**THIS IS A TEMPLATE/BASIC STARTING POINT. CUSTOMIZE THIS TEMPLATE WITH INFORMATION PERTINENT TO YOUR SETUP AND THE PROCEDURE YOU WILL BE USING/YOUR GROUP’S PERSONAL USE.**

STANDARD OPERATING PROCEDURE

Use this form to document the Health and Safety information associated with the procedure.

|  |  |
| --- | --- |
| **Procedure Title** | SOP for Tissue Culture |
|  |  |
| **Dept** |  |  | **Bldg/Rm** |  |  | **Supervisor** |  |

**Procedure Overview** (a brief description of the project)

The purpose of this Standard Operating Procedure (SOP) is to outline procedures that should be followed when performing work with tissue culture. All human and non-human primate derived tissue culture and related procedures must be performed following Biosafety Level 2 (BSL2) practices. Although not all cells utilized in this laboratory are human-derived, all tissue culture and related procedures are performed using BSL2 practices.

**Health and safety information for materials used (**briefly describe the hazards associated with the materials and/or equipment **OR** document your hazard assessment in Section I)

Human and non-human primate derived cell lines: These cell lines are considered Potentially Infectious Materials (PIMs) under the Occupational Safety and Heath Administration’s (OSHA’s) [Bloodborne Pathogens Standard](https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030). Human source material including human-derived cell lines are potential sources of bloodborne pathogens (BBPs), including but not limited to human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). The most probable routes of exposure for this work are percutaneous via a sharps injury (needle sticks, cuts), mucosal via contact with eyes, nose, and mouth (splash), or by contact with non-intact skin (scratches, abrasions, dermatitis).

Permissive cell lines that support viral replication may have a greater risk of contamination with viral pathogens. Well-established, tested, cell lines are generally considered safer, but the possibility of contamination by an advantageous pathogen must be considered.

Consider additional health and safety concerns related to additional materials (such as media supplements) or methods associated with your protocol(s). Include additional hazard control measures as required.

**Hazard Control Measures**

Please select which type of lab coat, eye protection, and hand protection will be used (Lab coat, eye and hand protection, and closed toe/heel shoes must be selected as required by Section D of the ISU Laboratory Safety Manual.)

All work with tissue culture must be performed in a BSL2 laboratory. This includes a room suitable for tissue culture and equipped with a certified Class II Biosafety Cabinet (BSC).

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| **[ ]**  | Latex gloves | [ ]  | Insulated gloves | [ ]  | Face Shield | [ ]  | Respirator |
| **[x]**  | Nitrile gloves | [x]  | Safety glasses | [x]  | Lab Coat | [ ]  | Fume hood |
| **[ ]**  | Neoprene gloves | [ ]  | Vented goggles | [ ]  | Apron | [x]  | Biosafety cabinet |
| **[ ]**  | Vinyl gloves | [ ]  | Splash goggles | [ ]  | Dust mask | [ ]  | Glove box |
| **[x]**  | Closed Toe/Closed Heel Shoes | [ ]  | Flame Resistant Lab coat |

***Other Control Measures***

Universal Precautions are observed to mitigate the risk of exposure to BBPs. This is an approach to infection control whereby human blood and other Potentially Infectious Materials (PIMs), including human-derived cell lines, are treated as if known to be infectious for BBPs.

* Eating, drinking, food storage, handling of contact lenses, applying cosmetics, mouth pipetting, and

tobacco are prohibited in the laboratory.

* Do not store food in freezers, refrigerators, cabinets, or any other areas where human blood and other

PIMs are stored or may be contaminated with human blood or other PIMs.

* Minimize splashing or spraying of human blood or other PIMs.
* Wash hands frequently, even if gloves have been worn.
* Use leak-proof and non-breakable containers for human blood and other PIMs
* Affix biohazard symbols to containers of regulated waste (such as sharps discards), refrigerators, and

freezers containing human blood and other PIMs.

* Human blood and other PIMs are placed in durable, leak-proof containers during collection, handling,

storage, and transport. Use secondary containment when these materials are transferred through hallways or between buildings.

* Keep waste containers near the area where work with human blood or other PIMs is being performed.
* Never overfill waste containers.
* Use extreme caution when working with sharp objects such as needles, razor blades, or broken

glass/ plastic, and dispose of in an appropriate sharps container.

