SCOTT & WHITE Healthcare | Academic Operations

INSTITUTIONAL BIOSAFETY MANUAL

Scott & White Healthcare
Academic Operations
Office of Biosafety
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1. INTRODUCTION

1.1. Overview:

This Biosafety Manual has been developed as part of the overall Scott & White Research Biosafety Program. It provides guidance in accomplishing the following goals:

- Protection of personnel from exposure to laboratory biohazards
- Protection of the environment from the release of laboratory biohazards
- Comply with all federal, state and local laws concerning biohazard use and disposal

Note: This is not a substitute for a laboratory-specific biosafety manual that is to be prepared and kept by each individual laboratory.

1.2. Review and Revision:

This Biosafety Manual will be reviewed on an annual basis and revised as necessary at that time. The review will be conducted by the Biosafety Officer and revisions will be approved by the Scott & White IBC. Any revision/change requests should be submitted to the Biosafety Officer.

1.3. Emergency Phone Numbers and Safety Office Contacts:

1.3.1. Emergency Phone Numbers

Ambulance/Fire/Police	24-2000
Employee Health Services	24-2934
Biological/Chemical Emergencies	254-771-4804 (Mon – Fri 8:00 am – 5:00 pm) 404-661-2647 or 24-2000 (after hours)

1.3.2. Safety Office Contacts

Francis J Novembre, PhD, Biosafety Officer	254-771-4804(office) 404-661-2647(cell)

1.4. Purpose:

The purpose of the Scott & White Biosafety Program is to:

- Ensure compliance with federal, state and local laws concerning the use, storage, and disposal of biohazards.
- Provide training in biosafety for PIs, laboratory workers and administrative personnel.
- Provide a guide for PIs and laboratory workers to safely assess the risk of biohazards present in the lab and to develop specific SOPs for working with biohazardous material.
- Provide a mechanism for registering and reviewing biohazardous agents and work that is being conducted at Scott & White.

1.5. Scope:

The Biosafety Program applies to all Scott & White research personnel whose occupational tasks or responsibilities include the handling and manipulation of biohazardous materials (including recombinant DNA, toxins, infectious agents, human-derived cells and tissues, non-human primate-derived cells and tissues, and nanomaterials).

1.6. Roles and Responsibilities:

Biosafety is a cooperative effort between Scott & White, its employees, and affiliate organizations. The Biosafety Officer, the Institutional Biosafety Committee (IBC), PIs, technicians, students, postdocs, and administrative personnel all must work in concert to minimize the risk of exposure, injury or illness associated with activities involving potentially biohazardous material.

The Biosafety Program is managed by the Biosafety Officer, with oversight provided by the IBC. Major policy changes require approval by the IBC.

Each person that may be involved in the use of biohazardous material has a responsibility to fulfill with regard to the implementation and maintenance of the Biosafety Program. This section outlines these responsibilities:

1.6.1. Institutional Biosafety Committee (IBC):

The IBC was chartered by Scott & White to oversee research with biohazardous material on the Temple campus, including any work with recombinant DNA. The responsibilities of the IBC are:

1. Approve and implement policy and procedures, which provide guidance for activities involving potentially biohazardous materials.
2. Ensure that biosafety policies, practices and facilities meet federal, state and local regulatory requirements and follow Institutional-accepted practices.
3. Review research proposals (Initial Review Application via IRIS) involving potentially biohazardous material or toxins (including recombinant DNA).
4. Reports any significant problems or violations of NIH Guidelines to the NIH Office of Biotechnology Activities as well as any significant research-related accidents and illnesses involving recombinant DNA research.

1.6.2. Biosafety Officer (BSO):

The responsibilities of the Biosafety Officer include:

1. Review activities and facilities for proper biohazard control; apply relevant laws, standards and guidelines.
2. Take measures necessary to ensure that all biohazardous activities comply with the policies and practices established by the IBC.
3. Provide specific training to PIs, supervisors, postdocs, students, and other laboratory workers in proper laboratory safety and other training that may be required per CDC, NIH, OSHA or other governmental agencies.
4. Report any significant problems, trends, and non-compliance violations of regulations, policies or practices to the IBC.
5. Assist the PI and laboratory staff in identifying potential biohazards and relevant risks (including protocols, techniques and practices), and in selecting potential alternatives, appropriate personal protective equipment (PPE) and use of other exposure controls.
6. Interact with PIs to determine weaknesses and deficiencies within the Biosafety Program and work to correct.
7. Respond to any exposures/accidents involving biohazardous material. Support follow-ups to these incidents and assist the PI with investigations.
8. Assist and advise facilities administrators on engineering controls for laboratory modifications and for new laboratory construction.

9. Advise the IBC, PIs, and laboratory workers on biohazard security, biosafety, and technical compliance issues.
10. Organize and conduct annual laboratory inspections and post-approval monitoring.

1.6.3. Executive Management

1. Management is responsible for maintaining Institutional safety and compliance with the Biosafety Program.
2. Management has the responsibility to support the BSO, IBC, and PIs in implementing the provision of the Biosafety Program within their divisions/departments.

1.6.4. Institutional Official (IO)

1. The IO is appointed by the CEO of Scott & White and acts as to ensure that research involving recombinant DNA is conducted in full compliance with the provisions of the NIH Guidelines.

1.6.5. Principal Investigators/Primary Supervisors

The PIs/Primary Supervisors are responsible for biosafety in their laboratory(ies). They shall:

1. Ensure that all work is conducted in accordance with established policies and guidelines described in this document.
2. Ensure that all employees under his/her supervision are adequately trained in good microbiological practices and have received safety training appropriate to the work conducted in the laboratory.
3. Develop, review and approve laboratory-specific and/or protocol-specific procedures, consulting with the BSO when necessary.
4. Provide training and relevant information to all employees regarding biohazards that are present in the lab.
5. Develop risk assessments for specific biohazards in the laboratory (in consultation with the BSO when necessary), and ensure that all employees have access to the assessments.
6. Ensure that employees are aware of any special requirements, such as vaccinations, required to work with and around specific biohazards.
7. Ensure prompt reporting of any job-related injuries, exposures or illnesses.
8. Inform the Department Chair and the BSO of any serious, or potentially serious, accidents, or incidents involving exposure to biohazardous materials; including accidental releases and illnesses.
9. Act, in a timely manner, upon requests and/or directives from the IBC or BSO concerning laboratory safety and work with biohazards.
10. Ensure that appropriate containment devices and other engineering controls are in place, operating correctly and certified (if necessary) for use; and ensure that employees have been trained to use such equipment in a proper manner.
11. Ensure that appropriate PPE is available, that the employees are adequately trained in donning and doffing, and that the PPE is being utilized.
12. Ensure that proper decontamination of the laboratory and/or equipment is conducted prior to any needed inspections, calibrations or repairs.
13. Ensure proper disposal of all biohazardous material, including any sharps.
14. Keep an up-to-date inventory of biohazards and respective amounts.
15. Maintain proper labeling of the labs.

1.6.6. Employees/Lab Workers

All employees performing work with biohazardous material must accept a shared responsibility for conducting their work in a safe manner. Ultimately, each individual is responsible for his or her own safety. Employees/lab workers must also:

1. Ensure that all work is conducted in accordance with established policies and guidelines described in this document and/or via specific laboratory SOPs.
2. Report all hazardous conditions to the PI and/or the BSO.
3. Report any job-related injuries, exposures, or illnesses to the PI and/or BSO and seek medical treatment immediately.
4. Refrain from operating any equipment or instrument without proper instruction and/or training.
5. Wear and maintain personal protective equipment necessary for each task.
6. Properly utilize engineering controls.
7. Participate in required training programs.

1.7. Biosafety Requirements

Appendix A shows a chart for determining requirements of PIs and lab personnel based upon the use of various agents in the laboratory. Details of requirements are further described in the subsequent sections.

1.7.1. Registration for the use of Biohazardous Materials

All Principal Investigators working with potentially hazardous biological agents (human or nonhuman primate cells, tissues, OPIM; viruses; bacteria; fungi; rickettsia; parasites; prions; toxins; recombinant/synthetic DNA; nanomaterials) are required to complete and submit an Initial Review Application via iRIS. The Biosafety Office must maintain accurate information regarding the use of biohazardous material by Scott & White personnel. Safety Office policy requires an annual update to the IBC registered/approved protocol and a new Initial Review Application to be submitted every three (3) years. Additionally, if any changes occur in agents, funding, personnel, or procedures, the PI is required to file an amendment to the application.

Most work with biological agents will not require official review of the Initial Review Application by the IBC. However, the IBC is required to review all work with recombinant/synthetic DNA molecules as directed by the NIH Guidelines for Research Involving Recombinant DNA Molecules (see below and OBA - NIH Guidelines). The IBC does reserve the right to review any research with agents listed in Risk Group 2 or higher. Also, prior to work starting in the laboratory, there are annual personnel training requirements that need to be fulfilled, as well as an annual laboratory inspection to be conducted by the Safety Office (see below).

The review process for registration is described in detail within the IBC policies and procedures (available on the IBC website).

1.7.2. Recombinant/Synthetic DNA Experiments

As indicated above, approval from the Scott & White IBC is required before initiation of most non-exempt recombinant DNA experiments. The PI should complete the Initial Review Application via iRIS

for exempt and non-exempt work with recombinant DNA. Exempt work does not require approval of the IBC, but the IBC does require registration of experiments.

1.7.3. Human Gene Transfer

Proposed clinical trials involving human gene transfer require the approval of federal agencies as well as the Institutional Biosafety Committee for Scott & White Healthcare Human Subject Research prior to initiation.

1.7.4. Human Blood, Body Fluids, Tissues, and Other Potentially Infectious Materials (OPIM)

The Occupational Safety and Health Administration has created the Bloodborne Pathogens Standard (29 CFR Part 1910.1030) to minimize or eliminate exposure to infectious agents that may be present in human blood, tissues or body fluids (bloodborne pathogens). The Standard applies to all employers that have employees that are occupationally exposed to human blood or OPIM. OPIM include:

- Human cell or tissue cultures
- Any unfixed tissue or organ, other than intact skin from a human
- Human body fluids, except urine, feces, saliva, or tears, unless visibly contaminated with blood
- Organ cultures
- HIV- or HBV-containing fluids
- Blood, organs, fluids or tissues from experimental animals infected with bloodborne pathogens

The employer is required to develop a bloodborne pathogen exposure control plan that outlines who may be occupationally exposed and how to minimize or eliminate exposures. All employees that are determined to be occupationally exposed are required to attend a bloodborne pathogen training session prior to beginning work, and then annually thereafter. Scott & White's exposure control plan is available online.

1.7.5. Research Animals

All experiments involving animals must be conducted in accordance with established federal laws and guidelines and must be approved by the Scott & White Institutional Animal Care and Use Committee (IACUC), prior to initiation. Animal research that involves a biological hazard must also be approved by the IBC.

1.7.6. Biological Safety Cabinets (BSCs)

BSCs are required for working with agents classified in Risk Group 2 and above and are required for use when work will involve the potential generation of aerosols and splashes. BSCs protect the worker, the material, and the environment from potential exposures. Personnel that utilize BSCs must be properly trained by the PI or the laboratory supervisor. Alternatively, training can be provided through the biosafety office if requested.

PIs should notify the BSO if planning on purchasing, moving, transferring or discarding a BSC. BSCs require annual certification.

1.7.7. Training

Training is an essential part of a comprehensive biosafety program. All laboratory workers should have general laboratory safety training and, if necessary any specialized training related to the work they are going to perform. The biosafety office provides general laboratory safety training for all lab workers.

Additionally, the biosafety office provides classes on BSL2 laboratory safety for those workers that will be working under those conditions, as well as those laboratory workers who will be present within a BSL2 laboratory. All laboratory workers need to demonstrate good microbiological practices. For specific work with specific agents, the PI is responsible for providing appropriate training. If so desired, the BSO can work with the PI on developing specific training regimens appropriate for the agent.

Personnel that work directly with animals will need additional safety training that deals specifically with animal work. The biosafety office will provide this training.

Finally, the biosafety office will also provide annual blood-borne pathogen (BBP) training to all laboratory workers who may be exposed to human blood, unfixed tissues, or other potentially infectious material (OPIM) from humans.

Table 1 below shows information on required training:

TABLE 1. Biosafety Training

Before initiating work involving:	You must satisfactorily complete the following training:	Training Options
Human blood, tissue, bloodborne pathogens, OPIM (including human cell lines and animals infected with bloodborne pathogens)	Bloodborne Pathogen Training: • Required before initiation of work and • At least annually thereafter	• Online training for Scott & White employees • Classroom course taught by the Scott & White Research Safety Office (Courses scheduled throughout the year)
Laboratory work at BSL1	Laboratory Safety Training: Chemical Safety Good Microbiological Practices • Required before initiation of work and • At least annually thereafter	Classroom course taught by the Scott & White Research Safety Office (Courses scheduled throughout the year)
Human or animal pathogens classified at BSL2	Biosafety Level 2 Training: • Required before initiation of work and • At least annually thereafter	Classroom course taught by the Scott & White Research Safety Office (Courses scheduled throughout the year)
Packaging, Shipping, Tranpsorting or Receiving Biohazards (Infectious agents, hazardous biological toxins, and human clinical specimens)	Shipping and Transport Training: • Required every 2 years	• Classroom course taught by the Scott & White Research Safety Office (Courses scheduled throughout the year) • Safety Office approved Shipping training guide
Work with research animals	Animal Safety Training	Classroom course taught by the Scott & White Research Safety Office (Course in development and will be scheduled during the year)

2. BIOSAFETY

2.1. GENERAL PRINCIPLES

Biological Safety, or biosafety, is defined as: The application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards. Biosafety defines the containment conditions under which infectious agents can be safely manipulated. The objective of containment is to confine biohazards and to reduce the potential exposure of the laboratory worker, persons outside of the laboratory, and the environment to potentially infectious agents. This can be accomplished through two main means:

- Primary Containment – the protection of personnel and the immediate laboratory environment through good microbiological technique (laboratory practice) and the use of appropriate safety equipment.

- Secondary Containment – the protection of the environment external to the laboratory from exposure to biohazardous material or other biohazards through a combination of facility design and operational practices.

Combinations of labaoratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Currently four Biosafety Levels (1-4) define the level of containment necessary to protect personnel and the environment. Biosafety Level 1 (BSL-1) is the least restrictive, while Biosafety Level 4 (BSL-4) requires a special containment laboratory or facility.

