

# BIOSAFETY AND BIOSECURITY MANUAL



Revised: 5/20/2005  
1/1/2006  
10/11/2006  
5/22/2007





## *Table of Contents*

1.0 Introduction .....	1
1.1 Purpose: .....	1
1.2 Program Statement: .....	1
1.3 Organizational Support: .....	1
1.4 Policy: .....	1
1.5 Scope: .....	2
2.0 Biohazard Risk Classification .....	2
2.1 Conventional Pathogens: .....	2
2.2 Unconventional pathogens ("Slow Viruses"): .....	3
2.3 Genetically Engineered Organisms: .....	3
2.4 Tissue Cultures: .....	4
3.0 Facility Standard Operating Procedures .....	5
3.1 Introduction: .....	5
3.2 Biosafety Regulations Applied to all Staff: .....	5
3.3 Staff Orientation: .....	5
3.4 Training and Documentation Review: .....	6
3.5 Medical Evaluations: .....	6
3.5.1 Biomedical Screening: .....	6
3.5.2 Immunization Requirements: .....	6
3.5.3 Pregnancy Information: .....	7
3.5.4 Eye-ware (glasses and contact lenses) .....	7
3.5.5 Personal Medications: .....	7
3.5.6 Training: .....	7
3.6 Facility Security Requirements: .....	8
3.6.1 General Security Provisions: .....	8
3.6.2 Visitor Procedures: .....	8
3.6.3 Contractors Procedures: .....	8
3.6.4 Buddy System: .....	8
3.6.5 After-hours Access: .....	8
4.0 Laboratory Operations and Safety Procedures .....	9
4.1 Introduction: .....	9
4.2 Entry/Exit Procedures: .....	9
4.2.1 Personnel: .....	9
4.2.2 Equipment and Materials: .....	9
4.2.3 Specimens: .....	10
4.2.4 Chemicals and Gases: .....	10
4.2.5 Heat Sensitive Equipment: .....	11
4.2.6 Animals: .....	11
4.3 Laboratory Work Practices: .....	11
4.3.1 Manipulations of Infectious Materials: .....	11
4.3.2 Handling of Hazardous (Non-infectious) Materials: .....	14
4.3.3 Use of Radioisotopes: .....	15
4.3.4 Small animal Manipulation: .....	16
4.3.4.1 Institutional Animal Care and Use Committee: .....	16
4.3.4.2 Uncontaminated Animal Handling: .....	16
4.3.4.3 General Animal Inoculation: .....	16

4.3.4.4 Infected Animal handling/Necropsy: .....	17
4.3.4.5 Disposal Procedures: .....	17
4.3.5 Working Safely in a Biological safety Cabinet (BSC):.....	17
4.3.5.1 Procedures for BSC work:.....	17
4.3.5.2 Burner Alternatives:.....	18
4.3.5.3 Material and Equipment Distributions.....	18
4.3.5.4 Recommended Best Practices in BSC's: .....	19
4.3.6 Safety Precautions for Laboratory Equipment: .....	23
4.3.6.1 Centrifuges: .....	23
4.3.6.2 Internal Transfer and Storage of Etiologic Agents:.....	23
4.3.6.3 Vacuum Apparatus:.....	24
4.3.6.4 Autoclaves:.....	24
4.3.6.5 Lypholiser: .....	26
4.3.7 Pipetting: .....	26
4.3.8 Needles and Syringes: .....	26
4.3.9 Containers Labeling: .....	27
5.0 Laboratory Decontamination Methods/ Procedures:.....	27
5.1 Introduction: .....	27
5.2 Specific Procedures: .....	27
5.2.1 Sharps Disposal: .....	27
5.2.2 General Laboratory Waste from BSL2/3 Facilities: .....	28
5.2.3 Chemical Waste .....	28
5.2.4 Organic Waste: .....	28
5.2.5 Laboratory Clothing: .....	29
5.2.6 Glassware: .....	29
5.2.7 Gas Cylinder: .....	29
5.2.8 Effluent Traps:.....	29
5.2.9 Laboratory Housekeeping and Cleaning: .....	29
5.2.10 Disinfection.....	29
5.3 Physical/ Mechanical Systems: .....	32
5.3.1 HEPA Housing/Duct Work:.....	32
5.3.2 Biological Safety Cabinets:.....	32
5.3.3 Cage Rack: .....	32
5.3.4 Renovation Waste (e.g. pipes, valves, ceiling tiles):.....	32
5.3.5 Laboratory Equipment: .....	32
5.3.6 Suite Decontamination: .....	33
6.0 Emergency Procedures.....	33
6.1 Introduction: .....	33
6.2 Spill Response: .....	33
6.2.1 Biological.....	33
6.2.2 Chemical: .....	35
6.2.3 Radiological:.....	36
6.3 Fire Management (CODE RED): .....	37
6.3.1 Authorities/Contacts: .....	37
6.3.2 Fire Prevention: .....	37
6.3.3 Fire Extinguisher Training and Use: .....	38
6.3.4 Fire Reporting: .....	38
6.3.5 Evacuation Procedures: .....	38
6.3.6 Suggested Maintenance Requirements of Fire Systems:.....	39