Work with biohazardous materials is performed using standard microbiological practices including;

* The laboratory has a door for access control.
* The laboratory is designed so that it can easily be decontaminated.
* Laboratory windows that open to the exterior are fitted with screens.
* Eating, drinking, food storage, handling of contact lenses, applying cosmetics, mouth pipetting, and use of

tobacco products are prohibited in the laboratory.

* Do not store food in freezers, refrigerators, cabinets, or any other areas where biohazardous materials are stored or may be contaminated with biohazardous materials.
* A sign is posted at the entrance to the laboratory when infectious materials are present. A door sign incorporating emergency contacts and hazard information for the lab using the following link: <http://www.ehs.iastate.edu/services/occupational/door-signage>
* Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
* Gloves are worn to protect against biohazardous materials, Disposable gloves are disposed of in an appropriate waste receptacle and are not washed or reused. Gloves are not worn outside of the lab. Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary.
* All PPE is removed before leaving the laboratory and disposed of or properly stored.
* A sink, soap, and paper towels are available in the lab
* Hands are washed after performing work and before leaving the lab
* An eyewash station is located within the laboratory.
* Work surfaces and equipment are decontaminated after using biohazardous materials.
* Mouth pipetting is prohibited. Mechanical pipetting devices are used.
* Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps
* Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to transporting the material.
* An effective pest management program is implemented.
* Animals and plants not associated with the work being performed are not permitted in the laboratory.
* An eyewash station is located within the laboratory.

In addition to universal precautions and standard microbiological practices

Work with human or nonhuman primate derived tissue culture is performed using BSL2 practices including;

* There is authorized access to the laboratory. Lab doors are self-closing and locked when the laboratory is unattended.
* Biosafety cabinets are certified annually and used appropriately.
* Use leak-proof and non-breakable containers for the storage of biohazardous materials.
* Biohazardous materials are placed in durable, leak-proof containers during collection, handling,

storage and transport. Use secondary containment when these materials are transferred through hallways or between buildings. The transport container has a universal biohazard label.

* Keep biohazardous waste containers near the area where work with biohazardous materials is being performed.
* Never overfill waste containers.
* Use extreme caution when working with sharp objects such as needles, razor blades, or broken

glass/ plastic, and dispose of in an appropriate sharps container

* An autoclave is available for decontaminating laboratory waste.
* Furniture is capable of being cleaned and decontaminated.
* Access to the laboratory is limited and doors are always closed to maintain BSL2 containment.
* All waste material is decontaminated prior to disposal.
* Vacuum lines are protected by liquid disinfectant traps and in-line HEPA filters or their equivalent.
* Any experiments of lesser biohazard are demarcated by space or time.
* Open flames are prohibited in the BSC.
* Procedures that generate aerosols are performed in the BSC including pipetting and vortexing.
* Work surfaces and equipment are decontaminated after using biohazardous materials.

All laboratory staff working with human-derived tissue culture should be enrolled in the Occupational Medicine Program specific to bloodborne pathogens. Participation requires the completion of a [Hazard Inventory](https://publications.ehs.iastate.edu/bbp/10/) form. Participation requires annual Bloodborne Pathogens Exposure Control training and offers personnel the choice of receiving Hepatitis B vaccination.

All laboratory staff working with human-derived tissue culture should work under the policies and guidelines established in the EH&S [Bloodborne Pathogens Manual](https://publications.ehs.iastate.edu/bbp/), which serves as the Exposure Control Plan for Iowa State University.

A sharps policy is implemented.

* Never recap, bend, or break needles
* If necessary to recap a needle, use a mechanical device, such as forceps, to handle the cap
* Handle other sharps such as broken glass/plastic, scalpels, razorblades, broken Pasteur pipets, and
* broken capillary tubes with mechanical devices whenever possible
* Dispose of sharps in appropriate sharps containers
* Avoid the use of sharps or breakable materials and use safer sharps devices whenever possible

**Methods**

**Cell Culture Specific Procedures**

See attached or Available…

**Basic Biosafety Cabinet (BSC) Procedures**

 **BSC Maintenance**

* BSCs must be cleaned and disinfected before and after each use
* All repairs, filter changes, and certifications must be performed by a qualified servicing company, by NSF certified technicians.
* BSCs must be recertified whenever they are moved, repaired, or have the filters changed

