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Occupational Safety & Health Environmental Health Laboratory Safety Industrial Hygiene Radiation Safety Hazardous Waste Pollution Prevention

ODU Biosafety Manual

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Office of Research
&
Environmental Health and Safety

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I. Purpose, Scope, and Responsibilities

PURPOSE

Old Dominion University has developed this Biosafety Manual to provide guidance to all faculty, staff, and students on the safe handling of biological materials and the proper safety practices to minimize the risk of exposure to potentially infectious materials. The practices and procedures listed in this manual will, when followed properly, reduce the likelihood of an occupational exposure to infectious agents, biological toxins, and/or recombinant/synthetic nucleic acid molecules. This manual is applicable to all faculty, staff, students, volunteers, and visitors at ODU who handle any of these types of materials. The successful implementation of this manual depends largely on those involved to be committed to maintaining a safe working environment and to be knowledgeable about laboratory safety.

The recommendations and requirements listed in this manual are derived from the Centers for Disease Control and Prevention (CDC) / National Institutes of Health (NIH) publication,

e. Plants work involving infectious agents and/or recombinant or synthetic nucleic acid molecules
f. Bloodborne pathogens or other potentially infectious material (human blood, fluids, tissues, blood products, or cell lines; including non-human primate derived materials and established cell lines).
g. Any exempt select agent or toxin below the permissible toxin amount.

   Note: At the present time, ODU is not authorized to possess, use, or transfer select agents or toxins, as defined by the select agent regulations, 7 CFR 331 (agriculture), 9 CFR 121 (animals and animal products), and 42 CFR 73 (public health).
h. Dual use research of concern (DURC)

Further information can be obtained on the ODU Biohazards webpage.

RESPONSIBILITIES

It is the responsibility of each member of the Old Dominion University community for implementation of the Biosafety Program. Principal Investigators bear primary responsibility for ensuring that their research complies with all applicable federal and state regulatory standards and ODU policies. All Principal Investigators shall be familiar with the regulations, policies, and guidelines applicable to that research. Failure to comply with established regulations and proc.396 08urmn2 (un)10 (i8 (ons (on c)4 (u)4 (s)- (de8 (o 14 (e)10 (e i(i8 ((i)-2 (c)4 p(bi)8 (l)1 (i)(m)-2 (a)4 on

Office of Research. The BSO is responsible for facilitating and managing the Biosafety Program. Specific responsibilities of the BSO are as follows:

a. Review and dissemination of research/teaching applications involving the use of biological materials to the Institutional Biosafety Committee.
b. Conducting periodic inspections to ensure laboratory standards are rigorously followed.
c. Report to the IBC and the University's administration any significant problems, including any violations of the *NIH Guidelines*, and any significant research-related accidents or illnesses of which the BSO becomes aware.
d. Assisting laboratories in developing laboratory specific emergency plans for handling spills and personnel contamination, and investigating laboratory accidents involving rsNA research.
e. Providing advice on laboratory security, as it relates to the protection and safeguarding of biological materials.
f. Providing technical advice to Principal Investigators and the IBC on research safety procedures for the purpose of assuring that the use of human etiologic agents conforms to the University policy and applicable governmental regulations.
g. In support of Principal Investigators, determine the necessity for health surveillance of rsNA research personnel, and require, if appropriate, a health surveillance program for the project.

5. Principal Investigator

The Principal Investigator (PI) is the faculty member in charge of a research project or class. PIs bear the primary responsibility for compliance with all applicable laws, regulations, and guidelines. A subordinate role on a research project does not excuse non-compliance. For a comprehensive list of the investigator responsibilities for conducting rsNA research, refer to the Investigator Responsibilities brochure. In addition, the specific responsibilities of the PI are as follows:

a. To determine whether experiments are covered by any statute, regulation, or guideline, and to comply with the applicable requirements before, during, and after the experiment.
b. To initiate and/or modify research or teaching activities which require BSO review and/or IBC approval until that research or the proposed modification thereof has been approved and has met all other requirements of all applicable references.
c. Develop, review, and approve laboratory-specific and/or protocol-specific procedures, consulting with the BSO when necessary.
d. Ensure that all personnel under his/her supervision are adequately trained in good microbiological techniques and have received required training for the procedures they will be conducting.
e. To provide personal protective equipment to all personnel based on the experimental procedures used in the lab.
f. To supervise the laboratory staff diligently to ensure that all required safety practices and techniques are employed.

g. Provide training and information to all employees under his/her supervision regarding laboratory-

Training in part is conducted online through the Collaborative Institutional Training Initiative (CITI, www.citiprogram.org), and includes, but is not limited to, Bloodborne Pathogen training, NIH Guidelines training, Basic Biosafety, Select Agent and Toxin training. In addition, all personnel working within any laboratory where hazardous materials are used and/or stored must complete the in-

IRBNet webpage. For additional support, contact the Office of Research at 757-683-3460.

1. Types of IBC Submissions

a. Annual renewals

All IBC protocols are approved for a period of 5 years, unless otherwise noted. IBC protocols must be renewed annually using the IBC Annual Renewal form which can be downloaded from the "Forms and Templates" section of IRBNet. The PI will receive automated reminders from IRBNet at 60, 30, and 7 days prior to the expiration date. The renewal will be reviewed by the IBC outside of a convened meeting, unless otherwise requested. The BSO will share the renewal with the committee for review upon submission into IRBNet and the committee will be given 5 business days to review the renewal form. Once approved, the protocol will be approved for another year. Failure to submit this renewal form may result in the suspension and/or termination of the protocol.

b. Amendments

Any changes to an approved IBC protocol must be reviewed and approved by the IBC prior to implementation into the protocol. An IBC Amendment Form must be

5. Dual Use Research of Concern (DURC) is defined in the United States Government (USG) Policy for Institutional

Import Permit Program (IPP).

9. Packing and Transportation Requirements of Biological Substances. Specimen shipments in the United States and internationally are regulated under either the Hazardous Materials Regulations (U.S.) or Dangerous Goods Regulations (international). As such, these specimens require packaging that meets Department of Transportation (DOT) (domestic shipments in the United States) Transportation of Hazardous Materials Regulations (HMR) and International Air Transport Association (IATA) (international air shipments worldwide) Dangerous Goods Regulations (DGR).

