

Vanderbilt University Institutional Biosafety Committee (IBC) Policy: Biosafety Best Practices for Use of Human-Derived Materials & Bloodborne Pathogens in Basic Research Applications

This document has been developed and implemented in support of the requirements of the Occupational Safety and Health Administration (OSHA)'s Bloodborne Pathogens (BBP) Standard codified at [29 CFR 1910.1030](#), specifically as they relate to the basic research use of **human blood and other potentially infectious materials (OPIM)** as defined by that rule. In conjunction with Vanderbilt University's [Bloodborne Pathogens Exposure Control Plan \(ECP\)](#), this policy addresses all exposure control aspects necessary to minimize exposure risk to materials regarded as potentially infectious to humans. Bloodborne Pathogens include, but are not limited to, hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV).

Bloodborne Pathogens–Risk Materials & Exposure Risk Defined

Materials that may be capable of transmitting bloodborne pathogens (as specifically addressed under the OSHA Bloodborne Pathogens Standard) include the following:

1. Human body fluids including: blood, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental settings, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids;
2. Any unfixed tissue or organ (other than intact skin) from a human (living or dead);
3. HIV-containing cell or tissue cultures, organ cultures, and HIV or HBV-containing culture media or other solutions;
4. Human cell/tissue/organ cultures not known to be free of bloodborne pathogens (see Attachment B). NOTE: Regardless of testing status, all human cells must be handled in accordance with BSL-2 containment practices as outlined in Appendix H of the [CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories", 6th edition](#) (see Attachment B);
5. Blood, organs, or other tissues from experimental animals infected with human bloodborne pathogens.

A person handling any of the above materials, or wastes contaminated with these materials in a basic research setting, is considered to have a reasonably anticipated risk of exposure to bloodborne pathogens. Examples of laboratory research activities that put a person "at risk" for exposure include:

- In vitro work with human cells;
- Processing of human blood or other BBP-risk body fluids*;
- Use of unfixed human tissues or anatomical parts in support of lab research;
- Administration or harvest of human cells in an animal model;
- Use of HIV-based viral vectors for in vitro or in vivo procedures;
- Handling of untreated wastes contaminated with human blood or OPIM (including disposal of liquid waste from cell culturing process and handling sharp containers used in an animal room where human cells are administered);
- Cleaning up a spill of human blood or OPIM.

*NOTE: Chemical, physical or other methods of inactivation of human-derived sample materials to eliminate infectious agent risk must be cleared with the VU Biosafety Officer before handling such materials to be in adherence with this policy.

A person who has a reasonably anticipated risk of exposure to BBPs must:

1. Be identified by their supervisor to the Occupational Health Clinic (OHC) for hepatitis B vaccination purposes;
2. Complete Bloodborne Pathogens training appropriate for their BBP exposure risk before being permitted to perform tasks with BBP-risk materials (contact [VU Biosafety](#) to identify options for completion);
3. Adhere to all exposure control and prescribed biosafety practices when working with BBP-risk materials;

4. Report any exposure incident (contact with mucous membranes or cut/scratch/puncture with a contaminated item) involving BBP-risk materials in accordance with document entitled ***Biomaterials Spill and Exposure Response*** located on the [VU Biosafety webpage](#) (report to OHC at 6th floor Medical Arts Building or Vanderbilt Adult Emergency Department for post-exposure followup; complete injury report through VU Risk Management incident reporting portal).
5. Complete refresher training at least annually, which features BBP exposure control topics that are relevant for that year in addition to key concepts covered in the initial training (contact [VU Biosafety](#) to identify options for completion if needed).

Basic Exposure Control Principles for All Laboratory Applications

All human blood and OPIM should be regarded as potentially infectious for BBPs when these materials are being handled in the basic research lab setting. This principle and approach for handling these materials is known as “universal precautions”. The following exposure control practices employ the universal precautions approach that is intended to protect those handling these materials as well as others who may be in the vicinity.