 **Prior to BSC use**

* Don appropriate Personnel Protective Equipment (See Hazard Control Measures, above)
* Open the BSC sash to the appropriate sash height.
* Turn on the cabinet blower and lights, and check the front and rear air intake grills of the cabinet to make sure they are not obstructed.
* Verify the cabinet is working properly.
* Do not use a BSC that is alarming, this indicates reduced airflow.
* Disinfect the cabinet working surface and walls with appropriate disinfectant, allowing for a minimum 5-minute contact time. If bleach or other corrosive disinfectant is used, wipe the surface and walls with 70% ethanol to remove residual disinfectant that may corrode the stainless-steel surface.
* Allow the blower to operate a minimum of 15 minutes before ascetic manipulations begin in the cabinet.

 **Use of BSC**

* Place only necessary materials onto the cabinet work surface. Waste receptacles should be placed inside the BSC.
* Disinfect media bottles and supplies with an appropriate disinfectant prior to placing them inside the BSC.
* Active work should flow from clean to contaminated areas across the work surface.
* Avoid rapid arm movements or frequent movement of arms into and out of the BSC.
* Use filtered tips for all manipulations.
* Connect aspiration bottles or suction flasks to an overflow collection flask filled with disinfection and to an inline HEPA filter.
* Do not cover/block air intake grills.
* (As applicable) Disinfect original cultures and liquid waste inside the BSC (see Waste Disposal Procedures, below).
* (As applicable) Collect original cultures and liquid waste inside the BSC to be autoclaved (see Waste Disposal Procedures, below).
* (Include lab-specific procedure(s) for contaminated solids and sharps) Collect contaminated disposable plastic ware, plastic sharps, glass sharps, and metal sharps separately. Items can be removed from the BSC after they have been decontaminated with an appropriate disinfectant or can be placed within a bag or sealed container in the BSC, which is sealed with disinfectant before removal from the BSC. (see Waste Disposal Procedures, below).

**Following BSC use**

* Disinfect media bottles and other supplies when removed from the biosafety cabinet.
* Disinfect work surfaces, micropipettes, and pipet aids used with appropriate disinfectant.
* Disinfect the cabinet working surface and walls with an appropriate disinfectant and allow for a minimum 5-minute contact time. If bleach or other corrosive disinfectant is used, wipe the surface and walls with 70% ethanol to remove residual disinfectant that may corrode the stainless-steel surface.
* Leave the BSC running for a minimum of 15 minutes.
* Turn off blowers and lights.
* Decontaminate any additional laboratory surfaces (such as benchtops), as applicable
* Remove PPE and wash hands with soap and water for a minimum of 20 seconds.

**Disposal and Disinfection**

 **General**

* Generated waste should be disposed of as outlined in the [Waste and Recycling Guidelines](https://publications.ehs.iastate.edu/warg/).
* Work surfaces and equipment are decontaminated routinely, and when work is concluded.
* Choosing the appropriate chemical disinfectant depends on the surface or item needing decontamination, as well as the organism requiring inactivation.
* Disinfect or autoclave reusable containers that have been in the biosafety cabinet.
* See the [Biosafety Manual](https://publications.ehs.iastate.edu/bsm/files/assets/common/downloads/publication.pdf?uni=c9ca461aeabe5a5b0eca89c3850e27d0) for more information about disposal and disinfection.
* The ISU [Sharps and Biohazardous Waste Disposal Flowchart](https://www.ehs.iastate.edu/sites/default/files/uploads/publications/factsheets/sharpsflowchart.pdf) is posted in the laboratory.

**Liquid Waste**

* (As applicable) Decontaminate all liquid biohazardous materials by treatment with an appropriate chemical disinfectant for sufficient contact time.
	+ Consult the SDS of the disinfectant to determine proper disposal.
	+ Remove as liquid chemical waste by submitting a [Waste Removal](https://www.ehs.iastate.edu/services/waste/wasteremoval) request.
* (As applicable) Decontaminate all liquid biohazardous materials by autoclaving.
	+ Median containing heat-liable antibiotics can be decontaminated by autoclaving. If the media contains no chemical constituents, the liquid may be disposed of by pouring them down the drain to the sanitary sewer
	+ Media containing heat-stable antibiotics can be decontaminated by autoclaving, however, it should be removed as chemical waste by submitting a [Waste Removal](https://www.ehs.iastate.edu/services/waste/wasteremoval) request.