6.4 Bomb Threat (CODE BLACK): .....	39
6.5 First Aid: .....	40
6.6 Accident Reporting (OSHA requirements): .....	40
6.7 Natural Disasters (CODE YELLOW): .....	40
7.0 Procedures for Repair and Renovation .....	40
7.1 Wall Repairs: .....	41
7.2 Ceiling Tile and Surface Repairs: .....	41
7.3 Floor Repairs: .....	41
7.4 Plumbing Re-fits: .....	42
7.5 Electrical Repairs: .....	42
7.6 Ventilation Repairs: .....	42
7.7 Casework and Benchtop Repairs: .....	42
7.8 Service Penetration Seals: .....	43
7.9 Laboratory Equipment Repairs: .....	43
8.0 Building Plans / System Performance Criteria .....	44
8.1 Principles of Containment: .....	44
9.0 Containment Laboratory Certification Requirements .....	44
9.1 Biological Safety Cabinets (BSC): .....	44
9.2 Directional Airflow and Failure Tests: .....	44
9.3 HEPA Filtration: .....	45
9.4 Autoclaves: .....	45
9.5 Review of Operating Procedures: .....	45
10.0 Transport of Biohazardous Material .....	45
10.1 Transport within or Between Labs: .....	45
10.2 Transport between Buildings: .....	46
10.3 Transport by Public Carrier: .....	46
11.0 Contacts and Inquiries: .....	46
12.0 Recommended Reading .....	47
13.0 Bio-security Issues .....	48
ANNEX "A" – Biological Safety Cabinets .....	55
Annex "B" – IACUC Protocol Document .....	63
Annex "C" – Hazard Vulnerability Analysis (HVA) .....	64
Annex "D" – Information System Policies .....	65
Annex "E" – MSDS's for Biological Agents .....	66
Annex "F" – Use of Viral Vectors .....	67
Annex "G" – Recombinant DNA Technology .....	72



## 1.0 Introduction

The University of Toledo has developed this manual for the employees involved with laboratory work which involves the use of biologically hazardous agents, organisms, animals and genetically modified organisms which may pose a risk to their health or wellbeing. The containment levels assigned to the various laboratories across the campus of the University of Toledo are in accordance with the CDC-NIH guidelines "[Biosafety in Microbiological and Biomedical Laboratories](#)" (BMBL) Current Edition. This manual explains the hazards associated working with the agents and the procedures and protocols required to minimize those threats.

A biohazard can be defined as any organism, and/or its toxin, that is known or suspected to cause human or animal disease. Biohazards include microorganisms such as viruses, bacteria, fungi, parasites and their toxic metabolites. Additionally, blood and body fluids, and certain types of nucleic acids such as DNA derived from pathogenic organisms, human oncogenes, and DNA from transformed cell lines are considered biohazards as well. Biohazards are assigned and grouped according to risk as defined in the BMBL and are explained in Section 2

### 1.1 Purpose:

The purpose of this manual is two-fold. First, it is developed as a practical guide and training tool for all staff working in or supporting the containment laboratories. Second, it assists the University to attain/retain the containment status (*biosafety levels 1-3, including animal biosafety levels*) associated with conducting programs with specific and registered organisms.