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used in this type of cabinet **only** if it exhausts to the outside via a canopy connection.

Figure 3: Class II Type A1 BSC - (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) common plenum; (F) blower

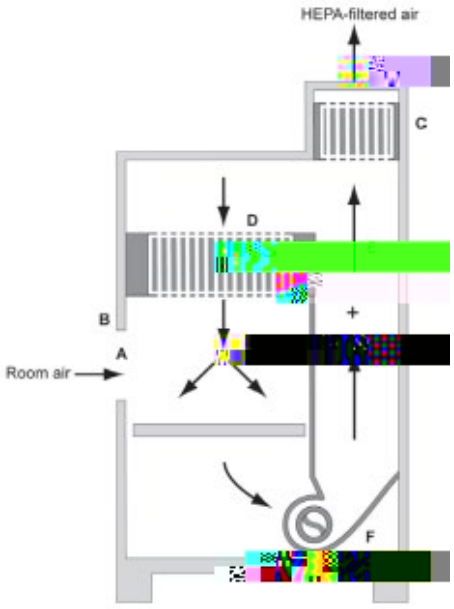
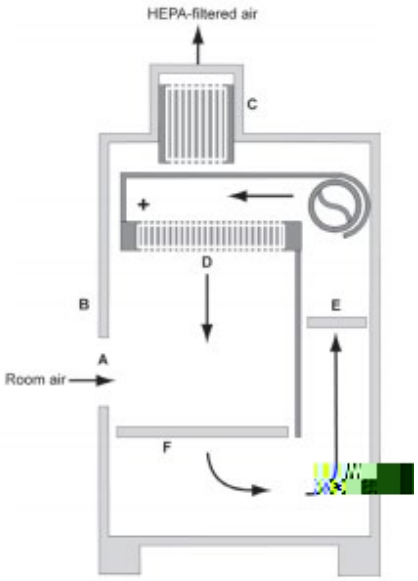


Figure 4: Class II Type A2 BSC- (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) positive pressure common plenum; (F) negative pressure plenum



Source: Biosafety in Microbiological and Biomedical Laboratories, 5th Edition

c. Class II, Type B1 (Figure 5)

The Type B1 cabinets maintain an average inflow velocity of 100 fpm at the face opening, recirculate approximately 30% of air through the supply HEPA filter, and discharge approximately 70% through an exhaust HEPA filter. Down-flow air that is pulled through the rear grille of the cabinet is exhausted directly through a HEPA filter and to the outside via hard ducting without recirculation within the cabinet or laboratory. This allows for manipulations of minute quantities volatile chemicals and radionuclides as long as the work is performed in the direct exhaust (rear) portion of the BSC.

d. Class II, Type B2 (Figure 6)

Type B2 cabinets maintain an average inflow velocity of 100 fpm at the face opening and are total exhaust cabinets, meaning no air is recirculated within the BSC or laboratory. All air that enters the cabinet is exhausted and passes through a HEPA filter prior to discharge to the outside. These cabinets are expensive to operate and their purchase and use should be justified by the research. Type B2 cabinets do not provide additional biosafety protection over other Class II BSCs.

Figure 5: Class II Type B1 BSC - (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure dedicated exhaust plenum; (F) blower; (G) additional HEPA filter for supply air

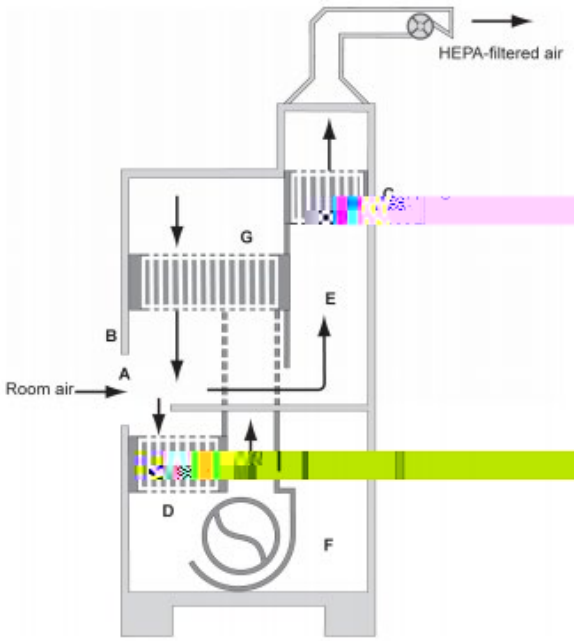
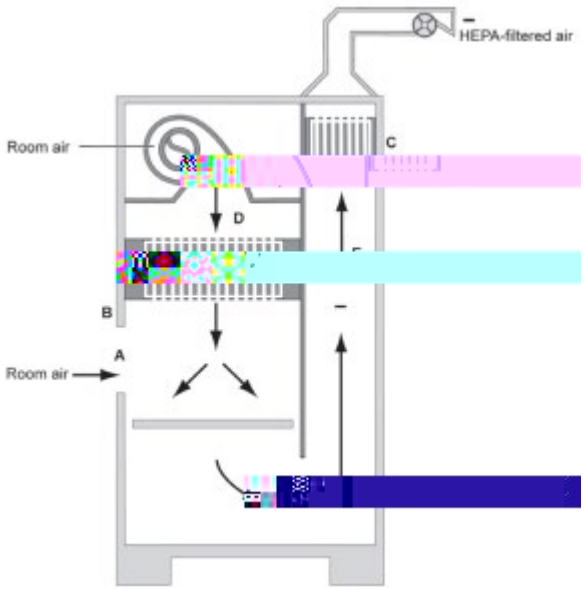


Figure 6: Class II Type B2 BSC - (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure exhaust plenum



Source: Biosafety in Microbiological and Biomedical Laboratories, 5th Edition

3. Class III Biosafety Cabinets (Figure 8)

The Class III BSC was designed for work with highly infectious microbiological agents and for the conduct of hazardous operations and provides maximum protection. They are a glove-box design (gas-tight containment) where both the supply and exhaust air is HEPA filtered. All exhaust air is discharge to the outdoors through double HEPA filters. Movement of materials in and out of the Class III cabinet requires passage through a dunk tank (filled with a chemical disinfectant) or double-door pass-through box (e.g., an autoclave) that can be decontaminated between uses. These cabinets are most appropriate for work involving Risk Group 4 agents.