The recommended biosafety level(s) for an organism or toxin represents the conditions under which the agent can ordinarily be safely handled. **The laboratory PI is specifically and primarily responsible for assessing risks and for appropriately applying the recommended biosafety level(s)** (see Assessments: Section 2.2).

The most important element in maintaining a safe work environment is strict adherence to good microbiological and laboratory practices and techniques. Most laboratory-acquired infections (90%) have occurred because of non-adherence to proper procedures, including good microbiological practices. Everybody working with infectious agents or potentially infected materials must be aware of the potential risks. In addition, they must be trained and proficient in the practices and techniques required for handling such material. It is the responsibility of the Principal Investigator or laboratory supervisor to provide and/or arrange for appropriate training of personnel in their laboratory.

2.2. BIOLOGICAL RISK ASSESSMENTS:

The assessment of risk is an essential element of safety in the laboratory. It is incumbent upon the PI to perform a risk assessment prior to beginning any work with the agent in the laboratory. Questions concerning the appropriate safety equipment, immunizations, training and waste disposal need to be addressed as well as safe procedures and practices. One of the most helpful tools utilized for risk assessment is the risk group characterization of agents (see above). However, simply relying on the risk group classification of an agent for an assessment does not address the complete picture for laboratory risk. Additional factors that must be considered include:

- Pathogenicity of the agent **and** the infectious dose
- Potential outcome of an exposure incident (local and community wide)
- Natural route of infection (as well as the ability to infect by other routes)
- Stability of the agent in the environment
- Concentration and volume of the agent being manipulated
- Availability of a suitable host
- Information from other laboratory, clinical and animal studies with the agent

- Activities planned in the laboratory for the agent
- Availability of appropriate engineering controls
- Any genetic manipulations of the agent that may alter its biology (including transmissibility, host range, therapeutic susceptibility, etc.)
- Availability of effective prophylaxis or post-exposure therapy

For most agents, guidelines, rules and regulations have clearly defined the procedures and practices to be followed in order to achieve safety in the work place. However, in cases of the newly isolated agents or toxins, or procedures not previously employed, further evaluation is needed due to the limited availability of information. Since individual judgment involves both personal and social values, opinions on what is "safe" vary significantly. In order to find a common ground for an acceptable risk assessment, the "rule of reason" needs to be applied. The following factors should be considered for the determination of what is reasonable:

1. **Custom of usage (or prevailing professional practice):** Many laboratory procedures involve the maintenance of sterility and cleanliness. These procedures are commonly considered safe, since adverse effects would have been obvious over time. (Caution: because a procedure has been used for many years does not necessarily imply that it is a good practice. An example is mouth pipetting, which was used for centuries and finally considered very unsafe.)
2. **Best available practice, highest practicable protection, and lowest practicable exposure:** It should be common practice in the microbiological laboratory to use the best available procedures with the highest level of protection. This not only provides for a safe work environment but also fosters excellence in scientific conduct.
3. **Degree of necessity or benefit:** The common question to ask is, are the benefits worth the risk? For example, there is no need to use a human pathogen causing severe gastroenteritis when general microbiological practices can be taught with a noninfectious organism.
4. **No detectable adverse effects:** This can be a very weak criterion since it involves uncertainty and should be applied accordingly.
5. **Principal knowledge:** At times, existing procedures are modified, involving the same or similar toxic chemicals or agents. For that reason, similar safety procedures should be applied. If new agents are isolated, we need to ask what we know about the close relatives. Many agents of known etiologic character are already categorized in risk groups, allowing for the selection of the appropriate biosafety level. New isolates from infected animals or humans with known infectious relatives warrant, at a minimum, the same level of protection.

Taking the above mentioned factors, as well as others, into consideration will allow for a reasonable approach to a new challenge. The BSO is available to assist in this process and should be contacted with questions. Once a risk assessment is completed, the results should be communicated to everyone involved in the process. If necessary, written standard operating procedures (SOPs) that are laboratory specific should be established.

Sandia National Laboratories provides a free tool to assist in risk assessments. It is termed the BioRAM and is available here: http://www.sandia.gov/ram/BIORAM.htm.

2.3. RISK GROUPS AND BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

2.3.1. Classification of Infectious agents on the basis of hazard (Risk Groups)

Globally, there are a number of systems in place to classify pathogens on the basis of risk/hazard. In general, these classification systems are based upon the relative pathogenicity of the organism, that is, how hazardous that organism is to humans/animals. For the USA, the current classification system is found in the NIH Guidelines for Research Involving Recombinant DNA Molecules (Risk Group Classification of Human Etiologic Agents). Biological agents are classified into one of four risk groups (RG; RG-1 through RG-4, with RG-4 being the highest hazard. Table 2 lists the basis for classification of these risk groups.

TABLE 2. Biological Risk Groups

Risk Group	Risk to the Individual and the Community	Examples
Risk Group 1 (RG-1)	A biological agent that is unlikely to cause disease in healthy workers or animals	E. coli K12 derivatives
Risk Group 2 (RG-2)	Agents that can cause human or animal disease but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment and for which preventative or therapeutic interventions are often available	Adenovirus, Hepatitis B virus, Herpes Simplex Virus 1 and 2, Influenza virus, Listeria monocytogenes, Clostridium tetani, Pseudomonas aeruginosa
Risk Group 3 (RG-3)	Agents that are associated with serious or lethal human or animal disease for which preventative or therapeutic interventions may be available (high individual risk, low community risk)	HIV, HTLV-1, VSV, Prions, Rickettsia, Mycobacterium tuberculosis
Risk Group 4 (RG-4)	Agents that are likely to cause serious or lethal human or animal disease for which preventative or therapeutic interventions are usually not available (high individual risk, high community risk)	Lassa virus, Ebola virus, Marburg virus

2.3.2. Biosafety Levels

Biosafety levels describe the conditions (engineering controls, administrative controls, PPE, and SOPs) under which organisms are manipulated. The following is a brief description of the biosafety levels as defined in the CDC-NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Edition manual 2009 (BMBL). For more detailed information regarding the requirements for the different containment levels, contact the Biosafety Officer or refer to the BMBL (see ***Table 3*** for a summary of Biosafety 1-3 containment criteria).

2.3.2.1. Biosafety Level 1

Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing. Biosafety level 1 (BSL-1) practices, safety equipment, and facilities are appropriate for work that is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. *Bacillus subtilis, Naegleria gruberi*, and infectious canine hepatitis virus is representative of those microorganisms meeting these criteria. Many agents not

ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Animal pathogens can infect other susceptible hosts, within same animal host species or different. Vaccine strains which have undergone multiple in vivo passages should not be considered avirulent simply because they are vaccine strains.

2.3.2.2. Biosafety Level 2

Primary hazards to personnel working with BSL-2 agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of biohazardous materials. Extreme precaution with contaminated needles or sharp instruments must be emphasized. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or devices such as a BSC or safety centrifuge cups. Other primary barriers should be used as appropriate such as splash shields, face protection, gowns, and gloves. Biosafety level 2 (BSL-2) practices, safety equipment, and facilities are applicable for work which is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human or animal disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, the Salmonella, and Toxoplasma spp. are representative of microorganisms assigned to this containment level. Biosafety Level 2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. This also applies to animal tissues or blood when the presence of an infectious agent is unknown. Personnel working with human-derived materials should refer to the Bloodborne Pathogens Exposure Control Plan for specific, required precautions. Secondary barriers such as hand washing and waste decontamination facilities must be available to reduce potential environmental contamination.

2.2.2.2.a. Biosafety Level 2+

BSL2+ is not specifically described in written documents, but refers to work done at BSL2, but with enhanced practices. These practices include, but are not limited to: Use of a double-door entry into the laboratory; use of disposable wrap-around gowns instead of lab coats; use of double-gloving of hands; all centrifugation should be conducted in closed containers using sealed buckets; laboratory should have negative pressure with respect to the hallway; sharps use is eliminated (including use of glass); and all waste is autoclaved prior to disposal.

2.3.2.3. Biosafety Level 3

Biosafety level 3 (BSL-3) practices, safety equipment, and facilities are applicable for work which is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Mycobacterium tuberculosis, St. Louis encephalitis virus, and Coxiella burnetii are representative of microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

At Biosafety Level 3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially

infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and a specialized ventilation system that minimizes the release of infectious aerosols from the laboratory.
BSL-3 facilities do not currently exist on the Scott & White campus, thus research with any BSL-3 agents is prohibited.

2.3.2.4. Biosafety Level 4

Biosafety level 4 (BSL-4) practices, safety equipment, and facilities are applicable for work with dangerous and exotic agents which pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route, and for which there is no available vaccine or therapy. Additionally, agents with a close or identical antigenic relationship to Biosafety Level 4 agents should also be handled at this level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at Biosafety Level 4. Agents in BSL-4 require very specific facilities only available at certain institutions.
Research involving agents requiring BSL-4 containment is strictly prohibited at Scott & White.

Table 3: Summary of Biosafety Levels

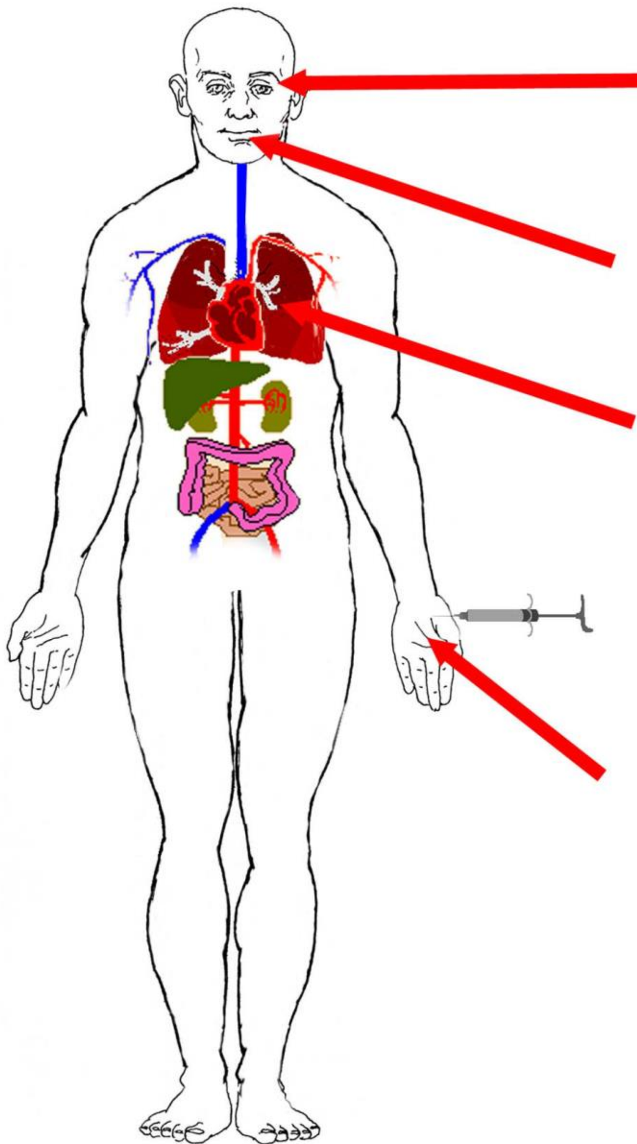
BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Laboratory bench and sink required
2	Agents associated with human disease Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practices plus: Limited access Biohazard warning signs Sharps precautions Biosafety manual needed defining any needed waste decontamination or medical surveillance policies	Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPEs: Lab coats, gloves, face/eye protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents	BSL-2 practices	Class I or II BSCs	BSL-2 plus:

	with the potential for aerosol transmission Disease may have serious morbidity and/or mortality	plus: Controlled access Decontamination of all waste	PPE: Protective laboratory clothing, gloves, respiratory protection as needed Decontamination of laboratory clothing before laundering Baseline serum	Physical separation from access corridors Self-closing, double-door access Exhaust air not recirculated Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of lifethreatening disease Aerosol-transmitted laboratory infections have occurred; or related agents with unknown risk of transmission	BSL-3 practices plus: Clothing change before entering Shower on exit All material decontaminated on exit from facility	Primary barriers: All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, airsupplied, positive pressure personnel suit	BSL-3 plus: Separate building or isolated zone Dedicated supply and exhaust, vacuum, and decontamination systems

Determining the actual Biosafety Level that should be utilized for experiments is part of the overall risk assessment (see below) undertaken initially by the PI. For example, HIV is a RG-3 agent, but in normal experimentation, HIV can be handled under BSL-2 levels (as long as large quantities (>10 liters) of virus are not prepared in the lab.

2.4. ROUTES OF INFECTION

When working in a biological research environment, it is not unreasonable to expect that a laboratory person working with infectious materials is more likely to become infected than members of the general community are. An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route and overcome the body's defense system. The following routes of infection have been reported for laboratory-acquired infections:



Mucous membranes: Exposures to mucous membranes of the eyes, nose, mouth and cuticles through splashes or splatters

Ingestion: Mouth pipetting, eating, drinking, smoking in the lab

Inhalation: Breathing in respirable sized aerosols (<5 mm), centrifuge leaks, spills, pipetting, etc

Percutaneous: Through intact or non-intact skin via needlestick, puncture with contaminated sharp object, animal scratch or bite, through wounds, abrasions, or eczema

Contact (indirect transmission): Via mucous membranes or non-intact skin from hands that have been in contact with a contaminated surface (i.e. benches, phones, computers, equipment, etc) or by failure to wash hands after working.

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Most of the laboratory-acquired infections reported in the literature point to spills, splashes and accidents involving needles or other sharp objects as the source of exposure. The general laboratory procedures outlined in this manual address those issues and provide guidance in handling infectious or potentially infectious materials. Table 4 lists protective measures that can be implemented to prevent exposure to the various routes of transmission.

Table 4. Protection for Routes of Infection

Route of Exposure	Protective Measures
Mucous Membranes: Exposure via the mucous membranes of the eyes, nose, mouth or cuticles due to splash/splatter	Wearing a full face shield Working in a biosafety cabinet or behind a protective shield Following good microbiological practices Wearing gloves
Ingestion: Mouth pipetting, eating, drinking, smoking in the lab	Good microbiological practices, no mouth pipetting
Inhalation: Breathing in respirable sized aerosols (<5 mm), centrifuge leaks, spills, pipetting, etc	Use of the Biosafety Cabinet, sealed rotors or canisters for centrifuges, safety containment equipment, HEPA filtered respirator, and good microbiological practices.
Percutaneous: Through intact or non-intact skin via needlestick, puncture with contaminated sharp object, animal scratch or bite, through wounds, abrasions, or eczema	Substitute plastic for glass. Use extreme precautions with sharps, dispose of immediately in rigid leak-proof sharps container, use animal restraints, cut-resistant gloves, sleeve covers, waterproof bandages, and double gloves, good work practices.
Contact (indirect transmission): Via mucous membranes or non-intact skin from hands that have been in contact with a contaminated surface (i.e. benches, phones, computers, equipment, etc) or by failure to wash hands after working.	Decontamination of work surfaces and hand washing. Good personal hygiene (avoid touching your face with glove or non-gloved hands), do not apply cosmetics within the laboratory.