Engineering Controls

Engineering controls include equipment that is designed to isolate or remove the bloodborne pathogen hazard from the workplace (i.e. sharps disposal containers, self-sheathing needles, blunt needles, plastic capillary tubes, biological safety cabinets, handwashing facilities, etc.). Specific considerations for use of engineering controls include:

- Assure that a handwashing sink and eye wash are readily available, functioning properly, and are appropriately stocked with soap and paper towels before working with BBP-risk materials;
- Select sharps options with safety features in accordance with the document entitled ***Using Biohazardous Sharps Safely*** available on the [VU Biosafety webpage](#);
- Select sharps disposal containers that have the appropriate capacity and opening configuration for the type of sharps waste that will be generated; refer to the documents entitled ***Using Biohazardous Sharps Safely*** and ***Biohazardous Waste Guide*** available on the [VU Biosafety webpage](#) for additional safety pointers;
- Use a biological safety cabinet for work with human cells (including administration in animals and harvesting from animals);
- Select disinfectants that are [EPA-registered for the destruction of HIV and HBV](#); NOTE: this is a specific requirement of the OSHA Bloodborne Pathogens Standard, and ethanol does not have this required designation;
- Select and use storage/transport and secondary containers that will effectively contain a spill as outlined in the documents entitled ***Transporting Biomaterials on Campus*** and ***Biohazardous Waste Guide*** available on the [VU Biosafety website](#).

Work Practices

1. Hand washing* must be performed:
 - After removal of gloves or other personal protective equipment;
 - When visible contamination with blood, body fluids, or other potentially infectious materials is present;
 - When work is completed and before leaving the laboratory.

*NOTE: Washing with soap and water is the most effective means of hand washing and should be carried out at a sink in the lab. Under field work conditions, if waterless hand cleanser or antiseptic towelettes are used due to lack of available running water, the person must follow up by washing with soap and water as soon as feasible.
2. Biologically-contaminated needles and other contaminated sharps must not be bent, recapped, or removed unless it can be demonstrated that there is no feasible alternative. In this event, such bending, recapping, or needle removal must be accomplished through use of a mechanical device or one-handed technique.
3. Immediately, or as soon as possible, after use, biologically-contaminated reusable sharps (i.e., reusable scalpels, knives) shall be placed in appropriate containers until properly cleaned and decontaminated. These containers must be:

- Puncture-resistant;
 - Labeled with the biohazard symbol;
 - Leak-proof on the sides and bottom;
 - Designed and used in such a manner that does NOT require individuals to reach by hand into the containers.
4. Disposable biologically-contaminated sharps (i.e., needles, glass Pasteur pipettes, capillary tubes) must be placed in appropriate containers (as described under “Engineering Controls”) immediately, or as soon as possible, after use. These containers must be:
- Permanently closable;
 - Puncture-resistant;
 - Leak-proof on the sides and bottom;
 - Labeled with the biohazard symbol.

During use, biohazardous sharps containers must be:

- Located as close as feasible to the immediate area where sharps are used, or otherwise can be reasonably found;
- Maintained upright throughout use;
- Replaced routinely and **not** overfilled. (Containers must be permanently closed and replaced when $\frac{3}{4}$ full).

Proper use of biohazardous sharps container lids is required. These practices include:

- Lids must be properly installed before a disposable biohazardous sharps container is put into use;
 - When not in use, or when moving a container from one location to another, sharps container lids must be closed (if design permits) to further eliminate the potential for exposure;
 - Container lids must be permanently closed before handling containers for disposal.
5. The use of glass in all operations should be minimized to the extent possible, including the replacement of glass Pasteur pipettes with alternatives that are not inherently sharp and less breakable. When broken, glass is a puncture and abrasion hazard that can lead to a high-risk exposure scenario if OPIM contamination is present. **Do not handle broken glass directly with hands.** Use mechanical tools such as a disposable broom and dustpan or tongs/forceps that can be cleaned and disinfected for reuse, etc., for retrieving broken glass. When purchasing reagents and lab supplies, consider options that are constructed of non-glass materials or have a safety coating that will minimize the potential for breakage and creation of jagged edges.
6. Eating, drinking, smoking, applying cosmetics or lip balm, handling contact lenses, and food/drink storage is prohibited in all laboratory areas. (A laboratory area is one where processing/manipulation of samples or storage of biohazardous waste takes place).
7. Mouth pipetting/suctioning of blood or other infectious materials is always prohibited.
8. Minimize splashing, spraying or other actions that generate droplets of blood or other potentially infectious materials. (A biological safety cabinet should be used or a combination of face protection, shielding, and isolation of the procedure to a limited access area of the lab should be substituted. Contact [VU Biosafety](#) for further guidance).
9. Specimens of blood or other potentially infectious materials must be placed in designated leak-proof containers, appropriately labeled for handling and storage.
10. Primary containers of potentially infectious materials must be placed in puncture-resistant, leak-proof, closable secondary containers for transportation outside of the work area (i.e., from lab to lab where a common hallway is used, etc.). Refer to documents entitled *Transporting Biomaterials on Campus* available on the [VU Biosafety webpage](#).

Personal Protective Equipment (PPE)

PPE is appropriate for protection against BBP occupational exposure only if it does not permit blood or other potentially infectious material to pass through or reach the individual's clothing, undergarments, skin, eyes, mouth, or other mucous membranes under normal conditions of use and for the duration of time that the protective equipment will be used. The following practices must be utilized to ensure that PPE is not contaminated and is in appropriate condition to protect individuals from potential exposure:

1. All PPE must be inspected periodically by the PI/supervisor and repaired or replaced as needed.
2. Reusable PPE (lab coats, safety glasses, face shields, etc.) must be cleaned or laundered and decontaminated as needed. Lab coats (and any personal clothing that becomes contaminated with blood or OPIM) must NOT be sent home with individuals for laundering. For assistance with identifying on-site laundry or commercial laundry services, please contact your departmental administrative office.
3. Single-use PPE that is contaminated with blood or OPIM to the extent where the material can drip or flake off of the item will be disposed of as biohazardous waste. (If performing cell culture operations, ALL used PPE must be collected and disposed of as biohazardous waste per BSL-2 procedures regardless of visible contamination status).
4. When using PPE, individuals must:
 - Inspect PPE prior to use to verify that it is in good condition;
 - Remove all PPE before leaving the work area;
 - Wear gloves when:
 - Hand contact with potentially infectious materials is anticipated;
 - Handling or touching contaminated items or surfaces;
 - Working with or performing any procedures with lab animals.
5. Replace disposable gloves as soon as possible after contamination or immediately when torn, punctured or otherwise rendered unable to function as an exposure barrier. NOTE: Treating gloves with ethanol during cell culture activities will degrade and compromise the glove material. Therefore, double-gloving is advised under these conditions and gloves must be changed frequently to prevent glove breakthrough and potential contact exposure.
6. Report any adverse reactions to glove material, or any known latex allergy, to the supervisor so that appropriate alternative protective devices can be provided.
7. Decontaminate reusable gloves (i.e., heavy gauge nitrile or vinyl) before reuse; if utility gloves are cracked, peeling, torn or exhibit other signs of deterioration, they must be discarded.
8. Wear eye protection and masks or face shields whenever there is a chance that a splash or spray may generate droplets of infectious materials.
9. Wear protective clothing (e.g. lab coat or fluid-resistant disposable smock) whenever splashes or aerosols of human blood or OPIM are anticipated.
10. Remove and replace compromised or moderately contaminated PPE as soon as feasible.
11. Wash hands after removal of PPE.

Housekeeping

Individuals working with potentially infectious materials must:

1. Clean and decontaminate all equipment and surfaces after contact with blood or other potentially infectious materials. Visible contamination must be removed before applying disinfectants* to surfaces to ensure product efficacy. Clean and disinfect:
 - Immediately (or as soon as feasible) when surfaces become contaminated;
 - After any spill of blood or potentially infectious materials;
 - At the end of the work shift, especially if the surface may have become contaminated during that shift.