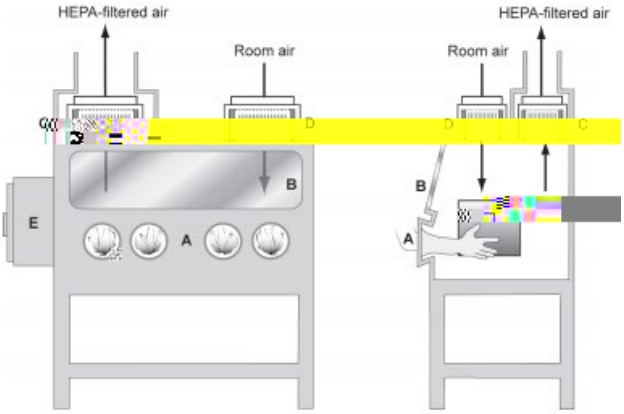


Figure 8: Class III BSC - (A) glove ports with O-ring for attaching arm-length gloves to cabinet; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) double-ended autoclave or pass-through box

Source: Biosafety in Microbiological and Biomedical Laboratories, 5th Edition

4. Guidelines for Safe Use of Biosafety Cabinets

The installation and use of a BSC is an indication that safe work practices are required in order to prevent contamination and infection. BSCs are engineered to provide excellent containment of microorganisms when used properly. They are not substitutes to good work practices and only serve to complement a safe worker in the laboratory. The following recommendations are geared towards Class II Type A2 BSCs, as they are the most commonly used BSC in laboratories, and are considered best practices that should be adhered to all times while working inside a BSC.

a. Preparing for Work

- Don applicable PPE (gloves, lab coat, safety glasses/goggles, etc.)
- If the BSC is off, turn it on and wait 5 minutes before using. Ensure there are no obstructions at either the front and/or rear air grilles.

BSC as they create airflow turbulence that may compromise personnel protection and sample sterility. Additionally, since BSCs recirculate air, the heat build-up may damage the HEPA filters. Instead, use electric devices such as loop sterilizers or incinerators, or disposable loops.

- If centrifuges, blenders, or other pieces of equipment are placed inside the BSC which may create air turbulence, they should be placed in towards the back of the

Figure 9: A typical layout for working “clean to dirty” within a Class II BSC. Source: Biosafety in Microbiological and Biomedical Laboratories, 5th Edition

- Clean all spills immediately
- Replace gloves whenever they are torn, punctured, or contaminated.
- nrsbfeoS

5. **BSC Certification Requirements**

   Biosafety cabinets are tested by the manufacturer in accordance with National Sanitation Foundation (NSF) Standard 49 criteria. All BSCs must be inspected and certified after installation, before use, and on an annual basis. The BSC must also be certified whenever it is moved and after any repairs are completed.

   The following companies can be used for BSC certification:

   - Environmental Safety Professionals (ESP) 800-688-7167 or (919) 217-2650
   - KeyStone 757-333-4988

   Annual certifications are required in order to ensure that the cabinet still performs as it did when it obtained NSF certification at the factory. NSF has established a certification program for certifiers to ensure a minimum level of competency and professionalism. It is recommended that the certifiers are trained service personnel according to National Sanitation Foundation (NSF) Standard 49, Annex F. A BSC that does not pass certification, or that is overdue for its certification, must not be used.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

PPE includes safety eyewear, laboratory coats, gloves, respiratory protection, and other equipment designed to supplement the containment provided by laboratory practices and engineering controls. PPE is considered the last line of defense after engineering controls, work practices, and administrative controls. Lab coats should be discarded or laundered periodically (e.g., weekly) or whenever they become contaminated. Cloth lab coats must never be brought home for laundering. Autoclaving these lab coats is an acceptable practice for decontaminating them for re-use. An alternative option is to establish a contract with a laundering facility who is trained to handle potentially contaminated PPE for cleaning and decontamination purposes. Disposable gloves are to be discarded after each use and must never be re-used.

FACILITY DESIGN

Facility design features include physical separation of laboratories from public access, sophisticated ventilation system to prevent airborne biological agents from migrating outside of the laboratory, location of autoclaves, security access controls, and other features developed into the design of the laboratory. These features protect both the personnel within the laboratory by reducing the potential for laboratory exposures, as well as the outside environment.

V.

Table 1: Biosafety Levels				
Biosafety Level	**Agents**	**Work Practices**	**Primary Barriers and Safety Equipment**	**Facilities (secondary barriers)**
1	Not known to consistently cause disease in healthy adults	Standard microbiological practices	• No primary barriers required. • PPE: laboratory coats and gloves; eye and face protection, as needed	Laboratory bench and sink required
2	• Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure.	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	• BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials • PPE: Laboratory coats, gloves, face and eye protection, as needed	BSL-1 plus: • Autoclave available
3*	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure •	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering	• BSCs or other physical containment devices used for all open manipulations of agents • PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory • Entry through airlock or anteroom • Hand washing sink near laboratory exit
4*				

	• Related agents with unknown risk of transmission			

*Work requiring BSL-3 or BSL-4 containment cannot be conducted at ODU

Table 2: Basis for the Classification of Biohazardous Agents by Risk Group	
Risk Group Classification	**Risk**
1	Agents that are not associated with disease in healthy adult humans
2	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available
3	Agents that are associated with serious or lethal human disease for which

facility safeguards. The Institutional Biosafety Committee will evaluate each application

or harboring infectious agents. Ideally, facilities for laboratory animals used for studies of infectious or noninfectious disease should be physically separate from other activities.

There are four escalating containment levels consisting of a combination of work practices, safety equipment, and facilities for experiments on animals infected with agents that produce, or may produce, human infection. These four combinations are designated Animal Biosafety Levels (ABSL) 1 - 4. A summary of the requirements for each animal biosafety level is found in Table 3 (pg. 25).

All vertebrate animal related activities must be reviewed and approved by the ODU IACUC prior to any work commencing. In addition to IACUC approval, all animal work involving infectious agents or acute toxins shall be reviewed by the ODU IBC. Contact the Office of Research for more information.