**Disposable Solids**

* Collect all non-sharp, disposable items (such as gloves, plastic ware, Kim wipes, etc.) contaminated with biohazardous materials in leak-proof autoclavable biohazard bags.
* Before decontaminating, place an autoclave indicator tape “X” over the biohazard symbol
* Decontaminate the waste by autoclaving for a minimum of 45 minutes before disposal
* After autoclaving, place the now decontaminated biohazard bag into a dark garbage bag, seal it, and place it in the regular trash.

**Laboratory Sharps-** Use separate containers to collect metal, glass, and plastic sharps

**Metal Sharps**

* Collect all metal sharps contaminated with biohazardous materials in autoclavable, leak-proof, puncture-resistant containers, which have been labeled with the universal biohazard symbol. Decontaminate the containers by autoclaving.
* After autoclaving, label the now decontaminated sharps waste containers with a “Non-Infectious Syringes and Metal Sharps Only” label.
* Collect metal sharps that have never been contaminated with biohazardous materials (e.g., used only with chemicals) in leak-proof, puncture-resistant plastic containers labeled with a “Noninfectious Syringes and Metal Sharps Only” label. Do not autoclave these containers, because they will melt.
* To dispose of metal sharps, submit a [Waste Removal](https://www.ehs.iastate.edu/services/waste/wasteremoval) request. EH&S will only pick up metal sharps waste that has been decontaminated.

**Glass Sharps**

* Collect all glass sharps contaminated with biohazardous materials in autoclavable leak-proof, puncture-resistant containers that have been labeled with the universal biohazard symbol. Decontaminate the containers by autoclaving.
* Collect glass sharps that have been autoclaved or that have never been contaminated with biohazardous materials (used only with chemicals) in a yellow tidy cat container in your laboratory for storage or into the yellow glass disposal bin on your building’s loading dock for disposal. Autoclaving of these containers is not necessary. Call FP&M at (515) 294-5100 for removal when the bin is full.

**Plastic Sharps**

* Collect plastic materials (pipette tips, plastic pipettes) that can poke out of bags and are contaminated with biohazardous materials in autoclavable, leak-proof, puncture-resistant containers which have been labeled with the universal biohazard symbol.
* Decontaminate the containers by autoclaving.
* After autoclaving, place the now decontaminated plastic sharps inside a garbage bag-lined cardboard box, seal, label “Plastic Sharps” and throw into the regular trash dumpster.

**Spill Cleanup Procedures**

 **Small spill within the BSC**

* Small spills within the operating BSC can be handled immediately, the blower must remain on.
* Remove any sharp contaminated objects from the spill area using mechanical means, never by hand.
* Cover small spills within the BSC with paper towels and apply an appropriate disinfectant starting from the outside working in. Allow appropriate contact time, usually 20 minutes. Collect towels at the edge of the spill and push them toward the center and dispose of them in a biohazardous bag or receptacle.
* Wipe down the cabinet interior and items inside of the cabinet with a towel dampened with disinfectant.
* Change gloves following decontamination.

 **Large spill within the BSC**

* Spills that result in the liquid flowing through the front or rear grill(s) require more extensive decontamination.
* Remove any sharp contaminated objects from the spill area using mechanical means, never by hand.
* Surface decontaminate and remove all items within the cabinet.
* Ensure the drain valve is closed and pour the disinfectant solution onto the work surface and through the grille(s) into the drain pan. Allow for appropriate contact time, usually 20 minutes. Attach a hose barb and flexible tube to the drain valve that is long enough to allow the open end of the tube to be submerged in disinfectant within a collection vessel. Empty the drain pan into a collection vessel containing disinfectant. Flush the drain pain with water, remove the drain tube, and close the drain valve.
* Change gloves following decontamination.
* Allow the cabinet to run for at least 10 minutes before resuming use.

Note: Alcohol is not recommended as a disinfectant for large spills, especially within a BSC, because large amounts of alcohol pose an explosion hazard.