***In accordance with the OSHA BBP Standard, disinfectants must be EPA-registered and capable of inactivating HIV and HBV. Freshly-made 1:10 (vol:vol) bleach solutions are also acceptable.**

2. Routinely inspect and clean all pails, bins, cans and other receptacles. These items must be properly decontaminated whenever visibly contaminated.
3. Pick up potentially contaminated broken glassware using mechanical means (such as tongs, forceps, or a dustpan and brush) and dispose of in a proper biohazardous sharps container. Do NOT handle broken contaminated glass with your hands!
4. Report and isolate spills of blood, body fluids, or any other potentially infectious materials. If you are properly trained for cleanup, then proceed with cleanup of spill. If you have not completed training for cleanup of spills or the size or nature of spill is beyond your cleanup capabilities, please contact VU for assistance. Refer to the document entitled: ***Biomaterials Spill and Exposure Response*** located on the [VU Biosafety webpage](#) for full details on these practices.
5. Collect and dispose of waste in accordance with the document entitled *Biohazardous Waste Guide* located on the [VU Biosafety webpage](#). For locations that use a medical waste contractor, wastes must be prepared and packaged in accordance with the Department of Transportation's (DOT) regulated medical waste (RMW) requirements for over-the-road shipment. Refer to RMW guidance documents on the Biosafety page of the [EHS website](#).

Labels & Signs

Biohazard labels consist of a red or fluorescent orange colored background with the biohazard symbol in a contrasting color, and the word "biohazard". The VU Biosafety Team maintains a supply of labels meeting these criteria, and these are available upon request.

The following items need to be labeled:

- Entrances to all laboratory areas where blood, cell cultures, or other potentially infectious materials are used;
- Containers of regulated waste;
- Refrigerators, freezers, incubators, or other equipment containing blood, cell cultures, or other potentially infectious materials;
- Biohazardous sharps disposal containers;
- Containers used to store, transport, or ship blood and other potentially infectious materials.

Activity-specific Considerations

HIV & HBV Research Laboratories & Production Facilities

In the context of the OSHA Bloodborne Pathogens Standard, a *Research Laboratory* is a laboratory producing or using research-laboratory-scale amounts of HIV or HBV. Research laboratories may produce high concentrations of HIV or HBV but not in the volume found in production facilities. A summary of the additional provisions that apply to an HIV or HBV research laboratory can be found in Attachment B. Labs in this category are expected to work closely with VU Biosafety to develop all necessary procedures and safety management systems to support compliance and reduce this elevated exposure risk.

NOTE: Labs whose activities require them to work with high titer human clinical specimens from known infected subjects (i.e., one with a titer anticipated to be 10^8 PFU/ml HIV or higher) are also expected to adhere to these practices.

Use of Human Cells in Basic Bench Research Applications

Under the OSHA Bloodborne Pathogens Standard, human-derived cells and cell lines may be excluded from regulation if they have been tested and determined to be bloodborne pathogen-free (see Attachment A). **However, the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th edition specifies that all human cells, regardless of testing status, are to be handled in a biological safety cabinet under BSL-2 containment conditions (see Attachment B).** Therefore, all human cells, regardless of testing status, should be handled in accordance with the provisions of this policy and associated BSL-2 guidance.

Use of Human Cells (and Tissues) in Animal Research Applications

Neither the OSHA BBP Standard, nor the CDC/NIH BMBL document specifically addresses the exposure potential associated with the introduction and use of human-derived materials in lab animal models. The fate and dissemination of the human-derived materials once administered to the animal may vary depending on the type of materials administered (for instance, a cell line tested and found to be negative for known BBP versus patient-derived blood, tissue or OPIM), how the materials are administered, the extent of immune compromise of the animal recipient of human-derived materials, and the degree of “humanization” of the animals on study. As animal model technologies and experimental systems continue to evolve, the Institutional Biosafety Committee (IBC) and VU Biosafety will collaborate with the research community on this matter to develop safety practices that are stratified according to risk but deemed appropriate to minimize individual exposure potential. As researchers prepare to conduct experiments in this category (for instance, implantation of human tumor cells into mice), it is vital that they contact VU and initiate a review and assessment of the potential for human-derived BBP to be shed by the animals or present at times of sampling or harvest of materials.