3. ANIMAL BIOSAFETY

3.1. BIOSAFETY AND ANIMALS-INFECTIOUS DISEASE WORK WITH VERTEBRATES

Laboratory facilities must provide containment for laboratory animals exposed to or harboring infectious agents. The containment provided and the biosafety level must be appropriate to the risk level of the infectious

agents involved. In addition to facility requirements, special equipment (e.g. filter cages, partial or isolation caging systems) may be used (refer to **Table 5 below**).

Laboratory animal facilities are simply a special type of laboratory. As a general principle, the biosafety level (facilities, practices, and operational requirements) that is recommended for working with infectious agents *in vivo* and *in vitro* is comparable. However, the animal room can present some unique problems. In the microbiological laboratory, hazardous conditions are caused by personnel or by the equipment being used. In the animal room, the activities of the animals themselves can present new hazards. Animals may generate aerosols, they may bite and scratch and they may be infected with a zoonotic disease.

Scott & White Research will follow the animal biosafety guidelines outlined in CDC-NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Edition manual. 2007 (BMBL). For more detailed information regarding requirements contact your Biosafety Officer or refer to the BMBL. **Table 5** summarizes the requirements of Animal Biosafety Levels (ABSL) 1-4.

The BMBL presupposes that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g. Guide for the Care and Use of Laboratory Animals and Laboratory Animal Welfare Regulations) and that appropriate species have been selected for animal experiments. In addition, the BMBL assumes that the company has in place an occupational health and safety program and references the recent publication of Institute of Medicine, Occupational Health and Safety in the Care of Research Animals. Scott & White Research has established guidelines for general laboratory animal handling in response to this publication. These guidelines can be found in the document ***Handbook of Policies and Procedures*** maintained on the Scott & White IACUC website by the Department of Comparative Medicine. All animal work shall be reviewed and approved by the Scott & White IACUC prior to work beginning. In addition to IACUC approval, all animal work involving infectious agents or acute toxins directed by Scott & White researchers must be reviewed and approved by the Biosafety Officer or designee of the IBC. Other policies and procedures may come into play when doing animal research. Please contact the IACUC Office for more information.

Table 5: Animal Biosafety Levels

ABSL	AGENTS	PRACTICES	SAFETY EQUIPMENT (PRIMARY BARRIERS)	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause disease in healthy human adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species	Standard animal facility • No recirculation of exhaust air • Directional air flow recommended • Hand washing sink is available
2	Associated with human disease	ABSL-1 practices plus:	ABSL-1 equipment plus:	ABSL-1 facility plus:

	Hazard: percutaneous exposure, ingestion, mucous membrane exposure	• Limited access • Biohazard warning signs • Sharps precautions • Biosafety manual • Decontamination of all infectious waste and of animal cages prior to washing	• Containment equipment appropriate for animal species • Class I, II, III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols PPE: • Laboratory coat, gloves, face and respiratory protection as needed	• Autoclave available • Hand washing sink available • Mechanical cage washer recommended
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious health effects	ABSL-2 practices plus: • Controlled access • Decontamination of clothing before laundering • Cages decontaminated before bedding removed • Disinfectant foot bath as needed	ABSL-2 equipment plus: • Containment equipment for housing animals and cage dumping activities • PPE: appropriate respiratory protection	ABSL-2 facility plus: • Physical separation from access corridors • Self-closing, double-door access • Sealed penetrations • Sealed windows • Autoclave available in facility
4	Dangerous/exotic agents that pose high risk of life threatening disease; aerosol transmission, or related agents with unknown transmission	ABSL-3 practices plus: • Entrance through change room where personal clothing is removed and laboratory clothing is donned • Shower on exiting	ABSL-3 equipment plus: • Maximum containment equipment (i.e. Class III BSC or partial containment equipment in combination with full	ABSL-3 facilities plus: • Separate building or isolated zone • Dedicates supply and exhaust, vacuum and

		• All wastes are decontaminated before removal from facility	body, air-supplied positive-pressure suit) used for all procedures and activities	decontamination systems • Other requirements outlined in text

PREVENTING TRANSMISSION OF ZOONOTIC DISEASES

Risks for Those Who Handle Animals and Their Tissues

Hazards associated with the handling of animals fall into three basic categories:

1. Physical injuries can occur from bites or scratches (rodents, rabbits, dogs, cats, swine, nonhuman primates and others), kicks or other direct injuries. The key to prevention of these types of injuries is proper animal handling training of personnel by the animal care staff or other qualified individuals.
2. Allergic hazards can be associated with breathing or contacting allergens found in animal dander or urine. Though some persons are much more susceptible than others, all employees can reduce their risk by wearing protective clothing (such as safety glasses, respirators, gloves and a lab jacket) when handling animals. Additional precautions may be posted on the animal room door.
3. There is the potential for transmission of zoonotic diseases between animals and humans. Although zoonotic diseases are not common in modern laboratory facilities, the prevention, detection and eradication of zoonotic diseases from the animal facility is a primary concern of the entire animal care staff. The risk for zoonotic diseases may be increased in farm situations. **Remember that infected tissues, body fluids/secretion/excretion as well as the living animals can frequently transmit zoonotic diseases.**

3.2. OVERVIEW OF ZOONOTIC DISEASES

Humans can be susceptible to infectious diseases that affect animals. Infections of animals may sometimes produce severe disease in humans even when the animals themselves show little, if any, signs of illness. A pathogen in the normal flora of a healthy animal may cause a serious disorder in a person exposed to it because the animal has developed resistance to these microorganisms, whereas humans with no previous exposure to the agent lack this protective immunity. Therefore, one should always be aware of possible consequences when working with each species of animal and take precautions to minimize the risk of infection. In the event that an employee becomes ill with a fever or some other sign of infection, it is important to let the physician know that he/she works with animals.

The Scott & White *Department of Comparative Medicine Handbook* provides general guidance for working with laboratory animals. This document is based on the National Research Council publication "Occupational Health and Safety in the Care and Use of Research Animals."

3.2.1. *Special Considerations for Pregnant Employees*

Employees who become pregnant (and who work with animals) should contact Employee Health Services as soon as possible for a consultation.

Toxoplasma is an infectious agent found primarily in cat feces and infected meat. It can infect the unborn fetus in women exposed during pregnancy who do not have immunity to the agent. Asymptomatic toxoplasma infection is common before childbearing years and many women have elevated antibody levels indicative of immunity. To help assess the level of immunity against this agent, serum samples can be tested prior to pregnancy. Cat feces should be avoided and gloves should be worn when working in areas potentially

contaminated with cat feces. Thorough hand washing after handling any potential source of infection is also necessary.

Listeriosis, a bacterial disease, can occur in small laboratory animals, farm animals and humans. Stress or pre-existing illness can contribute to susceptibility. Infection can cause acute febrile illness in pregnant women, followed by abortion, stillbirth, or seriously ill premature infants. It can be acquired by coming in contact with infected fetal membranes and feces, or ingesting milk, especially of stressed animals. It is primarily prevalent in farm animals, including sheep and goats.

3.3. Working with nonhuman primates, their tissues and blood

Nonhuman primates present a number of unique challenges in biosafety. Because of their size and agility, persons working with them are at a higher risk of exposures through biting, scratching, and contact with bodily fluids. Nonhuman primates also have species-specific viruses that, while being fairly benign to the nonhuman primate, can have significant morbidity and mortality effects on humans. Additionally, depending upon the species, nonhuman primates may be susceptible to infection with a number of human pathogens, thus can act as a transmitter to persons working with these animals.

3.3.1. Herpes B Virus

Although there are a number of nonhuman primate agents that can cause disease in humans, Herpes B virus (Macacine Herpesvirus 1) is the pathogen of most concern to persons working with macaque species or their tissues.

Herpes B virus is a neurotropic herpes virus indigenous to the macaque species (incl. rhesus, cynomolgus, pig tailed, and stump tailed). B virus infection in macaques is similar to Herpes simplex virus infection in humans—it can be associated with lesions or, in most cases, is sub-clinical. However, there is no correlation between presentation of lesions and active shedding of virus—macaques without lesions will also shed virus. In humans, herpes B virus presents as an acute ascending myelitis and encephalitis, and is usually fatal without early identification and treatment. The greatest risk for infection with B virus is through bites or scratches from macaques. However, it is now clear that mucous membrane exposure to bodily fluids can also result in transmission. Additionally, those working with fresh tissues (especially neural-derived tissues) and cells must also be vigilant in preventing exposures.

3.3.1.1. General Laboratory Requirements

Universal precautions as well as strict adherence to ABSL-2 and BSL-2 practices (at a minimum, depending upon the biohazardous agent being researched) and the use of appropriate PPE are necessary when handling nonhuman primates and their tissues, etc.

3.3.1.2. First Aid Following a Potential Exposure from a Nonhuman Primate

An exposure is defined as: a bite or scratch by a nonhuman primate, laceration or puncture wound caused by potentially contaminated equipment, mucous membrane exposure to potentially contaminated tissues, cells, and OPIM derived from nonhuman primates. **It is extremely important that all employees working with nonhuman primates, and materials derived there from, be educated on the proper procedures to follow in the event of an exposure**.

Following a potential exposure, the most important first step is to immediately wash the site with soap and water for 15 minutes. If you receive an exposure to the eye, immediately go to an eye wash station and flush the eyes for 15 minutes with water. Report the incident to your supervisor and to Occupational/Employee Health as well as the Biosafety Office.

3.4. WORK WITH DOGS OR CATS

Dogs and cats used in long-term studies may be vaccinated against rabies. Check with the Attending Veterinarian. Rabies vaccinations are provided to employees upon recommendation of Occupational/Employee Health.

Some dog and cat parasites are a potential risk to those handling infected animals. Examples include some roundworms, tapeworms, hookworms and mange mites. Ringworm, a fungal disease of dogs and cats, is also readily transmitted to humans. Cat Scratch disease is a zoonotic infection characterized by regional lymph node infection that can follow a scratch, bite or primary lesion caused by a cat. The agent involved is a *Bartonella sp.* While the prognosis is usually excellent and the disease in most cases is self-limiting, employees must report an infection or possible infection.

3.5. WORK WITH FARM ANIMALS (E.G., SHEEP, PIGS)

Q fever, a potentially serious human disease caused by the rickettsia, *Coxiella burnetti*, was formerly quite common in those drinking unpasteurized milk and in slaughterhouse workers exposed to freshly slaughtered ruminants (cattle, sheep and goats). It is known that the organism is shed from the placental membranes of sheep and goats. It can also be acquired by ingesting milk from infected animals. This route of exposure has been the cause of Q fever pneumonia and other associated symptoms in laboratory workers. Unless known to be free of the rickettsia, you should assume sheep to be infected and all personnel working where exposure is possible should take suitable precautions. Gloves, safety glasses, a mask and protective clothing are required for individuals working with pregnant sheep and goats. Infected persons can be effectively treated.

Erysipelas in pigs can be transmitted to humans causing a severe local skin infection. Therefore, pigs showing diagnostic "diamond back" lesions should be handled with care.

Similar in appearance, though less severe than erysipelas, skin lesions are also seen on the hands after contact with sheep and goats infected with contagious icthyma and vesicular stomatitis virus. Rabies can also be a threat from any unvaccinated cat or dog, or food animal, especially those on pasture or exposed to feral animals.

Cattle from commercial farms may be unsymptomatic carriers of salmonella, campylobacter, toxigenic *E. coli* (O157:H7), and cryptosporidia. These organisms are present in feces and some may also be shed in the milk. Calves with diarrhea may be shedding some of these organisms in high numbers.

Commercial swine may also carry salmonella and campylobacter. Aborted fetuses from swine and cattle, sheep and goats may be associated with a number of zoonotic pathogens such as Brucellosis, Leptospirosis, or Q fever. Aborted fetuses should be handled with extreme care and appropriate PPE (boots, mask, Tyvek coveralls, and gloves). *Baylisascaris procyonis* (raccoon large roundworm) is found wherever raccoons are found. This roundworm causes a highly pathogenic visceral larval migrans that is untreatable. Avoid contact with raccoon feces. If a raccoon latrine is found in a barn (haystack), use extreme caution. Use

respirator, gloves, Tyvek suit, and boots to remove feces from area and burn it. Heat is the only way to kill the eggs.

3.6. WORK WITH RODENTS

Contact with rodents requires precautions against such diseases as tapeworm infection, lymphocytic choriomeningitis virus (LCMV), salmonellosis and ringworm fungal skin infections. Additional concerns for investigators using some rodents are leptospirosis and bubonic plague. Specific attention should also be focused on the possibility of allergic reactions to rodent dander and urine. Care should be taken to limit exposure to soiled bedding as these can contain excreted chemical or biological hazards that have been given to the rodents and may be excreted in feces and urine (be sure to review the IACUC hazard forms on file for the specific study). Use of laminar flow, HEPA-filtered dump stations for bedding disposal is a first line of engineering control that should be utilized. Gloves, safety glasses and respirators not only limit exposure to soiled bedding, but also help prevent transmission of diseases through handling of rodents.

4. EXPOSURE CONTROL MEASURES

Lab supervisors and primary supervisors are responsible for ensuring that control measures are in place to reduce employee exposure to biohazards. When practical, engineering controls, administrative controls, and personal protective equipment (in that order) should be used to reduce the potential for exposures.

4.1. ENGINEERING CONTROLS

Engineering controls are methods of controlling employee exposures by modifying the source or reducing and controlling the quantity of contaminants released into the work environment. Examples include biological safety cabinets, fume hoods, glove boxes, and local exhaust. Engineering controls are the preferred primary control measure.

4.1.1. Ventilation

Ventilation Controls are engineering controls intended to minimize employee exposure to infectious agents, hazardous chemicals or toxic substances by removing air contaminants from the work site. There are two main types of ventilation controls:

1. General (Dilution) Exhaust: is where you have a room or building-wide system which supplies air from the outside and removes it at the same rate. Laboratory air is to be continually replaced, at a rate that prevents the concentration of toxic substances. General exhaust systems alone are inadequate for RG-3 agents or BSL-3 work.