PRIMARY HAZARDS OF LABORATORY ANIMAL RESEARCH

1. Primary Hazards

There are three primary hazards associated with conducting laboratory animal science:

a. Physical hazards such as bites, scratches, and kicks are ubiquitously associated with laboratory animal contact. Personnel working with larger animals might sustain crushing injuries if the animals kick, fall, or simply shift their body weight. These types of injuries are largely avoidable through proper training in animal-handling techniques. Following any breaks in the skin caused by equipment within the vivarium or by a laboratory animal, proper first aid is critical to avoiding infection. Immediately wash the site with soap and water for 15 minutes. If there was a splash or splatter of material into the eyes, flush the eyes for at least 15 minutes at the nearest eye wash. Report all incidents to EH&S and your immediate supervisor.

b. Allergic reactions to animals are among the most common conditions that adversely affect the health of workers involved in animal research. Allergic hazards can be associated with breathing or contacting

vivarium as well as improved veterinary care which has helped to ensure the availability of healthy research animal populations.

Although transmission of zoonotic diseases may be uncommon, it is still critical for all individuals working with or around animals to be aware of the potential hazards. Infections of animals may sometimes produce severe disease in humans even when the animals themselves show little, if any, sign of illness. It is therefore critical to always be aware of possible consequences when working with any species of animal and to take appropriate precautions in order to minimize the risk of infection. In the event of an illness, it is important to always inform the attending physician of the occupational exposure to laboratory animals.

Table 3: Animal Biosafety Levels	
Animal	

*Work requiring ABSL-3 or ABSL-4 containment cannot be conducted at ODU

IX. Laboratory Biosafety Practices

STANDARD LABORATORY PRACTICES

Prudent practices and solid laboratory techniques are of primary importance when it comes to laboratory safety. Both are based on sound technical knowledge, experience, common sense, and a positive attitude towards safety. Unlike administrative controls, when are behaviors dictated by regulation or laboratory policy, personal protective behaviors define an innate part of each individual worker's approach to the laboratory environment. These behaviors are the most important line of defense against preventing accidents in the laboratory.

The prudent biosafety practices outlined in Table 4 are to be adhered to all times, by all individuals, in all laboratories. Strict adherence to these basic principles will greatly reduce the likelihood of laboratory acquired infections.

Table 4: Prudent Biosafety Practices

Biosafety Practice	Routes of Exposure Blocked
Never mouth pipette	Inhalation, ingestion, skin and mucous membrane contact
Always manipulate infectious fluids carefully to avoid spills and the production of aerosols and droplets	Inhalation, ingestion, skin and mucous membrane contact
Always use a biosafety cabinet for aerosol generating procedures	Inhalation, ingestion, skin and mucous membrane contact
Always adhere to safe sharps practices - minimize the use of needles, syringes, and other sharps where possible and dispose of sharps properly	Percutaneous, inhalation
Always wear personal protective equipment - lab coat, gloves, safety eye wear, and other PPE as necessary, such as respiratory protection (e.g., N-95)	Inhalation, ingestion, skin and mucous membrane contact
Always wash hands upon completion of work	

- Never eat, drink, smoke, handle contact lenses, or apply cosmetics within the laboratory, or before washing hands after conducting any work with biological materials.
-

sources of hazard information for some toxins.

c. The BMBL also contains information on biological toxins and associated safe work practices.

WRITTEN STANDARD OPERATING PROCEDURES

In combination with the 5th edition of the BMBL and *NIH Guidelines*, this manual provides a general SOP for working with biological material. However, since this manual only touches on relatively general topics, each individual laboratory must develop SOPs specific to the biosafety concerns and procedures for that particular laboratory. A laboratory specific SOP should address the safe manipulation of the pathogens present in the laboratory, specific exposure control methods, and specific decontamination and waste handling requirements, amongst other relevant topics. There is no standard format required for these SOPs and laboratories are encouraged to use any format which effectively conveys the necessary biosafety information (including tables, pictures, and illustrations). The laboratory SOPs should complement this biosafety manual and should not duplicate the same general information.

SECURITY AND INVENTORY OF BIOLOGICAL AGENTS

Each PI is responsible for ensuring that their laboratory implements sufficient security measures and procedures in order to protect against loss, theft, release, or unauthorized access to their laboratory and biological materials. When working with permissible select toxin amounts, which are concentrations below the levels which classify them as select toxins and require federal oversight, it is critical to maintain an accurate inventory log. This log provides evidence that at no time has the amount of toxin within the laboratory been over the permissible amount. This inventory log should track the toxin from date received all the way through destruction. For more information on this topic, please contact the Office of Research.

PREVENTION OF AEROSOLS AND DROPLETS

Aerosols are liquid and solid particles suspended in the air. An aerosol with a diameter of 5 microns or less can remain airborne for a long period of time, spread wide distances, and is easily inhaled. Larger particles tend to settle more rapidly and contaminate skin, bench tops, keyboards, laboratory equipment, ventilation systems, and other surfaces within and surrounding the laboratory.

High energy procedures such as centrifuging, mixing, and pipetting can produce aerosols with diameters of 5 microns or less that stay airborne for extended periods of time, while lower energy procedures such as opening containers and streaking plates may produce larger droplets that settle quickly. Whenever aerosol producing procedures are conducted within the laboratory, a biological safety cabinet (BSC) must be utilized. A properly operating BSC

will contain the aerosols and droplets that are generated during work with infectious agents.

1. Examples of Aerosol Producing Procedures

a. Shaking or vortexing tubes, stirring

b. Centrifugation steps such as filling centrifuge tubes, removing plugs or caps from tubes after centrifugation, removing supernatant, resuspending sedimented pellets, breakage of tubes during centrifugation, and centrifugation itself

c. "Blowing out" pipettes, pipetting rapidly

d. Cell sorters

e. Sonication

f. Homogenizing, blending, grinding

g. Pouring liquids

h. Removing gloves

i. Necropsies

j. Cage cleaning/changing animal bedding

k. Opening lyophilized cultures, opening snap top tubes, breakage of culture containers

l. Flaming loops or slides

Note: Open flames (e.g., Bunsen burners) must never be used within a biosafety cabinet as they will disrupt airflow and could potentially damage the HEPA filter and cause a fire. Contact the BSO for alternative solutions to open flames.

m. Pulling needles out of septums, filling a syringe

2. Safe Work Practices to Minimize the Creation of and Exposure to Aerosols

a. *Proper Utilization of Pipettes*

Pipettes are used for volumetric measurements and the transfer of liquids that may contain infectious, toxic, corrosive, or radioactive agents/materials. Pipetting may lead to exposures when liquid from a pipette is dropped onto the work surface, forcefully/rapidly expelling the liquid directly into a tube, or when the last drop of an inoculum is blown out.