### 1.2 Program Statement:

This biosafety manual has been written for all laboratories across the campus of the University of Toledo, to allow for the safe handling of infectious microorganisms classified at Biosafety levels 1-3. Any new programs involving different microorganisms must receive approval from the office of Research and Sponsored Programs, the Institutional Biosafety Committee and the Safety and Health Department, or the Infection Control Committee, for the University Medical Center's Clinical Laboratories.

### 1.3 Organizational Support:

There are several University Committees and organizational units that have a mandatory role in the functioning of institutional laboratory space. Specific approvals are required when there are changes in programs, starting new projects, change in protocols, lab modifications or working with animals. All approval requests should be forwarded to the Office of Research and Sponsored Programs. All animal use requests must use the IACUC (Institutional Animal Care and Use Committee) protocol form RGA 401(Current Version). See attached Annex "B".

### 1.4 Policy:

The University of Toledo has established this manual and its contents as mandatory requirements for conducting research at Biosafety level 1-3-BMBL, CDC-NIH guidelines. Any exceptions must be requested in advance by the Safety and Health Department-Contact # (419-383-5069).

## 1.5 Scope:

This manual is developed primarily for the functioning of biomedical research and clinical laboratories at the University of Toledo, Toledo, Ohio. It includes references and protocols that are site specific. Any application of all or part of this manual for other areas or purposes at the outside of the University of Toledo is at the discretion of the user. Biosafety Level 3 laboratories require the implementation of a dedicated Biosafety and Security Manual any questions related to this should be directed to the Safety and Health Department 419-383-5069.

## 2.0 Biohazard Risk Classification

Risk classification is ultimately a subjective process that combines known consequences with several factors such as:

- severity of induced disease
- route(s) of infection
- virulence and infectivity of the microorganism
- antibiotic resistance patterns
- availability of effective medical treatment (e.g. antibiotic therapy) or vaccine
- presence of vectors (e.g. arthropods)
- whether the pathogen is indigenous to the United States
- possible effects on animals and plants
- volumes and methods of manipulation

Essentially, microbiological hazards are classified according to their impact on the individuals who manipulate them, upon their colleagues and other building officials, upon the surrounding community and upon the national interest of the country as a whole.

### 2.1 Conventional Pathogens:

The CDC-NIH guideline (Current Edition) [BMBL 4th Edition](#) lists various pathogens and establishes biosafety level assignments and associated precautions. These pathogens, should they be used, would require associated facility and protocols assigned to the appropriate biosafety level.

Example: \*\* *Coccidioides immitis* is a registered agent listed in 42 CFR Part 73 and requires that facilities meet either Level 2 Biosafety level (recommended for handling and processing clinical specimens, identifying isolates, and processing animal tissues; additionally animal biosafety level 2 practices and facilities are recommended for experimental animal studies when the route of challenge is parenteral.) Biosafety Level 3 practices and facilities are recommended for propagating and manipulating sporulating cultures already identified as *Coccidioides immitis* and for processing soil or other environmental materials known or likely to contain infectious arthroconidia.

The following table defines the biosafety level in accordance with the agent definition producing the hazard.

### Summary of Recommended Biosafety Levels for Infectious Agents

Biosafety level	Agent definition	Examples
1	Not known to cause disease in healthy adults	<i>Bacillus subtilis</i> , <i>Naegleria gruberi</i>
2	Associated with human disease, hazard= auto-inoculation, ingestion, mucous membrane exposure	Hepatitis B virus, Salmonellae, <i>Toxoplasma spp.</i>
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences.	<i>Mycobacterium tuberculosis</i> , <i>Burkholderia mallei</i> and <i>pseudomallei</i> , <i>Coxiella burnetii</i> , <i>Coccidioides immitus</i>
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol transmitted lab infections; or related agents with unknown risk of transmission.	Ebola virus, Marburg virus, Congo-Crimean hemorrhagic fever

#### 2.2 Unconventional pathogens (“Slow Viruses”):

There are some progressive neurological diseases caused by unconventional or slow viruses. Among the slow viruses, prions (proteinaceous infectious particles) have been associated with transmissible degenerative diseases of the central nervous system in humans (Creutzfeldt-Jacob, kuru) and animals (transmissible encephalopathy of mink and scrapie in sheep and goats). These unconventional viruses are resistant to destruction by chemical (10% formalin, glutaraldehyde, 70% ethanol, iodine) and physical (UV light, ionizing radiation, boiling) procedures. While there have been no documented cases of laboratory-acquired infections, the following precautions should be observed when handling neurological material from infected or potentially infected humans and animals:

- handle as risk group 3, or higher depending on the nature of the work and amount of agent being manipulated,
- handle formalin-fixed tissues and paraffin-embedded blocks as if still infectious,
- Handle various body parts such as the brain and spine separate from other parts,
- Utilize personal protective equipment to protect mucous membranes and eyes from exposure and disposable coveralls to protect everyday clothing
- Utilize local engineering controls including local exhaust and cabinets for handling specimens
- Keep up-to-date disinfection/sterilization protocols.
- The use of 1N NaOH has shown some utility in disinfecting surfaces contaminated with these slow viruses.

The Department of Research and Sponsored Programs (RSP) and appropriate University oversight committees must approve all work associated with unconventional, or “slow viruses” in advance.

#### 2.3 Genetically Engineered Organisms:

The term “biotechnology” describes a variety of techniques for manipulation of cells; biotechnology has long been used for purposes such as selective breeding of animals and food production (bread, yogurt, beer). More recently, in vitro incorporation of segments of genetic material from one cell into another (recombinant

DNA technology) has resulted in altered organisms, which can manufacture products such as vaccines, hormones, interferons and enzymes. Genetically engineered organisms are used for treatment of wastes, spills and plants can be made resistant to cold, disease, pests and drought. However, genetically engineered organisms can have adverse effects and potential for harm. Altered organisms may be directly pathogenic or toxic or, if released into the environment, might displace beneficial organisms, transfer undesirable genetic traits to wild species or mutate into a pathogenic form.

When working with recombinant technology, that has the potential for human, animal or environmental harm, the principal investigator must submit a request to the Department of Research and Sponsored Programs (RSP) for submittal to the Institutional Biosafety Committee (IBC) for review and approval. Consideration on the request should include the source of the DNA to be transferred, the vector and the host. When assessing the risk of, and containment level required by, a genetic engineering protocol, the following approach is recommended: if the components of a genetic manipulation are not hazardous, then the altered organism is unlikely to present a risk and no or few restrictions are required, but this must be confirmed by the IBC. (See also Annex "F" Use of Viral Vectors) However, if one of the components is potentially hazardous, a risk level appropriate for the known hazard is assigned and modified as required. Subsequent modifications depend on factors such as:

- expression of the transferred gene in the recombinant organism,
- ability of the vector to survive outside the laboratory environment,
- expected interactions between transferred gene, host and other factors.

## 2.4 Tissue Cultures:

Cell cultures derived from humans or animals known to be infected with a pathogen, as well as cultures known or suspected to contain infectious micro-organisms require to be assigned and have applied containment levels to the risk group appropriate as per the CDC-NIH guidelines, ["Biosafety in Microbiological and Biomedical Laboratories"](#). Tissue cultures, when used in animals, in multi-user facilities, can put other researchers and animals in the facility at risk.

In addition, mammalian cell cultures may carry unsuspected oncogenic, allergenic or infectious particles. It is impractical, if not impossible to screen such cultures for all potentially harmful micro-organisms. Even well-characterized lines with a history of safe use can become contaminated by adventitious, possibly infectious, micro-organisms. For this reason, it is prudent to treat all mammalian cultures as moderate risk agents-Risk group 2 (unless known to be higher) and to adopt containment levels and operating practices assigned to containment level 2 when working with them. In addition, when receiving organisms from other sites it is required by institutional policy to ensure that certain organisms and materials are indeed what was shipped and not a more hazardous agent. (Policy HM-08-031, Shipping, Packaging and Receipt of Hazardous Materials)

All biologic materials including attenuated strains, genetic materials, plasmids and other materials sent or received from other institutions must meet the following criteria:

- The sending institution must provide written assurances to the receiving institution that the material being sent is indeed that material and is not contaminated with any other biological or hazard that may present a risk to the receiving institution.
- These assurances may be made through documented culturing activities and identification techniques that can provide proof of the exact content of the materials being transferred (i.e. PCR etc.).
- Likewise the receiving facility is required to perform similar testing to provide confirmation of the materials received from the sender are as described on shipping documents.