2. Local Exhaust or Filtration: a ventilated, enclosed work space intended to capture, contain and exhaust or filter harmful or dangerous fumes, vapors and particulate matter. In the case of hazardous

chemicals this includes a fume hood. In the case of infectious agents biosafety cabinets should be used.

Other ventilation controls include the use of single-pass air, increased number of air changes, and the maintenance of negative pressure inside laboratories relative to outside corridors. All these controls provide for the mitigation of agent escape from the laboratory.

4.1.2. Biological Safety Cabinets (BSC)

BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets (BSCs), designated as Class I, II and III have been developed to meet various research and clinical needs. Biological safety cabinets use high efficiency particulate air (HEPA) filters in their exhaust and/or supply systems and are intended to be used when handling infectious, toxic or sensitizing materials.

BSCs should not be confused with other laminar flow devices or "clean benches"; in particular, horizontal flow cabinets, which direct air towards the operator. These benches protect the product but do not protect the operator. Laboratory personnel should be trained in the correct use and maintenance of biological safety cabinets to ensure that personnel and product protection (where applicable) is maintained.

When properly used in research involving the manipulation of biohazardous agents, biological safety cabinets are effective in containing and controlling particulates and aerosols, and complement good laboratory practices and procedures. The correct location, installation, and certification of the biological safety cabinet are critical to containing infectious aerosols.

All BSCs shall be inspected annually and certified by trained and accredited service personnel according to the NSF (National Sanitation Foundation) Standard 49, Annex F. Inspection and re-certification is required if the cabinet is relocated or after major repairs, filter changes, etc.

For general guidance on the safe and effective use of BSCs refer to the CDC\NIH document Primary
Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets available from
BMBL 5th edition **(http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf)**
NOTE: Before selecting any BSC for purchase, contact the Biosafety Officer for work-specific assessment and selection criteria.

A brief description of the different types of biosafety cabinets is as follows:

4.1.2.1. CLASS I BSC

The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. In the Class I BSC, unfiltered room air is drawn across the work surface and personnel protection is provided by this inward airflow. With the product protection provided by the Class II BSCs, general usage of the Class I BSC has declined. However, in many cases Class I BSCs are used specifically to enclose equipment

(e.g., centrifuges, harvesting equipment or small fermenters), or procedures (e.g. cage dumping, aerating cultures or homogenizing tissues) with a potential to generate aerosols.

4.1.2.2. CLASS II BSC

The Class II BSC provides personnel, environmental and product protection. Airflow is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet.

The Class II cabinet has four designs that differ in the amount of air that is recirculated and/or exhausted, and whether or not the BSC is hard-ducted to the ventilation system. All exhaust air must be exhausted to the outside and not recirculated back into the work environment. Any exception to this must be approved by the BSO.

All Class II cabinets are designed for work involving microorganisms assigned to biosafety levels 1, 2 and 3. Class II cabinets provide the microbe-free work environment necessary for cell culture propagation. Certain types of BSC may also be used for the formulation of nonvolatile antineoplastic or chemotherapeutic drugs. Care must be exercised when selecting the correct Class II cabinet design for these purposes. Biosafety Officer should be consulted to aid in the selection.

4.1.2.3. Class III BSC

The Class III BSC is designed for work with biosafety level 4 microbiological agents, and provides maximum protection to the environment and the worker. It is a gas-tight enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank (that is accessible through the cabinet floor) or double-door pass-through box (such as an autoclave) that can be decontaminated between uses. Reversing that process allows for safe removal of materials from the cabinet. Both supply and exhaust air are HEPA filtered. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge to the outdoors. Airflow is maintained by a dedicated independent exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure (usually about 0.5 inches of water pressure).

4.1.2.4. Biological Safety Cabinet Function and Operation

Biological safety cabinets (BSCs), when used properly, provide a clean work environment for research. Biological safety cabinets offer personnel, product, and environmental protection. The BSC provides primary containment for infectious materials. The efficacy of BSCs depends upon the behavior of the operator and the orientation of the unit in the facility.

The BSC isolates biohazards from personnel by confining the biohazardous material in the unit. The BSC removes aerosolized biohazardous material by moving air through high efficiency particulate air (HEPA) filters. The intake air is filtered through a HEPA filter before entering the BSC work area. Exhaust air also passes through a HEPA filter. Aerosols generated in the work area of the BSC are contained within the BSC.

Operating Procedures for Class II Biological Safety Cabinet:

♦ If used, turn off UV light; turn on fluorescent light and blower.
♦ Let blower run for at least 4 minutes.
♦ Disinfect all interior surfaces with 70% ethanol or suitable disinfectant.
♦ Gather items needed for your work and place them into cabinet; do not obstruct grills.
♦ Keep materials at least 4 inches inside work area.
♦ Work should proceed from clean to contaminated areas.
♦ After procedure, allow cabinet to run 2-3 minutes before removing all materials.
♦ Wipe down all work surfaces with 70% ethanol or suitable disinfectant.
♦ Turn off fluorescent light and blower if desired.

Many BSCs are equipped with germicidal ultraviolet (UV) lamps. Time of exposure, distance, presence of dust or debris and UV lamp intensity affect the germicidal effect of the UV lamp. The visible blueviolet glow of the UV lamp does not indicate there is germicidal effect. The UV lamp needs to be cleaned periodically to remove dust. UV lamps may damage eyes, skin, and laboratory equipment. UV lamps should be turned off while the room is occupied.

The Biosafety Office discourages the use of UV lamps due to the potential damage resulting from UV lamp use.

4.1.3. Bunsen Burners and Loop Sterilizers in the BSC

Bunsen burners are not allowed inside Biosafety Cabinets. Continuous flame gas burners shall not be used in BSCs. These burners can produce turbulence, which disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter. Sterilization of inoculating loops or needles in an open flame generates small particle aerosols, which may contain viable microorganisms. The use of a shielded electric incinerator or hot bead sterilizers minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available or recommended.

4.1.4. Chemical Fume Hoods

Chemical Fume Hoods are an important engineering control used to prevent exposure to hazardous materials. In conjunction with sound laboratory techniques, a chemical fume hood serves as an effective means for capturing toxic, carcinogenic, offensive, or flammable vapors or other airborne contaminants that would otherwise be released to the general laboratory atmosphere.

4.1.5. Safety Equipment

4.1.5.1. SAFETY SHOWERS

Safety showers provide an immediate water drench of an affected person. Standards for location, design and maintenance of safety showers are outlined in the Chemical Hygiene Plan.

4.1.5.2. EYEWASH STATIONS

Eyewash stations are required in all laboratories where injurious or corrosive chemicals are used or stored and where employees perform tasks that might result in splashes of potentially biohazardous materials. Standards for location, design and maintenance of emergency eyewash facilities are outlined in the Chemical Hygiene Plan.

4.1.5.3. HANDWASHING SINKS

Laboratory workers should have access to handwashing facilities for hygiene purposes. While gloves should always be used when working in the labs, it is important that workers frequently wash their hands during the day. Hands must be washed when gloves are removed and after any potential exposure. Hands should also be washed when leaving the laboratory.

4.2. ADMINISTRATIVE CONTROLS

Administrative Controls are methods of controlling employee exposures to infectious agents by adherence to appropriate work practices and by written procedures or policies. Examples include standard operating procedures or programs, training, signage, manuals and guidance documents.

At times unique programs, standard operating procedures or guidelines are required to address situations or achieve regulatory compliance. Programs like Waste Disposal, Bloodborne Pathogens Exposure Control Plan, Initial Review Application via iRIS), and CDC Select Agents are examples of such programs.

4.2.1. Training

Training requirements are important administrative controls instituted to minimize or prevent exposures to biohazards. Section 1.7.7 above outlines the training requirements for working with potentially biohazardous agents. Training must be completed prior to working in the laboratory and some training requires annual updates.

4.2.2. Waste Handling and Disposal

Labs can generate biohazardous waste and hazardous chemical waste that requires decontamination and/or disposal. All waste disposal should be coordinated through the Scott & White Biosafety Officer. Procedures for waste handling and disposal are contained elsewhere in this biosafety manual (see section 7.1, Decontamination and Disposal Methods).

4.2.3. Labeling and Signage

4.2.3.1. Biohazard Warning Sign

A biohazard label is required for all areas or equipment in which RG-2 or RG-3 agents are handled or stored, or where BSL-2 or BSL-3 procedures are required. The appropriate place for posting the label is at the main entrance door(s) to laboratories and animal rooms, on equipment such as refrigerators/freezers that contain hazards, incubators and transport containers. See below for examples of the biohazard symbol:

4.2.3.2. General Labeling and Signage Requirements – Hazard Communication

The following guidance is to be used when determining where biohazard signs and labels are to be used in laboratory and/or storage areas:

1. All laboratories where biohazards are stored or used are clearly labeled using the laboratory safety plaques (front door signs).
2. All other rooms and storage areas are clearly labeled with either the new laboratory safety plaques or other biohazard signage.
3. All biosafety cabinets where biohazards are used are clearly labeled.
4. All refrigerators, freezers, centrifuges, and incubators where biohazards are used are labeled.
5. All other pieces of equipment are evaluated by the laboratory occupants and assessed for risk. Items will be labeled with the biohazard symbol if they are at risk of being contaminated during the course of laboratory activities and are either:
   a. Being moved from the work area without adequate disinfection or decontamination, and/or;
   b. Being serviced in-place by Institutional or third-party serviceperson.
6. Pieces of equipment that do not fit these categories or risks do not need to be labeled.
7. Any equipment items that leave the laboratory for service or disposal must be handled according to the Institutional policy on biohazard/decontamination tagging.

4.2.3.3. Animal Rooms

The entrance to individual animal rooms must be posted with the Animal Area Biohazard Communication Form when a study is in progress. This form is provided to the PI after the Animal Use Protocol involving potentially infectious or otherwise hazardous materials derived from plant or animal sources is approved by IACUC and the Biosafety Officer.

4.2.3.4. Labeling and Storage of Biological Materials

The contents of all laboratory containers shall be properly identified. One of the overriding goals of prudent practice in the labeling and identification of materials is to avoid orphaned containers of unknown materials. The labels should be understandable to laboratory workers, members of emergency response teams, and others.

Sample Container Labeling and Storage Requirements:

1. All containers and/or racks are to be clearly labeled to identify the contents. Secondary containment should be considered where appropriate. If there is a large quantity of smaller containers of the same agent, labeling of the storage container, tray or cupboard will suffice.
2. If volatile materials are used (flammable, explosive potentials), they must be stored in equipment that is designed for this purpose (e.g. lab safe refrigerators).
3. Personal items, e.g., food and beverages, are **ABSOULUTELY** prohibited in lab refrigerators, cold rooms, freezers or incubators.

4.2.4. Laboratory Practices

4.2.4.1. Human Factors and Attitudes in Relation to Laboratory Accidents

For the purposes of safety, an attitude can be defined as an accumulation of information and experience that predisposes an individual to certain behavior. Human factors and attitudes result in tendencies on the part of the individual to react in a positive or negative fashion to a situation, a person or an objective. Laboratory supervisors and Principal Investigators should understand the importance of attitudes and human factors in their own efforts to control biohazards in their laboratory. Some observations that may be of help to supervisors are listed below:

♦ The lack of accident perception ability is often a significant factor in laboratory accidents.
♦ Inflexibility of work habits, that tend to preclude last minute modification when an accident situation is recognized, plays a part in the causation of some laboratory accidents.
♦ Working at an abnormal rate of speed is a significant causal factor.
♦ Intentional violations of regulations are a frequent cause of accidents. This is termed excessive risk taking.
♦ The performance of routine procedures such as diluting and plating cultures is the most frequent task being performed at the time of laboratory accidents.
♦ Working when one is very tired is more likely to create a higher potential for accidents.
♦ Working at a well-organized and uncrowded laboratory bench will help in the prevention of lab accidents. Each employee working with biohazardous agents must be consistently aware of the importance of the proper attitude in preventing accidents in the laboratory.

4.2.4.2. Biosafety Level 1

♦ Keep laboratory door closed when experiments are in progress.
♦ Use procedures that minimize aerosols.
♦ Do not smoke, eat, drink or store food in BL1 areas.
♦ Wear laboratory gowns or coats when appropriate.
♦ Do not mouth pipette. Use mechanical pipetting devices.
♦ Avoid using hypodermic needles.
♦ Wash hands after completing experimental procedures and before leaving laboratory.
♦ Disinfect work surfaces daily and immediately after a spill.
♦ Decontaminate all biological wastes before discard. Decontaminate other contaminated materials before washing, reuse, or discard.
♦ For off-site decontamination, package contaminated materials in closed, durable, leakproof containers.
♦ Control insect and rodent infestations.
♦ Keep areas neat and clean.

4.2.4.3. Biosafety Level 2

♦ Keep laboratory door closed.
♦ Post a universal biohazard label on equipment where infectious agents are used/stored.
♦ Allow only persons informed of the research to enter BL2 areas.

♦ Keep animals not used in BL2 experiment out of the laboratory.
♦ Do not smoke, eat, drink, store food or apply cosmetics in BL2 areas.
♦ Wear PPE (laboratory gowns or coats, gloves and full-face protection) when appropriate; do not wear PPE outside of the laboratory.
♦ Wash hands after removing PPE as well as before leaving laboratory.
♦ Change PPE when soiled or compromised.
♦ Do not mouth pipette. Use mechanical pipetting devices.
♦ Use procedures that minimize aerosol formation.
♦ Avoid using hypodermic needles.
♦ Substitute plastic for glass where feasible.
♦ Use biological safety cabinets to contain aerosol-producing equipment.
♦ Wash hands after completing experimental procedures and before leaving laboratory.
♦ Disinfect work surfaces daily and immediately after a spill.
♦ Maintain a biological spill kit within the laboratory.
♦ Report spills, accidents, near misses and disease symptoms related to laboratory acquired infection to the PI.
♦ Ensure that all biomedical waste containers are labeled with the biohazard symbol.
♦ Decontaminate all biological wastes before discard. Decontaminate other contaminated materials before washing, reuse, or discard.
♦ For off-site decontamination, package contaminated materials in closed, durable, leakproof containers.
♦ Control insect and rodent infestations.
♦ Keep areas neat and clean.