 **Spills outside of the BSC (For material with high organic content and low concentration of infectious microorganisms)**

* Remove potentially contaminated clothing and place them in a biohazard bag for autoclaving.
* Wash hands and exposed body parts with soap and water.
* Evacuate the laboratory for at least 30 minutes. IS
* Apply first aid or call 911, if necessary.
* Caution everyone not needed for spill cleanup to stay away from the spill area. Post signs if necessary.
* Don disposable gloves, eye protection, and a lab coat for spills smaller than 100mL. (based on risk assessment). Also, Don an N95 respirator for spills greater than 100mL. (based on risk assessment)
* Remove any sharp contaminated objects from the spill area using mechanical means, never by hand.
* Cover the spill with paper towels and apply an appropriate disinfect starting from the outside working in. Allow for the appropriate contact time, usually 20 minutes. Collect towels at the edge of the spill and push them toward the center and dispose of them in a biohazard bag or receptacle.
* Repeat the decontamination step. Place disposable materials into a biohazard bag. Spray non-disposable items with disinfectant.
* Remove PPE and wash hands thoroughly with soap and water.

**First Aid Procedures**

Exposure to bloodborne pathogens can occur when media containing human-derived tissue culture contacts the eyes, mouth other mucous membranes, or non-intact skin or when this material enters the body through an injury to the skin, such as a puncture wound with a contaminated object.

If exposure to human-derived tissue culture occurs or is suspected to have occurred, contaminated broken skin must be washed immediately with soap and water. If mucous membranes are involved, they must be flushed with water immediately for a minimum of 15 minutes. After thorough washing, apply first aid. Seek medical evaluation for the exposure

**All accidents and injuries occurring at work or in the course of employment must be reported to the employee's supervisor as soon as possible (even if no medical attention is required).**

**Report all incidents and exposures here:**

[**https://www.ehs.iastate.edu/services/occupational/accidents-injuries**](https://www.ehs.iastate.edu/services/occupational/accidents-injuries)

**References**

Biosafety in Microbiology and Biomedical Laboratories, 6th edition

 <https://www.cdc.gov/labs/BMBL.html>

ISU Biosafety Manual

 <http://www.ehs.iastate.edu/publications/manuals/bsm.pdf>

ISU Bloodborne Pathogens Manual

 <https://publications.ehs.iastate.edu/bbp/>

ISU Sharps and Biohazard Waste Procedures Factsheet

 <https://www.ehs.iastate.edu/publications/factsheets/sharps.pdf>

ISU Lab Safety Manual

 <http://publications.ehs.iastate.edu/labsm/>

OSHA’s Bloodborne Pathogens Standard (29CFR 1910.1030)

 <https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030>

 Spills of Biohazardous Materials, webpage and associated documents

 https://www.ehs.iastate.edu/research/biological/microbial/spill-cleanup

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| **Written By** |  |  | **Date** |  |
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| **Approved By** |  |  | **Date** |  |

 (PI or Lab Supervisor)

1. **HAZARD ASSESSMENT**

Use the hierarchy of controls to document the hazards and the corresponding control measure(s) involved in each step of the procedure.

Consider *elimination or substitution* of hazards, if possible.

***Engineering Control(s):*** items used to isolate the hazard from the user (i.e. fume hood, biosafety cabinet).

***Administrative Control(s****):* policies/programs to limit the exposure to the hazard (i.e. authorizations, designated areas, time restrictions, training).

***Required PPE***: indicate PPE including specific material requirements if applicable (i.e. flame resistant lab coat, type of respirator or cartridge).

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| --- | --- | --- | --- |
| **Hazard** | **Engineering Control(s)** | **Administrative Control(s)** | **Required PPE**  |
| ***Potential for Bloodborne Pathogens***  | ***Biosafety Cabinet*** | ***See “Other Control Measures” above*** | ***Nitrile Gloves, Safety glasses, Closed toe shoes,******Lab coat*** |
| ***70% Ethanol*** | ***Local ventilation*** |  | ***Nitrile Gloves, Safety glasses, Closed toe shoes,*** ***Lab coat*** |
| **INSERT SPECIFIC HAZARDS/CONTROLS HERE** |  |  |  |

According to ISU Policy, the minimum PPE that is required while working with laboratory hazards is lab coat or clothing protection, safety glasses or goggles, and fully enclosed shoes. Deviation from this policy should be noted and justified along with a risk assessment.

1. **TRAINING RECORD**

Use the following table to record the training associated with this Standard Operating Procedure.

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| --- | --- | --- | --- |
| **Print Name** | **Signature** | **Trained By** | **Date** |
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