and dispose of in a sanitary drain followed by excess water.

### Accidental Spills

For both BSL-1 and BSL-2 spills:

- Wear 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 container.
- Cover spills with absorbent material (e.g. paper towels, gelling substances) and add disinfectant (e.g. freshly prepared solution of 10% household bleach). Alternatively, wipe up gross contamination and add disinfectant (for at least 5-10 min., depending on disinfectant used), and perform a second application of disinfectant.
- Appropriately dispose of cleanup materials (see Disposal above).

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- Remove PPE, discard appropriately, and WASH HANDS immediately with soap and water.
- Complete an IBC Biological Hazard Spill report. Spills and exposure incidents must be reported by the PI and submitted to ORSP Research Compliance and BSO.
  - Please include:
    - Date of incident
    - IBC protocol number
    - Nature and description of incident
    - Estimated amount of spill/exposure
    - Personnel involved

Actions taken immediately following the incident

## **Personal Exposure**

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 (BSL-1 or BSL-2) 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 the DU Health and Counseling Center (303-871-2205). Complete an IBC Biological Hazard Spill report and (if necessary) a DU Injury 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/FrequentlyAskedQuestions/tabid/469/Default.aspx#4>, accessed 7 June 2008.
2. CDC/NIH. 2007. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. [Working with Human, NHP and Other Mammalian Cells and Tissues.](#)
3. National Institutes of Health (U.S.) (2002). NIH guidelines for research involving recombinant DNA molecules (NIH guidelines). Retrieved December 19, 2006, from the National Institutes of Health, Office of Biotechnology Activities web site: [http://www4.od.nih.gov/oba/rac/guidelines\\_02/NIH\\_Gdlnes\\_Ink\\_2002z.pdf](http://www4.od.nih.gov/oba/rac/guidelines_02/NIH_Gdlnes_Ink_2002z.pdf)
4. 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/FrequentlyAskedQuestions/tabid/469/Default.aspx#5>, accessed 7 June 2008.