4.2.4.4. Biosafety Level 2+

Biosafety level 2+ (BL2+) is the designation utilized for those biohazard experiments that require practices that are more stringent than standard BL2 procedures. Generally, BL3 practices are mandated in a space designed for BL2 work. It is preferred that the BL2 laboratory be self-contained with all equipment required for the experiment located within the laboratory. A biohazard door sign listing the agent in use, emergency contact, and entry requirements is posted on the door while BL2+ work is in progress and access is restricted to those involved in the experiment. When work is completed and equipment has been decontaminated, the sign is removed and the laboratory is returned to standard BL2 or BL1 use.

All manipulations of BL2+ material are conducted in a class II biological safety cabinet and secondary containment is utilized for centrifugation and other potential aerosol generating procedures.

Please consult the Biosafety Office prior to initiating any work at BL2+.

4.2.4.5. Cell Culture

♦ Wear a lab coat and gloves when working in the biosafety cabinet.
♦ Glassware and other contaminated items should be disinfected or autoclaved before washing, reuse or disposal.

♦ Glassware should be thoroughly cleaned and rinsed, by washing repeatedly with tap water and distilled water.
♦ Cell culture wastes must be decontaminated.
♦ Maintain a clean lab coat reserved solely for cell culture work.
♦ Avoid talking during culture manipulations as aerosols may be drawn into the work area.
♦ Place pipettes on a rack to avoid disrupting airflow when removed.
♦ Keep open tubes parallel to the airflow.
♦ After transferring inoculum always recap vials.
♦ Do not place tubes on work surface.
♦ Discard empty tubes immediately.
♦ Work with one specimen at a time; recap before going to the next.
♦ Autoclave verification should be performed routinely.

4.2.4.6. Transport of Biohazards on Campus (between labs or buildings):

♦ Must have two leakproof containers, including the following:
  A sealed primary container
  A sealed secondary container
  Absorbent (paper towels) between the primary and secondary containers suitable for the volume transported
  A biohazard sticker on the outside of the secondary container with agent name
  Lab address and phone number on the outside of the secondary container
  Utilize plastic containers whenever feasible. Avoid glass.
♦ Sealed plastic (not glass) primary vials can be transported within sealed, labeled plastic bags.
♦ If glass primary containers must be used, place containers within a sealed rigid plastic container with absorbent and padding to cushion vials during transport.
♦ Decontaminate the outside of the primary container before placing into the secondary container.
♦ Decontaminate the secondary container before leaving the laboratory.

4.2.4.7. **General Microbiological Practices**

4.2.4.7.a. Pipettes and Pipetting Aids
When pipetting, use the following precautions:
♦ Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used.
♦ Use disposable plastic pipettes instead of glass pipettes.
♦ Confine pipetting of biohazardous or toxic fluids to a biosafety cabinet if possible. If pipetting is done on the open bench, use absorbent pads or paper on the bench.
♦ Respiratory protection may need to be considered depending on the agent in use.
♦ Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.
♦ Never prepare any kind of biohazardous mixtures by suction and expulsion through a pipette, which create aerosols.
♦ Biohazardous materials should not be forcibly discharged from pipettes. Use “to deliver” pipettes rather than those requiring “blowout.”
♦ Do not discharge biohazardous material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.

♦ Avoid accidentally dropping hazardous material from the pipette onto the work surface. Place a disinfectant dampened towel or other absorbent material on the work surface, and autoclave before discard or reuse. Plastic backed bench paper is suitable for this purpose.
♦ Place discard pans for used pipettes within the biosafety cabinet.
♦ Contaminated pipettes should be placed horizontally into a pan or tray containing enough suitable disinfectant, such as hypochlorite, to allow complete immersion of the pipettes. Pipettes should not be placed vertically in a cylinder that, because of its height, must be placed on the floor outside the biosafety cabinet. Removing contaminated pipettes from the biosafety cabinet and placing them vertically in a cylinder provides opportunity for dripping from the pipette onto the floor, or the rim of the cylinder, thereby creating an aerosol, and the top of the pipettes often protrude above the level of disinfectant.
♦ After suitable contact time, excess disinfectant can be carefully poured down the sink. The pan and pipettes can be autoclaved together, and replaced by a clean pan with fresh disinfectant.

4.2.4.7.b. Syringes and Needles

The hypodermic needle is a dangerous instrument. To lessen the chance of accidental injection, aerosol generation, or spills, the use of syringes should be avoided when alternate methods are available. For example, use a blunt needle or cannula on the syringe for oral or intranasal inoculations and never use a syringe and needle as a substitute for a pipette in making dilutions.

The following practices are recommended for hypodermic needles and syringes when used for parenteral
injections:
♦ Use the syringe and needle in a biological safety cabinet only and avoid quick and unnecessary movements of the hand holding the syringe.
♦ Examine glass syringes for chips and cracks, and needles for barbs and plugs. This should be done prior to sterilization before use. Use needle-locking syringes only, and be sure that the needle is locked securely into the barrel. Replace glass syringes with plastic disposable syringes whenever possible.
♦ Whenever possible use safer needle systems.
♦ Wear latex gloves for all manipulations with needles and syringes.
♦ Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.
♦ Expel excess air, liquid and bubbles from a syringe vertically into a cotton pledget moistened with an appropriate disinfectant, or into a small bottle of sterile cotton.
♦ Do not use the syringe to forcefully expel a stream of infectious fluid into an open vial for the purpose of mixing. Mixing with a syringe is condoned only if the tip of the syringe is held below the surface of the fluid in the tube.
♦ If syringes are filled from test tubes, take care not to contaminate the hub of the needle, as this may result in the transfer of infectious material to the fingers.
♦ When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton pledget moistened with an appropriate disinfectant. If there is concern of the disinfectant contaminating sensitive experimental materials, a sterile pledget may be used and immediately discarded into a biohazard bag.

♦ When inoculating animals, position the hand that is holding the animal “behind” the needle or use a pair of forceps to hold the animal in order to avoid puncture wounds.
♦ Be sure the animal is properly restrained prior to the inoculation and be on the alert for an unexpected movements of the animal.
♦ Before and after injection of an animal, swab the injection site with an appropriate antiseptic.
♦ Discard syringes into **appropriate** sharps container. **DO NOT** bend, shear, recap or otherwise manipulate the needle. If recapping is unavoidable, use a one handed method or use a mechanical device. **DO NOT** discard syringes into a biohazard bag.

4.2.4.7.c. Sharps usage

In addition to needles/syringes, other sharps are often utilized in the laboratory, such as scalpels, blades, Pasteur pipets, etc. Laboratory personnel, along with PIs, should make every effort to find suitable substitutes for sharps instruments. For example, instead of using scalpels to generate single cell suspensions from tissues, one can use a tissue dissociator. It is important to minimize (and if possible eliminate) sharps usage when working with biohazardous agents. Please refer to the sharps usage policy for additional information (http://researchers.sw.org/ibc/reference-guidance).

4.2.4.7.c. Culture Plates, Tubes and Bottles

In the absence of definite accidents or obvious spillage, it is not certain that the opening of plates, tubes and bottles of other microorganisms has caused laboratory infection. However, it is probable that among the highly infective agents some infections have occurred by this means. Particular care is required when opening plates, tubes, or bottles containing fungi, for this operation may release a large number of spores. Such cultures should be manipulated in a biological safety cabinet.

To assure a homogenous suspension that will provide a representative sample, liquid cultures are agitated before a sample is taken. Vigorous shaking will create a heavy aerosol. A swirling action will generate homogenous suspension with a minimum of aerosol. When a liquid culture is re-suspended, a few minutes should elapse prior to opening the container to reduce the aerosol.

The insertion of a sterile, hot wire loop or needle into a liquid or slant culture can cause spattering and release of an aerosol. To minimize the aerosol production, the loop should be allowed to cool in the air or be cooled by touching it to the inside of the container or to the agar surface where no growth is evident prior to contact with the culture of colony. Following use of inoculating loop or needle, it is preferable to sterilize the instrument in an electric or gas incinerator specifically designed for this purpose rather than heating in an open flame. These small incinerators have a shield to contain any material that may spatter from the loop or needle. Disposable inoculating loops are available commercially. Rather than decontaminating them immediately after use with heat, they are discarded first into a disinfectant solution.

The practice of streaking an inoculum on rough agar results in aerosol production created by the vibrating loop or needle. This generally does not occur if the operation is performed on smooth

agar. It is good safety practice to discard all rough agar poured plates that are intended for streaking purposes with a wire loop.

Water of syneresis in Petri dish cultures usually contains viable microorganisms and forms a film between the rim and lid of the inverted plate. Aerosols are dispersed when opening the plate breaks this film. Vented plastic Petri dishes, where the lid touches the rim at only three points, are less likely to offer this hazard. The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar. Filter papers fitted into the lids reduce, but do not prevent dispersal. If plates are obviously wet, they should be opened in the biological safety cabinet.

Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of contaminated liquid, which may collect between the rim and the liner, is broken during removal of the closure. The practice of removing cotton plugs or other closures from flasks, bottles, centrifuge tubes, etc., immediately following shaking or centrifugation can generate aerosols and cause environmental contamination. The technique of shaking tissue cultures with glass beads to release viruses can create a virus-laden aerosol. Removal of wet closures, which can occur if the flask or centrifuge tube is not held in an upright position, is also hazardous. In addition, when using the centrifuge, there may be a small amount of foaming and the closures may become slightly moistened.

Because of these possibilities, it is good safety practice to open all liquid cultures of infectious or hazardous material in a biological safety cabinet wearing gloves and a long sleeved laboratory garment. Dried, infectious culture material may also collect at or near the rim or neck of culture tubes/flasks and may be dispersed into the air when disturbed. Containers of dry powdered hazardous materials should be opened in a biological safety cabinet.

4.2.4.7.d. Ampoules

When a sealed ampoule containing a lyophilized or liquid culture is opened an aerosol may be created.
Aerosol creation should be prevented or minimized; opening of ampoules should be done in biological safety cabinets. When recovering the contents of an ampoule, care should be taken not to cut the gloves or hands or disperse broken glass into eyes, face, or laboratory environment. In addition, the biological product itself should not be contaminated with foreign organisms or with disinfectants. To accomplish this, work in a biological safety cabinet and wear gloves. Nick the ampoule with a file near the neck. Wrap the ampoule in disinfectant wetted cotton. Snap the ampoule open at the nick, being sure to hold the ampoule upright. Alternatively, at the file mark on the neck of the ampoule, apply a hot wire or rod to develop a crack. Then wrap the ampoule in disinfected wetted cotton, and snap it open. Discard cotton and ampoule tip into disinfectant. The contents of the ampoule are reconstituted by slowly adding fluid to avoid aerosolizing the dried material. Mix contents without bubbling, and withdraw the contents into a fresh container. Some researchers may desire to use commercially available ampoules prescored for easy opening. However, there is the possibility to consider

that this may weaken the ampoule and cause it to break during handling and storage. Ampoules of liquid cultures are opened in a similar way.

Ensure that all hazardous fluid cultures or viable powdered infectious materials in glass vessels are transported, incubated, and stored in easily handled, nonbreakable leakproof secondary containers that are large enough to contain all the fluid or powder in case of leakage or breakage of the glass vessel. The secondary container must be labeled with a biohazard label bearing the name of the infectious material.

4.3. PERSONAL PROTECTIVE EQUIPMENT (PPE)

Multidisciplinary research conducted in Scott & White laboratories requires that personal protective equipment (PPE; protective clothing and safety apparatus/equipment) be used to protect the researcher from contact with infectious, toxic and corrosive agents, excessive heat, cold, fire and other physical hazards. Suitable PPE also protects the experiment from contamination. The extent and kind of clothing and equipment to be selected for any particular activity depends upon the research operations and levels of risk associated with the research. While PPE is an important component of any biological safety program, PPE is used with the understanding that PPE serves as a second line of defense. Good laboratory techniques, procedures and appropriate laboratory equipment are the primary barriers against potential exposure to hazardous agents.

For additional information you are urged to consult the Biosafety Office. In the event the Biosafety Office does not have a listing of the kind of protective devices you are seeking, efforts will be made to acquire the information needed.

4.3.1. Laboratory Clothing

Laboratory clothing serves to protect the wearer, the experiment, and environment against contamination. If proper precautions are not taken, contaminated clothing may carry infectious materials outside the laboratory and into other work areas, cafeterias, or the home. Infectious agents can remain viable on cotton and wool fabrics and be disseminated from these fabrics.

It is important that lab workers also wear laboratory-friendly street clothing. No shorts, short skirts, or open-toed shoes should be worn in the lab.

Both reusable and disposable clothing is available. Whichever is used, it must be durable, designed to provide protection and prevent exposure of the skin to harmful agents, as well as be compatible with the methods of decontamination employed.

Some additional points:
♦ Overt exposure to agents at all levels of risk should be followed by immediate decontamination of the PPE, and then a change into clean PPE to protect the worker, the experiments and the environment.
♦ Provisions should be made for PPE to be provided to visitors and maintenance or security personnel, if applicable.
♦ PPE worn within the laboratory should not be worn outside the facility to the library, cafeteria, or other places accessible to the public.
♦ Personnel should be encouraged to use disposable facial tissues instead of personal handkerchiefs.

♦ PPE should be placed in an appropriately designated area or container for storage, washing, decontamination or disposal.
♦ All PPE should be decontaminated before being sent to the laundry or discarded. Treat contaminated areas of PPE with an appropriate disinfectant. Lab coats with extensive contamination may be placed in a biohazard bag and autoclaved.
♦ DO NOT take PPE home to launder; select a laundry service that follows universal precautions.
♦ Change PPE as soon as feasible whenever it is compromised, soiled or torn.
♦ Wear appropriate sizes and keep an adequate supply of PPE available in the laboratory.
♦ Wash hands whenever PPE is removed.
♦ Do not touch door handles, elevator buttons, telephones, computers or other clean surfaces or items with gloved hands.
♦ Wear closed-toe shoes and long pants to guard against skin contamination or chemical exposure. Do not wear sandals or shorts in the laboratory.

4.3.1.1. Gloves

Gloves should be comfortable and of sufficient length to prevent exposure of the wrist and forearm. Depending upon intended use, the composition and design of the glove may vary to provide the desired level of flexibility, strength, impermeability, and resistance to penetration by sharp objects, as well as protection against heat and cold. Quality assurance is an important consideration.

No one glove can be expected to be satisfactory for all intended uses. Gloves may be fabricated of cloth, leather, natural and synthetic rubbers, or plastics. New formulations of synthetic rubber and plastic continue to be developed as research makes varied and changing demands on the protective capabilities of gloves. Changing applications lead to improved capabilities of impermeability, strength, flexibility, tactile sense and control. Within even the modest laboratory, the glove applications may be such that no less than four or five types of protective gloves need to be stocked and used.