As a given, all human-derived materials being administered in animal models must be handled and disposed of in accordance with the provisions outlined in this document. Note that the exact handling and processes will depend on risk factors. If implanted cells/tissues are to be harvested at the end of study, these must also be handled in accordance with the previously outlined practices. Sharps used for these procedures should be reviewed and selected based on the rationale outlined in the document entitled *Using Biohazardous Sharps Safely* available on the [VU Biosafety webpage](#).

As part of the Institutional Animal Care and Use Committee (IACUC) review process for each animal protocol, VU Biosafety reviews the protocols for human-derived materials administration activities. When identified, VU Biosafety requires the research team to prepare a safety practices document that summarizes the safety procedures to be followed for administration, sample collection and harvest activities. This document should be maintained with the animal protocol, and all individuals performing these procedures should adhere to the safety provisions outlined therein.

IBC Position/Recommendation for Specific Research Activities

The following table features 3 activities that elevate the BBP exposure risk associated with research and includes VU IBC’s latest recommendation for how to reduce the risk of these activities proactively. The IBC recognizes that not all proposed alternatives will be technically or scientifically feasible, but due diligence in documenting efforts to reduce exposure hazard is essential for regulatory compliance as well as metric generation for future risk reduction measures.

| Risk Activity | Risk Description | IBC Position/Recommendation |
|---|---|---|
| Cell shearing | Forcing cell material through a needle and syringe has a high likelihood of resulting in a needlestick for the person performing this task. | Use an alternate method for cell shearing/lysis that does not require the use of a sharp device; if a needle <u>must</u> be used, the lab should purchase a blunted needle of this purpose. If neither of these options are feasible due to technical or scientific reasons, a rationale will need to be prepared and submitted to the VU IBC for consideration. |
| Aspiration or transfer using glass Pasteur pipettes | Glass Pasteur pipettes are inherently sharp and easily broken during the handling and disposal process. As such, their use in conjunction with human cells increases the likelihood of a BBP exposure for those who use the pipettes and those who handle the biohazardous sharps containers. | Plastic serological pipettes are an available (and cost comparable) alternative for most applications with a greatly reduced sharp injury potential. If glass Pasteur pipettes must be used for technical or scientific reasons, a rationale will need to be prepared and submitted to the VU IBC for consideration. |
| Use of lentiviral vector systems | 1 st and 2 nd generation lentiviral vectors have a high potential for recombination that would result in the production of replication competent HIV. | 3 rd and 4 th generation lentiviral vector systems should be used whenever possible. If a 1 st or 2 nd generation system must be used, further risk assessment with VU Biosafety will be required to determine the need for additional biosafety practices. A rationale for use of the system will need to be prepared and submitted to the VU IBC for consideration. |

Attachment A: OSHA Letter of Interpretation

Regarding Applicability of BBP Standard to Human Cells

“... [T]he Bloodborne Pathogens Standard (BPS) provides protection to employees who have occupational exposure to human blood or other potentially infectious materials (OPIM). 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 considered to be OPIM and are not covered by BPS. Established human or 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 human immunodeficiency viruses are covered by the BPS. The final judgment for making the determination that human or other animal cell lines in culture are free of bloodborne pathogens must be made by a Bio-safety Professional or other qualified scientist with the background and experience to review such potential contamination and risk, in accordance with the requirements of the BPS. Documentation that such cell lines are not OPIM should be a matter of written record and on file with the employer for OSHA review.

All primary human cell **explants** from tissues and **subsequent in vitro** passages of human tissue explant cultures (human cell "strains" ***) must be regarded as containing potential bloodborne pathogens and should be handled in accordance with the BPS. Non-transformed, human cell "strains", characterized by documented, reasonable laboratory testing as described in the attachment, to be free of human immunodeficiency virus, hepatitis viruses, or other bloodborne pathogens may be exempted from the standard's requirements. However, if such tissue explants or subsequent cultures are derived from human subjects known to carry bloodborne pathogens, such as hepatitis viruses or human immunodeficiency viruses or are deliberately infected with bloodborne pathogens, they must be handled in accordance with the precautions noted in the BPS. Likewise, animal tissues, explants or cell cultures known to be contaminated by deliberate infection with human immunodeficiency virus or Hepatitis B virus are also subject to the BPS.