- Never mouth pipette. Always use a mechanical pipetting device.
- Always use filter-barrier or cotton-plugged pipette tips.
- Never forcibly expel liquid out of a pipette.
- Use "to deliver" pipettes rather than "to contain" pipettes, which require "blowout".
- Always discharge the liquid from a pipette down the sidewall of a container,

never directly into the liquid.

- Contaminated, broken, or intact Pasteur pipettes must always be discarded in a sharps container.
- Contaminated pipettes should be placed horizontally in a pan containing an appropriate disinfectant.

b. *Types of Centrifuges*

Centrifuges are considered high-risk equipment that can produce large amounts of aerosols due to air movement and leakiness of tubes. Centrifuges can be either bench top or floor units, they can have rotors or safety cups and buckets, and they can be low-, high-, or ultra-speed.

- Mini-centrifuges

  These types are typically low or high-speed units that are only capable of holding a smaller number of tubes (e.g. less than 12). The rotors in mini-centrifuges are typically not removable and should therefore be used within a biosafety cabinet when used with infectious materials.

- Benchtop centrifuges

  These units are the most common type found in laboratories. They are high-speed, can vary in size, can be refrigerated, and have either rotors or safety cups and buckets. These can be placed towards the back of a BSC for use inside the cabinet, but must be decontaminated before removed from the BSC for any reason.

  If not used within a BSC, the rotor or safety cups must be removed, taken to the BSC, and the samples containing infectious material must be loaded/unloaded only within the BSC. The rotors or safety cups must be surface disinfected before removed from the BSC and they must have lids with proper seals that have been approved for use with infectious material. These seals must be thoroughly inspected for cracks and wear before and after each centrifugation.

- Floor unit centrifuges

  These types of centrifuges are usually high-speed units for large volumes or ultra-speed centrifuges. For use with infectious materials, they must have rotors or safety cups with proper seals, as described above, and should ideally contain a HEPA filtered exhaust. Consider adding the HEPA filtered exhaust as an option when buying new centrifuges or for even retrofitting existing centrifuges.

  Samples must be loaded and unloaded in the rotor or safety cup within the BSC. Always ensure proper balancing of tubes prior to centrifugation.

- Ultra-centrifuges

  These units present an added safety issue due to the speed at which they operate.

  Proper balancing of tubes is crucial in ultra-centrifuges as any difference in weight between tubes might cause severe damage to the rotor. Broken rotors could possibly penetrate through the centrifuge wall into the outside environment (laboratory).

  If any suspicious noises are noticed during the operation of an ultra-centrifuge, evacuate the area and if possible, turn off the centrifuge. Notify the laboratory supervisor or EH&S of the situation. Do not attempt to open the centrifuge for at least 30 minutes before opening in order to minimize potential exposure to any aerosols that may have been created. If a spill is noticed within the centrifuge after opening the lid, *slowly* close the lid and evacuate the laboratory. See Section XIII for more detailed information regarding spill response procedures within centrifuges.

c. *Proper Utilization of Centrifuge Equipment*

- All laboratory personnel must be trained on the proper use and specification of all centrifuges within the laboratory.
- High-speed and ultra-centrifuges must have an individual log book kept adjacent to the centrifuge where information such as rotor type, rotor run duration, speed, o-ring condition, any defects, and operator's name and date should be included.
- Always inspect tubes and bottles for cracks or stre s52 (t)-2 1 Tf1.5 0uif ths iom0785j7 (n i)-Tvv

not needed, use a vacuum aspirator with appropriate in-line reservoirs and filters to remove the supernatant.

d. *Inoculation Loops*

- Flaming inoculating loops can result in spatter and release of aerosols and droplets. Use of an electric incinerator will effectively control spatter resulting from sterilization of inoculating loops. Alternatively, the use of disposable loops that are discarded after each and every use can be utilized to prevent this issue.

  Note: Open flames (e.g., Bunsen burners) must never be used within a biosafety cabinet as they will disrupt airflow and could potentially damage the HEPA filter and cause a fire. Contact the BSO for alternative solutions to open flames.

e. *Use of Absorbent Materials*

- Always cover work surfaces with absorbent paper to collect splashes and splatters, and to minimize the spread of contamination. The absorbent paper should be changed regularly, such as at the completion of work, always after a spill, and at least daily.

PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE) must be provided without cost to personnel. PPE is considered to be the last-line of defense against prevention of exposure in the laboratory, and should never be a substitute for the use proper engineering controls and

lab coats can be re-used, but must be discarded when they become contaminated or whenever they are torn, ripped, punctured, etc.

b. *Gloves*

Gloves must be selected on the basis of the hazards involved and the activities to be conducted.

Standard latex examination gloves provide protection against microbiological hazards, but do not generally provide adequate protection against certain liquid chemicals. Additionally, some people may have a latex allergy or be more prone to irritant contact dermatitis when using latex gloves. Nitrile gloves are a suitable alternative to latex gloves that provide similar dexterity, protection, and increased chemical resistance, without the latex allergy hazard.

- Gloves must always be worn when working with biohazardous and/or toxic materials and chemicals.
- When working within a biological safety cabinet with infectious materials, gloves

Figure 10: This image shows one technique for proper glove removal, which avoids contamination of bare skin and/or clothing.

practices require that personal clothing or the equivalent, such as scrubs, should cover all exposed skin. It is also important to avoid loose, dangling jewelry that may get caught in equipment or make incidental contact with hazardous materials.