Disposable (single use) gloves provide a barrier between infectious agents and the skin. Glove use is a basic precept of preventing infectious agent transmission. Breaks in the skin barrier of the hand (damaged cuticles, scrapes, micro-cuts, dermatitis, etc.) are common.

Gloves shall be removed and hands washed before exiting the laboratory. Use the one glove method, or an appropriate secondary container, when transporting materials through common use areas.
The Biosafety Office can provide information on gloves needed for various tasks, such as working with animals, dry ice, heat, acids, etc. Consult OEHS with details of your work to receive further information about the type and availability of gloves that will best meet your requirements.

Considerations for the selection and use of gloves:
♦ Gloves are not 100% leakproof; change gloves periodically and when soiled and always wash hands after removing gloves or other PPE.
♦ Gloves will not prevent needle sticks or other puncture injuries.

♦ Check gloves for visible tears before use.
♦ Avoid wetting examination gloves as water or disinfectants will encourage wicking and leaking
♦ Do not reuse examination gloves; discard contaminated gloves in a biohazard bag immediately after use.
♦ Double glove or use household utility gloves when cleaning spills. Household utility gloves may be decontaminated and reused (replace when compromised.)

Procedure for removing gloves:
Grip the outside of one glove at wrist with the other gloved hand, pull glove off and gather in palm of gloved hand. Place index or middle finger of the ungloved hand on wrist of gloved hand, slide finger under the glove opening and pull glove off inside out.

When removing PPE, remove lab coat or solid front gown first, then remove gloves (aseptically), remove face protection last to avoid touching your face with contaminated hands. If wearing double gloves, remove outer gloves before removing lab coat or solid front gown.

4.3.1.2. Shoes

Shoes worn in the laboratory must be closed-toe. Protective shoes are required for certain work activities.
When working with infectious agents it is advisable to wear shoe covers, which can be decontaminated (autoclaved) before disposal, over street shoes. For work in tissue culture laboratories it may be necessary to change from street shoes to specific laboratory shoes for protection of cultures from contamination.

4.3.1.3. Gowns, Lab Coats, Jumpsuits, Aprons, and Other Protective Clothing

Gowns, lab coats and jumpsuits protect the wearer's clothing and skin from contamination. As with all PPE, the type of clothing needed is dependent upon a proper risk assessment and of the task being performed and the degree of potential exposure anticipated.

Solid front wrap-around clothing (ex. Disposable infectious disease gowns) offers better protection than pull-over type clothing or clothing with front closures, such as lab coats. Lab coats are not 100% leakproof; one should always change PPE when soiled, and always wash your hands after removing any PPE. Lab coats or other protective clothing will not prevent needle sticks or other punctures. Many workers prefer not to button up front closing jackets, which leaves street clothing exposed. If front closing jackets must be worn, strict measures shall be implemented to assure the clothing is closed at all times when performing procedures or tasks that may cause exposure.

Long sleeved garments with snug fitting cuffs are preferred over open or short sleeves. Snug fitting cuffs prevent splashes, splatters and aerosols from making contact with exposed skin on the lower arms. Longer single-use gloves can be pulled over snug fitting cuffs to seal out any infectious materials.

Plastic, vinyl or rubber aprons are usually worn over other protective clothing when extra protection is desired. Aprons are necessary for protection against liquids spilling or splashing on clothing. It is recommended that appropriate aprons be worn to protect against the potential harmful effects of liquid waste. Aprons may also be used to provide protection from steam and hot water in locations such as animal handling facilities, autoclave rooms and laboratory glasswashing rooms.

4.3.1.4. Face and Eye Protection

Protection of the face and eyes is of prime importance in laboratories due to the potential for foreign material, both liquid and solid, to splash on the head, face and eyes or contact lenses. A variety of face shields, head covers/hoods, protective goggles, and lenses are available from safety supply houses. The selection is dependent upon materials of construction, fit, comfort, and compatibility with the work and the overall facial area requiring protection.

Some of the considerations for selection and use of face and eye protection are indicated below:

♦ Face shields and hoods protect the face and the neck from flying particles and sprays of hazardous material; however, they do not provide basic eye protection against impacting objects.

♦ Shields should cover the entire face, permit tilting back to clean the face if desired, and be easily removed in the event of an accident.

♦ If an eye hazard exists in a particular operation or experiment, the soundest safety policy would be to require that eye or face protection, or both, be worn at all times by all persons entering or working in the laboratory.

♦ Contact lenses do not provide eye protection. It is recommended that contact lenses not be worn when working around chemicals, fumes, and other hazardous material and dust particles since these items may become trapped in the space between the contact lens and the cornea. When contact lenses are worn, eye protection, such as tight fitting goggles, must be worn.

4.3.1.5. Respiratory Protection

Protection of the respiratory system is a major concern of any biological safety program because infectious organisms can readily enter the human body through the respiratory tract. The possibility of this occurring depends on the type and infectious dose of the particular organism. For some, as few as one to ten organisms, when inhaled, may cause infection. Particles with an effective aerodynamic diameter of between 0.5 and 5.0 μm (the respirable fraction) are most effective at penetration and retention in the deep pulmonary spaces. Particles larger than 5 micrometers are generally trapped in the upper respiratory tract and eventually cleared or swallowed.

Engineering controls, such as the use of biological safety cabinets, should always be considered as a first line of defense against respiratory infection when working with infectious

organisms. Respirators should only be considered as a second line of defense after feasible engineering controls have been put into place and additional controls are still needed.

Respirators vary in design, application, and protective capability. Respirators can be placed into two categories:
♦ air purifying
♦ supplied air

By far, the most commonly used respirators in laboratories are air purifying respirators. These protect by purifying the existing breathing air through a filter (for particulates) or cartridge (for gases and vapors). Dust masks that have been approved by NIOSH are also considered to be air purifying respirators. These are ranked by their filtering efficiencies and by whether they can be used in an environment containing oil aerosols.
Approved dust masks will have one of the following designations – N95, N99, N100, R95, R99, R100,
P95, P99, or P100. N95 masks are currently in use at Scott & White. Proper selection of cartridges and respirators is very important and should not be made without input from the Biosafety Office and Occupational/Employee Health. New regulations concerning respirators require initial and annual training and fit-testing, and well as medical surveillance of all respirator wearers. Please make sure that the Biosafety Office and Occupational/Employee Health is notified whenever the use of a respirator is being considered. The Occupational/Employee Health Office can assist in evaluating the procedure, selecting the proper respirator, and provide the required training and fit testing. The Employee Health Office must also be notified so that medical surveillance and clearance can be issued prior to wearing the respirator. Powered air purifying respirators (PAPR) can also be used instead of N95-type masks. Please contact the Biosafety Office if considering these for use. Medical clearance for PAPR use also needs to be obtained.

4.3.1.6. Selection of PPE

The Biosafety Office is your first contact regarding the use of PPE. We can provide recommendations for ordering the correct PPE to use in your laboratory. Table 6 below also provides some guidance on proper selection of PPE.

Use the following PPE to minimize exposure via mucous membrane or non-intact skin:

♦ For face protection, wear safety glasses and a mask, or a chin length face shield whenever splashing, splattering or droplets may be anticipated (any work with liquids on the open bench). An impact resistant face shield should be used when operating the autoclave. Impact resistant face shields will protect the user’s face against splatters of hot liquids or broken glass fragments.
♦ Gloves and a lab coat are worn to protect the skin and clothing from contact with potentially infectious materials. Wear gloves that are long enough to extend over the sleeves of the lab coat and cover wrists. Consider double gloving when working with cultures of infectious agents or handling spills. Thicker household utility gloves can be worn for cleaning blood or BL2 spills. Utility gloves can be decontaminated and reused until the integrity of the glove is compromised.

Temperature resistant gloves should be worn to protect hands from physical damage when working with very hot (autoclave) or cold (liquid nitrogen tank, -70°C freezer) materials.

♦ Sleeve covers are worn over lab coat and gown sleeves to provide protection to the sleeves and wrists from contamination when working in the biological safety cabinet. Disposable sleeve covers have tight fitting grips at both ends.

♦ Waterproof bandages are worn to cover any wounds or non-intact skin before gloving. It is preferred to double glove when skin is damaged or non-intact. Inform your supervisor of any severe skin conditions or wounds. Avoid working with BL2, BL3 or other potentially infectious materials if non-intact skin cannot be adequately covered.

♦ Solid front gowns provide more protection to clothing and skin than lab coats. Solid front gowns are worn for high hazard infectious agent work. The tight fitting cuffs of the gown help to minimize wrist contamination.

♦ Impervious lab coats, gowns or aprons are worn when heavy contamination or soiling is likely.

♦ Head covers are worn to protect the hair and scalp from splatter or droplets when working with heavy contamination or when contact with the head is likely. When choosing a head cover make sure it is impervious to liquids (some head covers are not impervious).

♦ Shoe covers are worn over the shoes to protect shoes from contamination when working in heavily contaminated areas (such as large spills, crime scenes, morgues, cadaver dissection areas, surgical operation areas).

♦ Gowns, head and shoe covers also help keep contaminants from entering the sterile area in clean rooms and surgical suites.

Use the following PPE to minimize exposure via cuts, slices, or scratches:

Kevlar gloves and sleeves are cut resistant and will help guard against slices, scratches or cuts, but will not prevent direct puncture or needlestick injuries. Steel mesh gloves also protect against slices, cuts, and scratches but will not eliminate punctures. Neoprene and other abrasive resistant gloves are cut resistant, but significantly reduce dexterity.

Use the following PPE to minimize exposure via aerosols:

HEPA filtered respirators (air purifying or powered air purifying) are worn to prevent exposure to potentially infectious aerosols when cleaning spills of concentrated infectious material or responding to centrifuge incidents. Employees who wear a respirator must enroll in the Occupational/Employee Health program prior to using these respirators.

Table 6. Proper Selection of PPE Based Upon Biosafety Level

PPE	Biosafety Level 1	Biosafety Level 2	Biosafety Level 3
Gloves	Required	Required	Double Gloves Required
Lab Coat	Required	Required	Solid front protective clothing such as back fastening gown with tight fitting cuffs must be worn to protect street clothing and skin from contact with infectious agents
Face Protection		Wear protective eyewear and surgical mask or chin length face shield whenever splashing, splattering or spraying is anticipated to prevent contact with mucous membranes of eyes, nose and mouth. Researchers may choose to augment eye protection by performing experiments behind a protective splash shield	Face protection is not required when performing all work inside a biological safety cabinet. However, if there is a potential for splashing or splattering (such as during container transport, face and eye protection must be worn.
Respiratory Protection			The use of respiratory protection such as a PAPR or N95 mask will be recommended or required by the Biosafety Officer on a case by case basis. PIs should do a primary risk assessment to gauge the need for such protection. The use of a PAPR is required for response and cleanup of a BSL3 spill. All those persons that wear a respirator must be enrolled in the Employee Health program, be medically cleared, and be certified for use.
Other		Other PPE such as Tyvek coveralls, booties, sleeve guards, plastic aprons and household rubber gloves can be recommended on a case by case basis. As a general rule, persons should use additional protective clothing when performing procedures that have the potential for generating splashes, splatters or sprays of infectious material.	Other PPE such as Tyvek coveralls, booties, sleeve guards, plastic aprons and household rubber gloves can be recommended on a case by case basis. As a general rule, persons should use additional protective clothing when performing procedures that have the potential for generating splashes, splatters or sprays of infectious material.

4.4 Laboratory Biosafety Manual

Laboratories conducting research utilizing Risk Group 2 agents or higher are required to assemble a laboratory biosafety manual that is specific for their laboratory. The 5th edition of the BMBL requires that the following be included in a laboratory biosafety manual:

- Lab-specific biosafety policies (e.g. hand washing, food storage, restricted access, daily surface decontamination procedures, emergency spill and clean-up procedures, use of PPE, etc.).
- Project-specific safety SOPs
- Fact sheets on organization safety policies (e.g. Lab sharps policies, Disposal policies, etc.)
- Exposure control plans
- Relevant sections of the BMBL regarding agents utilized in the laboratory
- Documentation of training for all laboratory personnel
- Copies of IBC permits and Inspection Results

In addition to these requirements, the Office of Biosafety requires the inclusion of contact information for the PI and other laboratory personnel.

5. LABORATORY EQUIPMENT

5.1. Procedures for Centrifugation

All centrifugation of biohazardous agents (Risk Group 2 and above) shall be done using centrifuge safety buckets or sealed centrifuge tubes in sealed rotors. If a small centrifuge (microfuge) is used and centrifuge safety cups are not available, the centrifuge should be operated in the biological safety cabinet.

Each person operating a centrifuge should be trained on proper operating procedures.

Keep a log book detailing operation records for centrifuges and rotors to assist in determining service requirements.

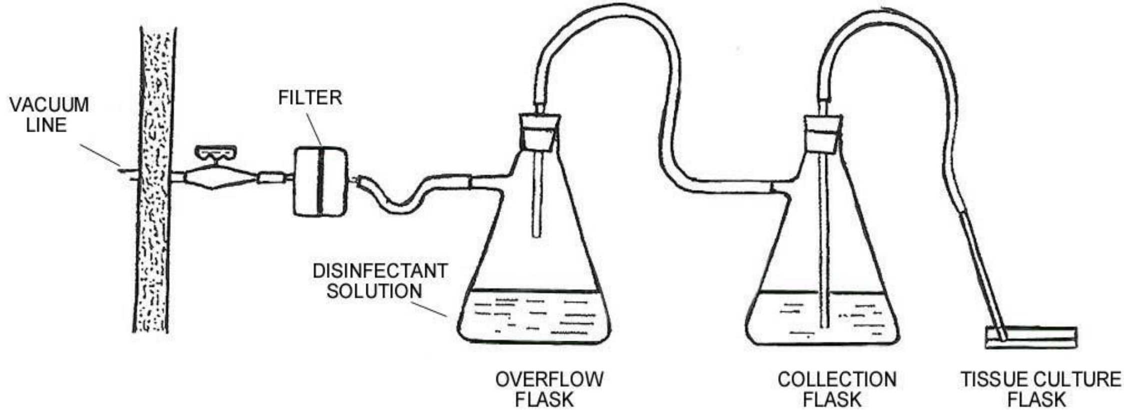
The following procedures for centrifugation are recommended:

- ♦ Examine tubes and bottles for cracks or stress marks before using them.
- ♦ Fill and decant all centrifuge tubes and bottles within the biological safety cabinet. Wipe outside of tubes with disinfectant before placing in safety cups or rotors.
- ♦ Never overfill centrifuge tubes as leakage may occur when tubes are filled to capacity. The maximum for centrifuge tubes is 3/4 full.
- ♦ Always cap tubes before spinning.
- ♦ Place all tubes in safety buckets or sealed rotors. Inspect the "O" ring seal of the safety bucket and the inside of safety buckets or rotors. Correct rough walls caused by erosion or adhering of matter and remove debris from the rubber cushions.
- ♦ Wipe exterior of tubes or bottles with disinfectant prior to loading into rotor or safety bucket.
- ♦ Never exceed safe rotor speed.
- ♦ Stop the centrifuge immediately if an unusual condition (noise or vibration) begins.
- ♦ Wait five minutes after the run before opening the centrifuge. This will allow aerosols to settle in the event of a breakdown in containment.
- ♦ Decontaminate safety carriers or rotors and centrifuge interior after each use.
- ♦ Open safety buckets or rotors in a biological safety cabinet. If the rotor does not fit in the biological safety cabinet, use the fume hood.