- These procedures will prevent the inadvertent sending and receipt of potentially contaminated materials or the movement of materials that are not labeled properly as to their content.

\*\*Please refer to Institutional Safety & Health Policy #HM-08-031, Shipping, Packaging and Receipt of Hazardous Materials.

## 3.0 Facility Standard Operating Procedures

### 3.1 Introduction:

Selection of an appropriate biosafety level for work with a particular agent or animal study depends upon a number of factors (see [Section V](#), Risk Assessment). Some of the most important are: the virulence, pathogenicity, biological stability, route of spread, and communicability of the agent; the nature or function of the laboratory; the procedures and manipulations involving the agent; the endemicity of the agent; and the availability of effective vaccines or therapeutic measures.

Agent summary statements in this section provide guidance for the selection of appropriate biosafety levels. Specific information on laboratory hazards associated with a particular agent, and recommendations regarding practical safeguards that can significantly reduce the risk of laboratory-associated diseases, are included. Agent summary statements are presented for agents which meet one or more of the following criteria: the agent is a proven hazard to laboratory personnel working with infectious materials (e.g., hepatitis B virus, M. tuberculosis); the potential for laboratory-associated infections is high, even in the absence of previously documented laboratory-associated infections (e.g., exotic arboviruses); or the consequences of infection are grave.

### 3.2 Biosafety Regulations Applied to all Staff:

All procedures involving the manipulation of infectious materials at the BSL2-3 levels should be conducted within biological safety cabinets or other physical containment devices, by personnel wearing appropriate personal protective clothing and equipment. This manual and its incorporating regulations are designed to be non-discretionary, however at various times and for unique situations variations and exceptions may be necessary. When these occur and depending on whether it may become a common practice, inform the Biosafety Officer and he/she will evaluate the situation and consider a revision to the manual.

### 3.3 Staff Orientation:

New staff at the University of Toledo should be required to complete a formal **orientation and training** program. Laboratory personnel working with the organisms classified as BSL2 or higher must be fully trained in biosafety procedures.

Some segments of this program should take place on the days immediately following employment, and other segments will undoubtedly take longer.

The orientation program is the responsibility of several people including the Principle Investigators, animal veterinarians, the Biosafety Officer, the staff member's supervisor and his or her "buddy" (see 3.6.4).

The training segment is initially the responsibility of the Biosafety Officer, with specific instruction being provided by the new staff member's supervisor as he or she settles into the job.

### 3.4 Training and Documentation Review:

It is the responsibility of the laboratory director to ensure that this manual and the policies required for the safe operation of the laboratory are adhered to. He/she must also provide for training and review all safety related documentation for and with the employees.

It is mandatory for employees to attend all training and understand and comply with all the requirements established by the supervisor.

These include:

- Accepted Biosafety Practices
- Biosecurity Program
- Emergency Response Activities

### 3.5 Medical Evaluations:

#### 3.5.1 Biomedical Screening:

Biomedical screening of staff is part of the Laboratory's Occupational Safety and Health Program. All tests and results are strictly confidential. (See Safety and Health policy # HM-08-016 - Industrial Hygiene Monitoring and Medical Surveillance). Under certain circumstances **Blood serum samples** are collected from new appointees and other workers intending to stay at least a month in the secure area. (See Safety and Health Policy # HM-08-021 Universal Precautions for Laboratories.)

The analysis of these samples will only be conducted in the event a staff member becomes ill during the course of his or her work and where it is considered, on medical advice, that there is a possibility that he/she has been infected by a micro-organism used at the institution. Under no circumstances will such analysis be conducted without the knowledge and permission of the person concerned. Upon resignation or termination, blood samples are taken and serum frozen for future reference. Serum samples will be held indefinitely after an employee leaves the University of Toledo.

#### 3.5.2 Immunization Requirements:

Immunization is carried out for those people who work directly with, or who may be exposed to, certain micro-organisms that can infect humans. Hepatitis B immunization is arranged for staff working directly with human blood, and tetanus immunization is offered to staff working in higher-risk environments, or having contact with research animals. Individuals working with animals suspected of carrying rabies will be offered the rabies vaccine.