STORAGE AND LABELING OF BIOLOGICAL AGENTS

Biological agents must be stored using leak proof and sealed containers. These containers must be clearly labeled with the identity of the agent. It is recommended that all samples be stored in secondary containers to help prevent leakage. Secondary containers should list the

b. Cages and animal rooms used for housing animals infected with human cells or tissues

protocol involves human blood, blood products, or other potentially infectious materials (OPIM) covered by the OSHA Bloodborne Pathogen Standard. Bloodborne Pathogen (BBP) training through Environmental Health and Safety or CITI is mandatory for all employees who come in contact with blood or OPIM during the course of their work. It is the position of the CDC and OSHA that all cell lines of human origin are considered potentially infected with bloodborne pathogens, and these materials are to be handled using a minimum of BSL-2 containment and procedures. All persons working with human and non-human primate cell lines are therefore required to complete BBP training before beginning work and on an annual basis. For more information, refer to the ODU Safety Training webpage. The CITI aspect of this training must be completed annually.

d. NIH Recombinant DNA (rDNA) Guidelines (CITI)

This course must be completed by all individuals listed on an approved IBC protocol if the protocol involves rsNA work. Completion of this course is required every three (3) years.

e. *Additional CITI Training Courses*

Each laboratory should maintain training records which include the names of personnel in the laboratory and their most recent dates of laboratory safety training, including CITI, EH&S offered training, and the laboratory specific training offered by the PI or other competent laboratory member. Training records are also kept electronically by the Biosafety Officer in the Office of Research for all individuals listed on an approved protocol.

To avoid complacency in the laboratory, training should be a continuous process for all members of the laboratory. As new hazards and procedures are introduced into the laboratory, all individuals must receive an update to their training.

XI. Decontamination and Sterilization

DEFINITIONS

1. **Decontamination**
   A process or treatment which renders a device, instrument, or work surface safe to handle. A decontamination procedure can range from sterilization by autoclave to simple cleaning with soap and water. Sterilization, disinfection, and antisepsis are all forms of decontamination.

2. **Sterilization**
   The use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores. However, sterilization may not destroy prions.

3. **Disinfection**
   A less lethal process than sterilization which eliminates nearly all recognized pathogenic microorganisms, but not necessarily all microbial forms on inanimate objects. Effectiveness is influenced by the kinds and numbers of organisms, the amount of organic matter, and the object to be disinfected and chemical exposure time, temperature, and concentration**.**

TYPES OF DECONTAMINATION

Decontamination of cultures and objects contaminated by biological agents must be routinely performed within microbiological laboratories. This is a vital component of laboratory safety practice and serves to protect laboratory personnel, as well as others, from infection, and prevents the release of infectious organisms to the outside environment. The reduction of cross-contamination in the laboratory is an added benefit of decontamination.

Depending on the circumstances and tasks, decontamination of a surface (e.g., lab bench) is accomplished with a disinfectant, while decontamination of biomedical waste is done by

Both VHP and chlorine dioxide are oxidizers that are toxic and corrosive that require specialized equipment and training before using. If this level of decontamination is needed, it is typically recommended to hire a professional.

4. **Radiation**

Gamma and X-ray are two principal types of ionizing radiation used in sterilization. Their application is mainly centered on the sterilization of prepackaged medical devices.

Ultraviolet (UV) radiation is a practical method for inactivating virusV)1 (tiv)2 (a)livB(a)ly (p)eh iations stre a pro /AMC30.2702 Tc -0.002 Tw -35.44 -1.825Td[U)4-/Pa77let (u is 3(a)4 (l)h(r)5 (a)6 4 .

XII.

Note: If you choose to not seek medical care, you must still complete the BBP-2 form and select "Employee refuses post-exposure medical care". Complete remaining applicable portions of the form (name, UIN#, and signature). If you choose not to seek immediate medical care, you may do so anytime thereafter.

d. As soon as possible following the exposure, complete the BBP-1 form. This form can also be found in Appendix E of ODU's BBP Exposure Control Plan, and within the Forms and Pamphlets section of the ODU EH&S website. Once complete, forward a copy of the completed BBP-1 form to EH&S via campus mail or fax (757-683-6025).

e. Contact the University's Worker's Compensation representative at 757-683-3051 to file a report of injury.

a. Treatment with cytotoxic chemotherapeutic agents

b. Treatment with adrenocorticosteroids

c. Treatment with immunosuppressive agents/drugs or certain antibiotics

d. Disease processes that suppress the immune system

e. Pregnancy

Any individual who is aware of being immunodeficient/immunosuppressed are able to receive an appropriate medical evaluation and all communications are confidential. These individuals should be counseled as to the advisability of working in areas where the potential for exposure to potentially hazardous organisms is present. Any limitations or restrictions relating specifically to the immunodeficient/immunosuppressed condition and laboratory work shall be reported to the employee's supervisor.

XIII. Spill Response Procedures

The hazard associated with a biological spill is a function of the volume of the spill, the pathogenicity of the agent, and its concentration within the spilled material. When a spill occurs, the appropriate response should consider the protection of employees; preventing release of viable biological agents outside of the laboratory environment; and cleanup and disinfection of the area. A minor biological spill is one that the laboratory staff is capable of handling safely without any assistance from EH&S and/or the Biosafety Officer. All other biological spills are considered major and EH&S and/or the Biosafety Officer should be notified to assist.

Since biological spills will happen within the laboratory environment, it is important that each laboratory on campus be prepared to handle the situation. Laboratories working with biohazards must have a basic biological spill kit ready to use at all times. For most instances, the basic kit can be assembled from items already available within the laboratory. It is preferred to have the contents of the spill kit all in one location, such as within a dedicated bucket or drawer, where it is easily accessible to everyone in the lab, prior assembly is not always necessary. Each spill kit should contain the following items:

- Appropriate disinfectant for the biohazardous agents present in the laboratory
- Absorbent Material, such as paper towels
- Waste container (e.g., biohazard bags and sharps containers)
- PPE (e.g., extra lab coat, gloves, eye, and face protection)
- Disposable brush and dustpan or tongs/forceps for picking up broken glass

Alternatively, pre-assembled spill kits may be purchased online.

1. Biological Spill inside Laboratory

a. Clear spill area of all personnel, remove PPE and exit the area.

b.

NOTE: Always inspect tubes for cracks and stress marks before centrifuging. Check rotors and seals, specifically the "o" rings on sealed rotors. Ensure all tubes are rated properly for the desired speed at which they will be centrifuged.