♦ If construction of the centrifuge permits, the centrifuge chamber is to be connected to a vacuum pump with a HEPA filter installed between the centrifuge and the vacuum pump.

5.2. Vacuum Line Chemical Traps and Filters

Vacuum line chemical traps and filters prevent suction of infectious and non-infectious materials into the vacuum lines. A typical setup is illustrated below:



Considerations and Limitations of Vacuum Line Chemical Traps and Filters:

- ♦ Add full strength chemical disinfectant to chemical trap flasks. Allow the aspirated fluids to complete the dilution. (For example: Start with 100-ml household chlorine bleach, aspirate 900-ml fluids and discard.)
- ♦ Vacuum line filters shall be examined and replaced if clogged or if liquid makes contact with the filter. Used filters shall be discarded in the medical waste stream.
- ♦Vacuum trap lines should be contained within the Biological Safety Cabinet. However, if space is at a premium, they may be kept outside of the hood, contained within a large tray that would be able to hold the contents of the flasks should they spill. The flasks must contain disinfectant.

5.3. Blenders, Mixers, Sonicators, and Cell Disruption Equipment

Hazardous aerosols are created by most laboratory operations involving blending, mixing, stirring, grinding or disrupting biohazardous materials. Even the use of a mortar and pestle can be a hazardous operation. Other devices that may produce aerosols are ball mills, colloid mills, jet mills, tissue grinders, magnetic mixers, stirrers, sonic cleaning devices, ultrasonic cell disintegrators, and shakers.

Adequate decontamination is essential prior to sonic cleaning due to possible aerosol generation. Wherever sonicators are used in the cleaning process; such as in dishwashers, animal cage washers, etc.; all items should be sterilized prior to cleaning.

The laboratory practices generally required when using equipment that may generate aerosols with biohazardous materials are as follows:

- ♦ Operate blending, cell disruption, and grinding equipment in a biological safety cabinet;
- ♦ Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl. In the absence of a leakproof rotor, inspect the rotor for leakage prior to operation. A preliminary test run with sterile water, saline, or methylene blue solution is recommended prior to use;

- ♦ If the blender is used with infectious material place a towel moistened with an appropriate disinfectant over the top of the blender. Sterilize the device and residual contents promptly after use;
- ♦ Glass blender bowls are undesirable for use with infectious material because of the potential for glass bowls to break;
- ♦ Blender bowls sometimes require supplemental cooling to prevent destruction of the bearings and to minimize thermal effects on the product;
- ♦ Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud;
- ♦ Grinding of infected tissues or materials with any open device is best done within a biological safety cabinet.

5.4. Microtome/Cryostat

Due to the very sharp blade and the nature of the materials used with the microtome/cryostat, training is essential in the use of the equipment and in the hazards of the materials used with the equipment. Users should be informed of the need to prevent cuts and scrapes as well as protect the eyes, nose, mouth and skin from exposure to the materials being used.

New personnel must be trained in the proper use and maintenance of the equipment, and demonstrate proficiency prior to use.

If using human or nonhuman primate tissue, microtome/cryostat users are required to attend Bloodborne Pathogens training. Fixatives take time to penetrate tissue; the fixatives may not inactivate pathogens deep in the tissue. Freezing and drying do not inactivate most pathogens, especially within the context of tissue samples. So, as with fixative use, the pathogens that may be present in the tissue should be considered capable of causing infection.

Microtome/cryostat users shall also attend Chemical Safety Laboratory Personnel training due to the fixatives and dyes used in histology.

When purchasing new units the available safety features should be taken into consideration prior to deciding on a manufacturer or model. Some available safety features are:

- ♦ Auto-decontamination cycle.
- ♦ Easy blade release for installing and changing blades.
- ♦ Retractable knife/blade to permit safe entry into chamber for cleaning, retrieving specimens, etc.
- ♦ Disposable blades.

6. EMERGENCY PROCEDURES (Biological Exposures)

Refer to Scott & White Emergency Procedures for more information on chemical and radiological spills, fire, evacuations and tornadoes. Also, refer to the Spill Response section in this document (section 9) and the Scott & White IBC policy on biohazardous spills (Appendix Z).

There are four basic steps to exposure response: 1) Immediate care; 2) Reporting; 3) Medical attention; and 4) Follow-up.

6.1. Immediate Care

An exposure is defined as a specific contact (eye, mouth, other mucous membrane, respiratory tract via inhalation, non-intact skin, or parenteral) with potentially infectious materials that results from the performance of a person's duties. A person who sustains a known or potential exposure incident must **immediately** stop work, remove gloves and treat the affected area immediately as indicated below:

6.1.1. Percutaneous Injury

If you experience a needle stick or cut (scalpel, scissors, razor blade, etc.) while working with biohazardous agents, immediately stop your work, remove your gloves and wash the affected area for 15 minutes with soap and water.

6.1.2 Splash to the Face

If you are splashed in the face with a potential biohazard, proceed to the eyewash and flush the affected area for 15 minutes.

6.1.3 Aerosol Exposure

If you experience an aerosol exposure (such as from a large spill), immediately leave the room. Remove your PPE carefully and make sure to turn potentially exposed areas of the PPE inward. Post a spill sign on the entrance to the lab and inform others of the spill—the lab should be evacuated for at least 30 minutes. Wash hands well with soap and water and wash exposed area(s) well also with soap and water (unless the exposed areas are the eyes—then flush the eyes at the eyewash).

In all exposure cases, the person experiencing the exposure must report to the Scott & White Employee Health Clinic (Building 19) after the primary response for a proper medical evaluation.

6.2 Incident Reporting

The person experiencing the exposure must report the incident immediately to his/her supervisor. An incident report also needs to be filed with the Biosafety Office within 24 hours. If the exposure incident involves work with research animals, it must also be reported to the Scott & White IACUC. The Biosafety Office may initiate an investigation, in cooperation with the PI, based upon the incident report for the sole purpose of identifying ways to mitigate such an exposure in the future.

6.3. Medical Attention

Those experiencing exposures are urged to report to Employee Health (Bldg. 19) for an evaluation after they have received any first aid necessary at the site of the exposure. Employee health services will review the exposure and determine if any additional treatment is necessary.

It should be emphasized that the reporting of accidents to the principal investigator or laboratory supervisor is the responsibility of the employee who has the accident. Please also report incidents that did not result in an

exposure (near miss) to the Biosafety Office. Evaluation of near misses can lead to alternative work practices and implementation of engineering controls to minimize future incidents.

Whenever an injury involves a sharp and human material (body fluid, tissue, cell line, etc.) the Biosafety Office must perform an investigation to determine if a safe sharps device is available to prevent future occurrences of the injury. If safe sharps devices are available they must be evaluated by the biosafety office in conjunction with the Group or Department. The incident must also be recorded on the Sharps Injury Log, maintained by the Worker's Compensation Office. The confidential log will include the type and brand of device involved in the incident; the Department or work area where the exposure incident occurred; and an explanation of how the incident occurred.

6.4 Follow-up

The person experiencing the exposure may need follow-up contact with the medical professional for additional treatment or testing. In addition, the Biosafety Officer will follow-up with the person to fully investigate the exposure and provide any assistance in ensuring that such an exposure does not occur again.

7. DECONTAMINATION AND DISPOSAL

7.1. Decontamination Methods

Decontamination is an important aspect for consideration in laboratory biosafety. It is first important to understand the principles of sterilization and disinfection. An item is considered to be sterile when it is completely free of all living microorganisms and viruses—something is either sterile or it is not. A sterilization procedure is one that kills all microorganisms, including high numbers of bacterial endospores. Disinfection is, in general, a less lethal process than sterilization. It eliminates nearly all recognized pathogenic microorganisms, but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. Physical and chemical means of decontamination are available to researchers at Scott & White. These include three main categories: heat; liquid decontaminants; and vapors and gasses. It is important for the PI to perform a risk assessment with regard to the use of specific decontaminants, especially liquids, as these can vary in effectiveness, depending upon the biohazardous agent in use.

7.1.1. Heat

The application of heat, either moist or dry, is considered the most effective method of decontamination/sterilization. Steam at 121°C, under pressure (autoclaving) is the most convenient method of rapidly achieving sterility under ordinary circumstances. Sterility can be achieved in as little as 30 minutes (depending upon load size). Dry heat, at 160°C to 170°C for two to four hours is suitable for sterilization of impermeable non-organic materials such as glass, but is not reliable in even shallow layers of organic or inorganic material that could act as insulation. Finally, incineration is also a method of heat decontamination. Incineration provides a complete combustion of waste to render it nonpathogenic. This is an ideal method for human and animal pathological wastes.

The primary hazard with the use of heat in decontamination/sterilization is the potential for serious burns when handling hot solids and liquids. Autoclaves are especially hazardous due to the presence of steam, which can be forcefully ejected if the equipment is improperly used.

7.1.2. Liquid Decontaminants

For the most part, the usefulness of liquid decontaminants is limited to surface decontamination and as decontaminants of liquid wastes prior to disposal in the sanitary sewer system.

There are a great number of liquid decontaminants on the market, under a number of different names. However, they can be placed into the following categories: Halogens; Acids and Alkalies; Heavy Metal Salts; Quaternary Ammonium Compounds; Phenols; Aldehydes; Ketones; Alcohols; and Amines. It is important to note that all compounds do not have the same effects on all agents and that these compounds can fail to disinfect. Thus, liquid disinfectants must be properly evaluated for efficacy. The following considerations should be taken into account when choosing a disinfectant: Target organism; Temperature of effectiveness; Contact time; pH; Humidity; Concentration; and penetrability and reactivity of organic material at the site. Small variations in these factors may make large differences in the effectiveness of some liquid decontaminants. An additional consideration to take into account is that the more active the decontaminant is, usually the more corrosive it is.

It is important to handle liquid decontaminants, especially concentrated solutions, with care. These have the potential to induce significant damage to exposed areas.

The charts on the next two pages provide some guidance with regard to choosing an appropriate liquid disinfectant(s) for use in the laboratory. The first chart lists a number of liquid disinfectants and their effectiveness against various microorganisms. The second chart lists characteristics of various liquid disinfectants

The Antimicrobial Spectrum of Disinfectants

Chemical Disinfectants

Note: Removal of organic material must always precede the use of any disinfectant.

susceptibility of microorganisms to chemical disinfectants: most susceptible (top) → most resistant (bottom)

	Acids (hydrochloric acid, acetic acid, citric acid)	**Alcohols** (ethyl alcohol, isopropyl alcohol)	**Aldehydes** (formaldehyde, paraformaldehyde, gluteraldehyde)	**Alkalis** (sodium or ammonium hydroxide, sodium carbonate)	**Biguanides** (chlorhexidine®, Nolvasan®, Chlorhex®, Virosan®, Hibistat®)	**Halogens** hypochlorite	**Halogens** iodine	**Oxidizing Agents** (hydrogen peroxide, peroxyacetic acid, Trifectant®, Virkon-S®, Oxy-Sept 333®)	**Phenolic Compounds** (Lysol®, Osyl®, Amphyl®, TekTrol®, Pheno-Tek II®)	**Quaternary Ammonium Compounds** (Roccal®, Zepharin®, DiQuat®, Parvosol®, D-256®)
mycoplasmas	+	++	++	++	++	++	++	++	++	+
gram-positive bacteria	+	++	++	+	++	+	+	+	++	++
gram-negative bacteria	+	++	++	+	++	+	+	+	++	+
pseudomonads	+	++	++	+	±	+	+	+	++	−
rickettsiae	±	+	+	+	±	+	+	+	+	±
enveloped viruses	+	+	++	+	±	+	+	+	± [a]	±
chlamydiae	±	±	+	+	±	+	+	+	±	−
non-enveloped viruses	−	−	+	±	−	+	±	±	−	−
fungal spores	±	±	+	+	±	+	+	±	+	±
picornaviruses (i.e. FMD)	+	N	+	+	N	N	N	+	N	N
parvoviruses	N	N	+	N	N	+	N	±	N	−
acid-fast bacteria	−	+	+	+	−	+	+	±	±	−
bacterial spores	±	−	+	±	−	+	+	+ [b]	−	−
coccidia	−	−	−	+ [c]	−	−	−	−	+ [d]	−
prions	−	−	−	−	−	−	−	−	−	−

LEGEND

- ++ highly effective
- + effective
- ± limited activity
- − no activity
- N information not available

a–varies with composition
b–peracetic acid is sporicidal
c–ammonium hydroxide
d–some have activity against coccidia

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DISCLAIMER: The use of trade names does not in any way signify endorsement of a particular product. For additional product names, please consult the most recent Compendium of Veterinary Products.

ADAPTED FROM: Linton AH, Hugo WB, Russel AD. Disinfection in Veterinary and Farm Practice. 1987. Blackwell Scientific Publications; Oxford, England; Quinn PJ, Markey BK. Disinfection and Disease Prevention in Veterinary Medicine, In: Block SS, ed., Disinfection, Sterilization and Preservation. 5th edition. 2001. Lippincott, Williams and Wilkins: Philadelphia.