All laboratory work with primary human tissues or body fluids is covered by the BPS.”

DEFINITIONS

* A human cell **line** is defined as **in vitro** or animal passaged (e.g., nude mouse) cultures or human cells that fulfill traditional requirements of a cell line designation. That is, the cells are **immortalized** cells, transformed by spontaneous mutation or natural or laboratory infection with an immortalizing agent such as Epstein-Barr virus (EBV). EBV is a bloodborne pathogen. It should be noted that human cervical carcinoma cells or other transformed human cell lines like HeLa cells are sometimes adulterated with laboratory pathogens accidentally introduced by cultivation with other cell cultures, or physically contaminated by other cell cultures handled in the same lab. In order to handle human HeLa cells, without having to comply with the requirements of the bloodborne pathogens standard (BPS), human HeLa cells should be documented to be pure HeLa cells and shown to be free of bloodborne pathogens by testing.

**Characterization of human cells, for inclusion or exclusion from compliance with the BPS, would include screening of the cells lines or "strains" for viruses characterized as bloodborne pathogens by the Standard, including human immunodeficiency viruses, hepatitis viruses or EBV, if the cells are capable of propagating such viruses. Most cell lines are screened for human mycoplasmas and are free of bacterial and mycotic contaminants. Testing may include antigenic screening for viral or agent markers, co-cultivation with various indicator cells that allow contaminants to grow, or using molecular technology (polymerase chain reaction or nucleic acid hybridization) to identify latent viruses capable of infecting humans such as herpes viruses(e.g., EBV), or papilloma members of the Papovavirus group, etc. Cell lines that are procured from commercial vendors or other sources with documented testing to be free of human bloodborne pathogens and which have been protected by the employer from environmental contamination may be excluded from the BPS.

*** Human cell **strains** are defined as cells propagated **in vitro** from primary explants of human tissue or body fluids which have finite lifetime (non-transformed) in tissue culture for 20-70 passages. Human cell "strains" must be handled as potential biohazards unless characterized by testing to be free of bloodborne pathogens (i.e., WI-38 cells are often so documented).

Link: https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=INTERPRETATIONS&p_id=21519

Attachment B: CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (Excerpt Regarding Human Cells and Tissues)

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 present 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 468 Biosafety in Microbiological and Biomedical Laboratories 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 (of BMBL 6th edition) 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/laws-regs/standardinterpretations/1994-06-21>

Attachment C: Specific Requirements for HIV and HBV Research Laboratories under the OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030(e))

This regulatory excerpt outlines the provisions that apply to research laboratories engaged in the culture, production, concentration, experimentation, and manipulation of HIV and HBV. It does not apply to clinical or diagnostic laboratories engaged solely in the analysis of blood, tissues, or organs. **These requirements apply in addition to the other requirements of the Standard.**

Standard & Special Practices

Research laboratories and production facilities will adhere to the following practices:

- All regulated waste shall either be incinerated or decontaminated by a method such as autoclaving known to effectively destroy bloodborne pathogens.
- Laboratory doors shall be kept closed when work involving HIV or HBV is in progress.
- Contaminated materials that are to be decontaminated at a site away from the work area shall be placed in a durable, leakproof, labeled or color-coded container that is closed before being removed from the work area.
- Access to the work area shall be limited to authorized persons. Written policies and procedures shall be established whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements, and who comply with all entry and exit procedures shall be allowed to enter the work areas and animal rooms.
- When other potentially infectious materials or infected animals are present in the work area or containment module, a hazard warning sign incorporating the universal biohazard symbol shall be posted on all access doors. The hazard warning sign shall include the name of the agent, special entry requirements and responsible person contact information.
- All activities involving other potentially infectious materials shall be conducted in a biological safety cabinet (BSC) or other physical containment devices within the containment module. No work with these other potentially infectious materials shall be conducted on the open bench.
- Laboratory coats, gowns, smocks, uniforms, or other appropriate protective clothing shall be used in the work area and animal rooms. Protective clothing shall not be worn outside of the work area and shall be decontaminated before being laundered.
- Special care shall be taken to avoid skin contact with other potentially infectious materials. Gloves shall be worn when handling infected animals and when making hand contact with other potentially infectious materials is unavoidable.
- Vacuum lines shall be protected with liquid disinfectant traps and high-efficiency particulate air (HEPA) filters or filters of equivalent or superior efficiency, and which are checked routinely and maintained or replaced as necessary.
- Hypodermic needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) shall be used for the injection or aspiration of other potentially infectious materials. Extreme caution shall be used when handling needles and syringes. A needle shall not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe shall be promptly placed in a puncture-resistant container and autoclaved or decontaminated before reuse or disposal.
- All spills shall be immediately contained and cleaned up by appropriate professional staff or others properly trained and equipped to work with potentially concentrated infectious materials.
- A spill or accident that results in an exposure incident shall be immediately reported to the laboratory director or other responsible person.

- A biosafety manual shall be prepared or adopted and periodically reviewed and updated at least annually or more often if necessary. Personnel shall be advised of potential hazards, shall be required to read instructions on practices and procedures, and shall be required to follow them.

Containment Equipment

Research laboratories will adhere to the following provisions regarding containment equipment:

- Certified biological safety cabinets (Class I, II, or III) or other appropriate combinations of personal protection or physical containment devices, such as special protective clothing, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals, shall be used for all activities with other potentially infectious materials that pose a threat of exposure to droplets, splashes, spills, or aerosols.
- Biological safety cabinets shall be certified when installed, whenever they are moved and at least annually.
- Each laboratory shall contain a facility for hand washing and an eye wash facility which is readily available within the work area.

Training Requirements per 1910.1030(g)(2)(ix)

In addition to all other training requirements outlined in the OSHA BBP Standard, the following apply for those working with HIV or HBV in research labs.

For those with no experience:

- The employer shall provide a training program to employees who have no prior experience in handling human pathogens. Initial work activities shall not include the handling of infectious agents. A progression of work activities shall be assigned as techniques are learned and proficiency is developed. The employer shall assure that employees participate in work activities involving infectious agents only after proficiency has been demonstrated.

For those with experience:

- The employer shall assure that employees have prior experience in the handling of human pathogens or tissue cultures before working with HIV or HBV.
- The employer shall assure that employees demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the facility before being allowed to work with HIV or HBV.

NOTE: Research labs working with HIV, HBV or any other pathogens infectious to humans will also need to follow all applicable biocontainment provisions outlined in the [CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories", 6th edition](#) and the [NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules](#). Additionally, work with HIV and other Risk Group 3 materials may be subject to additional biosafety or biosecurity requirements. Contact [VU Biosafety](#) for further information.

Policy Revision History

The original policy on this subject was approved by the Vanderbilt University (VU) and Vanderbilt University Medical Center (MC) Institutional Biosafety Committees (IBCs) on January 24, 2017.

This policy was rewritten as a Vanderbilt University IBC Policy in August 2022 to reflect current responsible parties, institutional guidance documents and biosafety standards. The policy was endorsed by the VU Institutional Biosafety Committee on 09/27/2022. Minor updates related to internal reference documents incorporated by BSO (10/03/2023).

The VU IBC reviewed proposed edits prepared by VU Biosafety and revised this policy on January 28, 2025. A summary of the revisions includes the following:

- Updated the policy title to be consistent with other biomaterial-specific policies,
- Standardized terminology related to biologically-contaminated sharps and biohazardous sharps containers,
- Added section addressing research activities that elevate risk (cell shearing, transfers with glass Pasteur pipettes, lentiviral vector use) and the IBC's current recommendations for these scenarios,
- Updated reference document titles as needed and links throughout document.

The policy will be reviewed for updates at least annually and when determined appropriate by the VU Biosafety Officer and IBC Chair.