4. **Biological Spill inside an Incubator**
   a. If a spill has occurred inside an incubator that is contained within the incubator, slowly close the door and notify the laboratory supte.9Mvisor and EH&S of the spill.
   b. If the spill has leaked outside of the incubator into the open laboratory, notify others in the laboratory to evacuate for at least 30 minutes and call the laboratory supte.9Mvisor and EH&S.
   c. Before re-

production of biologicals and antibiotics likely to have been contaminated by organisms likely to be pathogenic to healthy humans are regulated medical wastes.

- Human blood and human body fluids. Wastes consisting of human blood or human body fluids or items contaminated with human blood or human body fluids.
- Tissues and other anatomical wastes. All human anatomical wastes and all wastes that are human tissues, organs, or body parts are regulated medical waste.
- Sharps. Sharps likely to be contaminated with organisms that are pathogenic to healthy humans, and all needles, syringes with attached needles, suture needles, and scalpels are regulated medical wastes. This includes sharps generated through veterinary practice.
- Animal carcasses, body parts, bedding and related wastes. When animals are intentionally infected with organisms likely to be pathogenic to healthy humans for the purposes of research, in vivo testing, production of biological materials or any other reason; the animal carcasses, body parts, bedding material and all other wastes likely to have been contaminated are regulated medical wastes when discarded, disposed of or placed in accumulated storage.
- Any residue or contaminated soil, water, or other debris resulting from the cleanup of a spill of any regulated medical waste.
- Any solid waste contaminated by or mixed with regulated medical waste.

Proper handling and disposal of RMW is necessary to prevent infection of personnel (laboratory workers, custodians, laboratory visitors, etc.) and release to the environment. OSHA and Virginia DEQ regulations require that RMW be properly labeled, stored, and disposed of.

3. **Plastic Pipettes** (Serological Pipettes)
   Pipette collection:
   - Collect Plastic Pipettes in a leakproof, Poly container, double-bagged (Examples: Pipette washers tubes, 5-gallon buckets or Small trash can)
     - not mixed with other solid wastes
   - When the bags are full, remove bags, form a "log/Roll shape", close bags and place into a sterilization tray and dispose of at an approved autoclave location or Vendor provided waste cart (IRPII).
   - Prior to disposal of bagged pipettes, be sure the bags are marked as follows:
     PI's name
     Room Number

   Pipettes and will be accepted for treatment per location schedule:

   - BSSF: schedule waste drop-off
   - CHEM: availability of Autoclave
   - Health Sci: Discretion of Autoclave operator

4. **Solid Regulated Medical Waste**
   Solid RMW includes microbial agents, tissue culture, and other contaminated material such as cultures, pipettes, gloves, and any other item falling under the RMW definition listed above. These materials must be collected in double red, autoclavable biohazard bags (*serological pipettes must be bagged separately from any other solid waste*) that are labeled with the universal biohazard symbol. References to autoclavable bags can be found on the ODU EH&S Regulated Medical Waste Guidelines webpage.

Unauthorized Material

<u>The following materials should never be autoclaved – Unauthorized Material:</u>

- Flammable, reactive, corrosive, toxic, even if mixed with biological waste (not including small spills/general disinfecting)
- Solvents or volatiles
- Radioactive materials
- Non-absorbed Bleach or any liquids containing bleach (sodium hypochlorite)
- Mixed waste materials (Radiological, Chemical)
- Liquids in sealed containers
- Resin plastics that will melt (e.g., high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyethylene terephthalate (PET), or polyethylene terephthalate glycol-modified (PET-G))
- Animal carcasses/anatomical remains.

These items will be refused at RMW drop-off locations and PI/GA's will be told to contact EH&S for proper disposal.

Approved biohazard bags must be:

a. Red in color
b. Contain the universal biohazard label (at least 2" tall)
c. Contain the words "Biohazard" or "Potentially Infectious Material"
d. Contain the word "Autoclave Bag" or "Autoclavable"
e. Made of Polypropylene
f. At least 2.0 mil thick
    - Examples, as per the BSSF: Fisherbrand No. 01-828D

Unless specifically used to clean-up a biological spill, absorbent material (e.g., paper towels) should never be placed in the RMW bags. Absorbent material used to routinely clean a work surface before and after use, should be placed in the regular trash.

Loosely seal the bags with twist ties, rubber bands, or autoclave or laboratory tape when the bag is 3/4 full. Bags must **never** be sealed air-tight, as this will cause the bags to expand during autoclave, and will also restrict steam from penetrating into the bag, significantly reducing the effectiveness of the autoclave.

All bags must be double bagged and placed in approved, autoclavable sterilization trays. Each lab is required to purchase their own sterilization trays and are responsible for retrieving them once their waste has been autoclaved.

BSSF recommends the following sterilization tray:

- Thermo Scientific Nalgene Large Polypropylene Sterilizing Pan (Catalog #13-359-

and Safety (757-683-4495).

Old Dominion University is legally required to comply with the Department of Transportation (DOT) and/or the International Air Transport Association (IATA) regulations as they apply to shipping dangerous goods and hazardous materials. The intent of the packaging and transportation regulations is to prevent accidental exposure of personnel who may handle the material during its shipment. There, certain general criteria apply to all possible transportation scenarios.

Federal regulations have outlined specific shipping requirements for DG/hazardous materials. Depending upon the mode of transportation and destination, these shipments are regulated by the 49 Code of Federal Regulation parts 171-180 and /or International Air Transport Association (IATA). To comply with shipping regulations, hazardous materials must be properly classified, documented, packaged, and handled.

The following controls must be in place for the transportation of any biological materials:

- Emergency procedures (e.g., spill clean-up & disinfection protocols) and contact information must be known to the person carrying the materials
- Container must be appropriate for the material being transported and must be properly labeled
- Material must be packed for that it will stay upright during transportation
- Proper PPE must be worn during the packaging of the material
- Hands should be washed after handling materials, after the removal of gloves.
- The person packaging the material must ensure that the exterior surfaces of each package are free of any potential contamination by the packed material.