Characteristics of Selected Disinfectants

For More Information, see the 'Disinfection 101' document at www.cfsph.iastate.edu

Disinfectant Category	Alcohols	Aldehydes	Biguanides	Halogens: Hypochlorites	Halogens: Iodine Compounds	Oxidizing Agents	Phenols	Quaternary Ammonium Compounds (QAC)
Sample Trade Names	Ethyl alcohol Isopropyl alcohol	Formaldehyde Glutaraldehyde	Chlorhexidine Nolvasan® Virosan®	Bleach	Betadyne® Providone®	Hydrogen peroxide Peracetic acid Virkon S® Oxy-Sept 333®	One-Stroke Environ® Pheno-Tek II® Tek-Trol®	Roccal® DiQuat® D-256®
Mechanism of Action	•Precipitates proteins •Denatures lipids	•Denatures proteins •Alkylates nucleic acids	•Alters membrane permeability	•Denatures proteins	•Denatures proteins	•Denature proteins and lipids	• Denatures proteins • Alters cell wall permeability	• Denatures proteins • Binds phospholipids of cell membrane
Advantages	•Fast acting •Leaves no residue	•Broad spectrum	•Broad spectrum	•Broad spectrum •Short contact time •Inexpensive	•Stable in storage •Relatively safe	•Broad spectrum	• Good efficacy with organic material • Non-corrosive • Stable in storage	• Stable in storage • Non-irritating to skin • Effective at high temperatures and high pH (9-10)
Disadvantages	•Rapid evaporation •Flammable	•Carcinogenic •Mucous membranes and tissue irritation •Only use in well ventilated areas	•Only functions in limited pH range (5–7) •Toxic to fish (environmental concern)	•Inactivated by sunlight •Requires frequent application •Corrodes metals •Mucous membrane and tissue irritation	•Inactivated by QACs •Requires frequent application •Corrosive •Stains clothes and treated surfaces	•Damaging to some metals	• Can cause skin and eye irritation	
Precautions	Flammable	Carcinogenic		Never mix with acids; toxic chlorine gas will be released			May be toxic to animals, especially cats and pigs	
Vegetative Bacteria	Effective	Effective	Effective	Effective	Effective	Effective	Effective	YES—Gram Positive Limited—Gram Negative
Mycobacteria	Effective	Effective	Variable	Effective	Limited	Effective	Variable	Variable
Enveloped Viruses	Effective	Effective	Limited	Effective	Effective	Effective	Effective	Variable
Non-enveloped Viruses	Variable	Effective	Limited	Effective	Limited	Effective	Variable	Not Effective
Spores	Not Effective	Effective	Not Effective	Variable	Limited	Variable	Not Effective	Not Effective
Fungi	Effective	Effective	Limited	Effective	Effective	Variable	Variable	Variable
Efficacy with Organic Matter	Reduced	Reduced	?	Rapidly reduced	Rapidly reduced	Variable	Effective	Inactivated
Efficacy with Hard Water	?	Reduced	?	Effective	?	?	Effective	Inactivated
Efficacy with Soap/ Detergents	?	Reduced	Inactivated	Inactivated	Effective	?	Effective	Inactivated

? Information not found

Disclaimer: The use of trade names does not in any way signify endorsement of a particular product.
For additional product names, please consult the most recent Compendium of Veterinary Products.

References: Linton AH, Hugo WB, Russel AD. Disinfection in Veterinary and Farm Practice. 1987. Blackwell Scientific Publications; Oxford, England; Quinn PJ, Markey BK. Disinfection and Disease Prevention in Veterinary Medicine, In: Block SS, ed., Disinfection, Sterilization and Preservation. 5th edition. 2001. Lippincott, Williams and Wilkins: Philadelphia.

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7.1.3 Vapors and Gases

For many years, formaldehyde gas was the gold standard for large-scale decontamination (Biological Safety Cabinets, rooms, etc). Now, there are several alternatives to formaldehyde, which has been recently designated a carcinogen. Ethylene oxide has been used as an alternative for a number of years, and is primarily utilized in surgical and clinical areas. However, mutagenic potential has been noted for this compound as well. More recently, vaporized hydrogen peroxide (VHP) and chlorine dioxide gas (CD) have been increasingly used for large-scale decontaminations.

Formaldehyde gas (achieved by heating paraformaldehyde flakes in a pan; 0.3 g/cubic foot) is still utilized for small space decontaminations (such as for a BSC). It does require humidity and temperature control (relative humidity of 80% is optimal; temperature of at least 20°C). These procedures usually take from four hours to overnight to complete dependent upon the size of the area to be decontaminated. The formaldehyde gas is neutralized with ammonium carbonate. Formaldehyde is low-cost, but the disadvantages include that it is a carcinogen and it can polymerize on surfaces due to changes in temperature, humidity or concentration.

Vaporized Hydrogen Peroxide (VHP; 0.5 – 10 mg/liter effective concentration) is a powerful oxidizing agent and is sporicidal. It can be used for BSCs, rooms, isolators, ambulances and in sterilizers for medical and dental equipment. It requires less time than formaldehyde, but still has a requirement for temperature and humidity (15-25°C, from 25-70% humidity, depending on the system utilized). Advantages include the shorter time for decontamination, no harmful breakdown products (result is water and oxygen), it is compatible with electronics, and it is not carcinogenic. Disadvantages include that it is toxic, it is a vapor--so it cannot get everywhere like a gas can, and can be trapped in porous materials such as wood.

Chlorine Dioxide Gas (1-5 mg/liter) is another alternative to formaldehyde for instrument and space decontamination. It is toxic, but it is non-carcinogenic and is not a reproductive hazard. It will effectively penetrate water. And because Chlorine Dioxide is a gas, it will penetrate places that a vapor cannot. It is approved by the NSF for BSC decontamination. Disadvantages include that it must be generated onsite; it is an oxidizer and can be incompatible with uncoated ferrous metals and latex rubbers; and it is rapidly broken down by light.

Both VHP and Chlorine Dioxide Gas have high initial capital equipment costs associated with the instrumentation purchase. However, there are companies that use these technologies and provide decontamination services on a fee-for-service basis.

7.2 Disposal of biohazardous waste.

7.2.1 Solid waste. All laboratories should have an appropriate number of biohazardous waste receptacles. It is required that these receptacles have covers to prevent possible aerosol exposure when waste is disposed. Red biohazard bags should be placed in the receptacles for the collection of solid waste materials. When the bags are ¾ full, they should be removed from the receptacles and tied loosely. The bag should then be placed into a second red biohazard bag and that bag similarly tied.

7.2.1.1 Autoclaving. One option for decontaminating biohazardous waste is to utilize the autoclave. You should refer to the manufacturer's guidelines on the use of the autoclave, but a cycle of 1 hour at 121°C, 15-18 PSI is adequate to decontaminate solid waste. Bags should not be tightly sealed, so that the steam can penetrate and decontaminate everything. Verification of autoclave efficacy needs to be done on a weekly basis using biological indicators. All records of autoclave operation (temperature recordings, biological indicator verification, etc.) should be

maintained for at least three years. Autoclaved waste can then be discarded in the normal trash (but the red biohazard bags should not be).

7.2.1.2. Contractor disposal. As a second option, Scott & White Healthcare contracts with Medsharps to collect, haul away and incinerate medical and biohazardous waste. After the waste is collected in the laboratory, simply place them in the leak-free boxes that Medsharps supplies, and bring them to the common area for collection after sealing the full boxes. Medsharps will take them away for incineration. This option is much less taxing on the laboratory worker as no validations need to be performed.

7.2.2 Liquid waste. Any biohazardous liquid waste should be decontaminated by laboratory personnel. An appropriate disinfectant should be chosen by the laboratory, based upon the agent being utilized (please see the charts earlier in this section for aid in determining an appropriate disinfectant, or contact the Biosafety Office for assistance). The Biosafety Office usually recommends household bleach as the disinfectant of choice for most agents. In this case, a final concentration of 10% bleach is used for treating liquid waste (example—100 mls of undiluted bleach is added to 900 mls of liquid waste to give a 10% final concentration). The bleach should be allowed to work for at least 30 minutes, then the mixture can be disposed of down the sanitary sewer. If using a different disinfectant, incubation times may vary before disposing.

8. Transportation of Biological Materials

8.1 Transportation of Biological Materials between laboratories. Whenever one is transporting biological materials between laboratories (including between a Scott & White laboratory and a Texas A&M laboratory on campus), the material must be packaged in a leak-proof secondary container. This is to ensure that if the item falls and the primary receptacle breaks, the material will be contained within the secondary packaging.

8.2 Shipping of Biological Materials. Whenever biological materials are being shipped to another location, it is required that the person(s) packing and preparing the materials for shipping must be trained and certified for the proper methods. Shipping of hazardous materials is covered by specific US Department of Transportation guidelines that must be followed exactly.

Methods for shipping hazardous biological materials will not be covered in this biosafety manual. However, there are four critical steps involved in shipping biological materials: Classification, Packaging, Labeling/Marking, and Documentation. The biosafety office provides training for those wishing to ship such materials. The training is required to be completed every two (2) years. Please call the biosafety office to inquire about scheduling training.

8.3. Exportation of Biological Materials. The export of infectious material may require a license from the Department of Commerce. The Biosafety Office will review each export of biological or chemical materials to determine if an export permit is required. If a permit is required the Biosafety Office will assist with obtaining the permit

8.4. Importation of Biological Materials. Certain materials require import permits prior to entering the United States. Most agencies have indicated that if there is doubt whether a permit is required it is best to submit the permit application and the agency will decide. If you have any questions please call the Biosafety Office or the appropriate agency.

9. Spill Response

This guide outlines the basic procedures for dealing with some of the biological spills that you may encounter in your research laboratory. All lab personnel should refer to the relevant spill response within their laboratory-specific Biosafety Manual procedures before initiating their experiments.

9.1 Composition of a Basic Spill Kit

Microbiological and biomedical research laboratories should prepare and maintain a biological spill kit. A spill kit is an essential safety item for labs working with microbiological agents classified at Biosafety Level 2 or higher and for groups working with large volumes (> 1 liter) of Biosafety Level 1 material. A basic spill kit should include:

♦ Concentrated household bleach
♦ A spray bottle for making 10% bleach solutions
♦ Forceps, broom and dust pan, or other mechanical device for handling sharps
♦ Paper towels or other suitable absorbent
♦ Biohazard autoclave bags for the collection of contaminated spill clean-up items
♦ Utility gloves and medical examination gloves
♦ Face protection (eye wear and mask, or full face shield)

Additional personal protective equipment, such as Tyvek jump suits and powered air-purifying respirators (PAPR's), may be required for response to spills in Biosafety Level 3 laboratories.
Representatives from the OEHS Occupational Health and Safety section are available if you have any questions regarding biological spill response procedures or decontamination (785 - 3550). All spills in a BL3 laboratory shall be reported to OEHS immediately.

9.2 Biosafety Level 1 (BSL1) Spill

♦ Notify others in the area, to prevent contamination of additional personnel and environment.
♦ Remove any contaminated clothing and wash exposed skin with disinfectant.

9.2.1 Clean-up of BSL1 Spill

♦ Wearing gloves, lab coat, and face protection, cover spill with paper towels, pour concentrated disinfectant around the spill allowing it to mix with spilled material. Allow suitable contact time.
♦ Pick up any pieces of broken glass with forceps and place in a sharps container.
♦ Discard all disposable materials used to clean up the spill into a biohazard autoclave bag.
♦ Wash hands with soap and hand-washing disinfectant.

9.3 Biosafety Level 2 (BSL2) Spill

♦ Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door, and post with a warning sign.
♦ Remove contaminated clothing, turning exposed areas inward, and place in a biohazard bag.
♦ Wash all exposed skin with soap and water.
♦ Inform Supervisor, and, if assistance is needed, consult the Biosafety Office (785-3550).

9.3.1 Clean-up of BL2 Spill

♦ Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up
materials (disinfectant, paper towels, biohazard bags, and forceps).
♦ Put on protective clothing (lab coat, face protection, utility gloves, and booties if necessary). Depending on the nature of the spill, it may be advisable to wear a HEPA filtered respirator instead of a surgical mask.
♦ Cover the area with disinfectant-soaked towels, and then carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use more concentrated disinfectant as it is diluted by the spill. Allow at least a 20 minute contact time.
♦ Pick up any sharp objects with forceps and discard in a sharps container. Soak up the disinfectant and
spill using mechanical means, such as an autoclavable broom and dustpan, since there may be sharps under the paper towels, and place the materials into a sharps container. Smaller pieces of glass may be collected with cotton or paper towels held with forceps. If no sharps were involved in the spill discard the materials into an autoclave bag.
♦ Wipe surrounding areas (where the spill may have splashed) with disinfectant.
♦ Soak up the disinfectant and spill, and place the materials into a biohazard bag.
♦ Spray the area with 10% household bleach solution and allow to air-dry (or wipe down with disinfectant-soaked towels after a 10-minute contact time). Place all contaminated paper towels and any contaminated protective clothing into a biohazard bag and autoclave.
♦ Wash hands and exposed skin areas with disinfectant or antiseptic soap and water.

9.3.2 Blood Spills

For blood or other material with a high organic content and low concentration of infectious microorganisms:
♦ Wear gloves, eye protection, and a lab coat.
♦ Absorb blood with paper towels and place in a biohazard bag. Collect any sharp objects with forceps or other mechanical device and place in a sharps container.
♦ Using a detergent solution, clean the spill site of all visible blood.
♦ Spray the spill site with 10% household bleach and allow to air-dry for 15 minutes.
♦ After the 15 minute contact time, wipe the area down with disinfectant-soaked paper towels.
♦ Discard all disposable materials used to decontaminate the spill and any contaminated personal protective equipment into a biohazard bag.
♦ Wash your hands.

Table A. PI Requirements for Biosafety Compliance

	Required Registration					Required Training				
If using items below requirements indicated with an X	Scott & White IBC registration	Scott & White IBC Full Review	Scott & White IRB	Scott & White IACUC	FDA, NIH	NIH Guidelines	Bloodborne Pathogens	General Laboratory Safety	BSL-2	Hazardous Shipping
Recombinant DNA work (exempt)	X					X		X		
Recombinant DNA work		X				X		X		
Human Materials (Cells, Tissues, Cell Lines, etc)	X		X**				X	X	X	
Infectious Agents, BSL-2	X	X*					X***	X	X	
Animal Use with Recombinant DNA		X		X		X		X		
Animal Use with Human Materials	X			X			X	X	X	
Animal Use with BSL-2 Agents	X	X*		X			X	X	X	
Human Gene Therapy		X	X		X	X	X	X	X	
Infectious Agents, Dry Ice Shipping										X

*Certain agents may require full IBC review
**Review by the Scott & White IRB may not be required in some cases
***Bloodborne pathogen training may not be required in some cases