Anyone who offers for shipment any hazardous materials **must** receive the appropriate DOT/IATA training **prior** to shipping any materials. Examples of these materials include:

- Laboratory chemicals, cryogenic materials, and samples containing flammable, toxic, explosive, radioactive, oxidizer, and/or corrosive materials
- Paints, stains, refrigerants, aerosols, medicines, pesticides, disinfectants, fuels
- Equipment containing hazardous materials, such as mercury, compressed gases, batteries (wet, lithium, and dry batteries containing sodium, potassium hydroxide)
- Dry ice
- Biological/infectious materials

1. **Transportation of Materials on Campus**
   All individuals must adhere to the following requirements for the transportation of biological materials within on campus (e.g., between laboratories).

   a. At a minimum, all laboratory materials must be transported within a secondary container that is shatterproof and leak-proof. The secondary container must also be

easy to decontaminate and absorbent material should be placed inside the secondary container to absorb any spills.

b. Materials must never be carried in hands or pockets.

c. Label information must include the biohazard symbol (if risk group 2 or above) and the PIs name and laboratory on the outside of the secondary container.

d. The container must be impervious(s)-1 (kgr)3 (i)-2 (e)4 (d di)-2 (r)3 (e)-6 (c)4 (t)-2 (l)-2 (y t)-2 (o t)-2 (he)4 (

Contact ODU EH&S for guidance and assistance. EH&S has personnel with the appropriate DOT/IATA training to ensure the proper shipment of all hazardous materials. Individuals must submit a Dangerous Good Shipping Request Form at least 3 days in advance to allow ample time to obtain the supplies that may be needed for packaging and shipping.

Note: If shipping a hazardous material that is a commercially available product, it is more cost-effective in many cases to purchase the material at the location to which you wish to ship it. For example, if you are planning field-research, which required the use of hazardous chemicals, you may find it easier and less expensive to have a vendor ship the chemicals directly to the location where you will be working.

Once the proper packing has been obtained, EH&S staff will pick-up the material for shipping and will package, label, and document the shipment in accordance with all applicable regulatory requirements. Any costs related to the packaging and shipping of the material will be the responsibility of the shipper.

Penalties for non-compliance with the rules are significant and could result in fines up to $500,000 and jail sentences up to 5 years.

ODU's Mail Service Department cannot handle/transport hazardous materials.

Appendix A - Policy on the Use of Human and Non-Human Primate Cells and Tissues

1. Purpose

The purpose of this Institutional Biosafety Committee (IBC) policy is to describe the procedures and precautions that must be taken to protect personnel from exposure to human bloodborne pathogens (BBP), specifically those found in human cells and tissues, and to prevent environmental contamination, to provide an environment for high quality research while maintaining a safe work place, and to comply with all applicable federal, state, local, and university requirements.

2. Definitions

2.1. *Primary Cells*

Cells, tissues, organs, or other human/non-human primate derived materials taken directly from a subject, which is not immortalized.

2.2. *Established Cell Lines*

An “immortalized” or “continuous” cell line which has acquired the ability to proliferate indefinitely either through random mutation or deliberate modification.

2.3. *Biosafety Level 1 (BSL-1)*

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment.

2.4. *Biosafety Level 2 (BSL-2)*

BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment.

2.5. *Animal Biosafety Level 1 (ABSL-1)*

Animal Biosafety Level 1 is suitable for work in animals involving well-characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment.

2.6. *Animal Biosafety Level 2 (ABSL-2)*

ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

NOTE: Research requiring BSL-3/ABSL-3 or BSL-4/ABSL-4 containment cannot currently be conducted at ODU.

3. Background

In 1991, the Occupational Safety and Health Administration (OSHA) issued the Bloodborne Pathogen (BBP) standard to protect employees who have occupational exposure to human blood or other potentially infectious materials. While human blood, most body fluids, unfixed human tissues, and organs were clearly included within the scope and application of the standard, the inclusion of human cell lines was ambiguous.

In 1994, OSHA issued an interpretation of the applicability of the BBP standard towards human cell lines. According to the interpretation, human cell lines are considered to be potentially infectious and within the scope of the BBP standard unless the specific cell line has been characterized to be free of hepatitis viruses, HIV, Epstein-Barr virus, papilloma viruses, and other recognized bloodborne pathogens. The US Department of Health and Human services publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 5th edition, also describes the risks associated with handling human cells, tissues, blood, and body fluids and states that it is the responsibility of the institution to conduct a proper risk assessment based on the origin of the cells or tissues (e.g., species and tissue type), as well as the source (e.g., recently isolated or well-characterized).

The main risk presented by cell cultures is their ability to sustain the survival and/or replication of a number of adventitious agents. The major agents of concern are viruses, but other agents such as *Mycoplasma* spp. should also be c6uiMycshould ay

protection of the immune system.

4. Policy

Based upon the regulations, guidelines, and recommendations for handling human and non-human primate cells and tissues, the ODU IBC has adopted the following policy:

4.1. *Biosafety Level*

- All human and non-human primate cells and tissues, including both primary material and established cell lines, must be handled using BSL-2 work practices and containment. All experiments involving these materials require IBC approval prior to initiation of work.

  Note: The biosafety level designated to ATCC cell lines is strictly for the purposes of safe shipment, not laboratory containment, according to whether or not it is known that the cell line is harboring a known virus or any portion of a virus which causes human disease. ATCC further recommends handling all human and primate cultures under BSL-2 containment (Biosafety level for ATCC cultures).

4.2. *Animal Biosafety Level*

- All primary cells/tissues from human or non-human primate origin must be handled at ABSL-2, unless rigorously tested and shown to be free of human pathogens of concern. These pathogens include:
  - Mycoplasma spp., Human immunodeficiency virus 1 (HIV1), Human immunodeficiency virus 2 (HIV2), Human T-lymphotropic virus 1 (HTLV1), Human T-lymphotropic virus 2 (HTLV2), Hepatitis virus A, Hepatitis virus B, Hepatitis virus

- Established cell lines that do not

Appendix C –

Guidelines for Disposal of Regulated Medical Waste (per Autoclave location)

BSSF / MGB

- https://www.odu.edu/sites/default/files/documents/bssf-medical-waste-guidelines.pdf

Health Sciences

- Discretion of the autoclave operator

Chemistry

- https://odumain.prod.acquiasites.com/sites/default/files/documents/chem_medwaste_guidelines.pdf

IRP / CBE

- https://odumain.prod.acquiasites.com/sites/default/files/documents/irocbe_%20medwaste_guidelines.pdf