The immunization programs will be conducted by qualified people and with the full consent of the employees. An employee refusing to receive a vaccination or suspected of having a communicable disease that puts them at risk, may be reassigned to an alternate work assignment.

After vaccination, blood samples may be required for antibody tests to determine the effectiveness of the vaccination. It may be necessary to monitor the antibody levels at intervals, and in some cases staff may be required to repeat a vaccination or booster.

Vaccination schedules are to be arranged by consulting with the Biosafety Officer (#419-383-4521 - Michael Valigosky) and University Health Services.

### **3.5.3 Pregnancy Information:**

It is important that female employees who normally work with infectious agents advise their supervisor immediately when they become aware that they are pregnant. The supervisor must notify the Safety and Health Department. (See Safety and Health Policy # HM-08-028 Teratogens Safe Work Practices.)

Pregnant women are ADVISED of any known dangers involved with continuing work with a particular agent. If evidence is available that indicates a risk to the fetus as a consequence of handling a particular infectious agent, transfer to another work area that does not involve handling that agent can be arranged. However, in most cases, the person will be expected to make her own decision about this. Although conclusive evidence of harmful effects on the fetus as a result of exposure of the pregnant woman to chemicals is, in most cases, lacking, pregnant women are nevertheless advised not to handle concentrated acids or alkalis, glutaraldehyde, formaldehyde or epoxy resins used as embedding agents. Only small amounts of volatile solvents should be used, and only when an exhaust system is available to prevent inhalation of the solvents.

Pregnant women should not handle carcinogenic, mutagenic, teratogenic or known toxic materials. Supervisors are responsible for making staff aware of any special toxicity problem associated with materials used in their laboratory. For further advice, consult with the Biosafety Officer.

Pregnant women may handle radioactive materials; however it is recommended that the exposure level does not exceed 10% of the normal allowable levels. For further advice, you should consult the Radiation Safety Department (#4301). (See Safety and Health Policy HM-08-008 Radiation Worker Pregnancies.)

### **3.5.4 Eye-ware (glasses and contact lenses)**

Staff requiring glasses for use within the Containment Area may be supplied with a pair of prescription safety glasses that must be kept within the Contained Area.

Contact lenses are not recommended in laboratory areas unless medical documentation can be provided that shows wearing glasses will either hamper the individual's ability to perform their job or that their vision can not be effectively corrected with glasses. Prescription safety glasses are a viable option and the employee should contact the Safety and Health Department at x419-383-5069.

All employees working in BSL1, 2, or 3 laboratories and animal rooms should wear splash goggles if there is potential for splashes to the eyes or mucous membranes, when shielding offered by cabinets or other engineering controls are insufficient in their protection of the worker.

### **3.5.5 Personal Medications:**

Personal medicines may not be taken into the contained spaces but must be kept in your locker. They are not to be removed from the contained area. Medicines should only be consumed outside the containment facility. Personal medications should not be consumed in laboratories and animal rooms.

### **3.5.6 Training:**

Proper instruction is the key to a successful training program. The Safety and Health Department will

arrange for and organize training in Biosafety, Biosecurity, Radiation Safety and Emergency Hazard Response. It is the responsibility of all supervisors to allow their employees to attend and it is the responsibility of all employees to attend the training and take the information received back to their work site for implementation.

### 3.6 Facility Security Requirements:

#### 3.6.1 General Security Provisions:

It is the responsibility of all staff to ensure the security of the biomedical research laboratory. The physical security measures are an integral part of the biocontainment capabilities of the lab. Doors should always remain locked and access granted to only qualified and necessary individuals. **Should any security breaches or threats be apparent, please immediately notify Campus Police at x77 (HSC) or x2600 (Main campus).**

#### 3.6.2 Visitor Procedures:

Visitors to the biomedical research laboratories must be pre-arranged. Casual visits are not encouraged. If you are expecting a visitor, you should contact the Principle Investigator and let them know the name of the visitor and the date and expected time of arrival.

#### 3.6.3 Contractors Procedures:

Contractors working inside the biomedical research laboratories are subject to regulations similar to visitors. Prior notification must be given so that arrangements can be made for material and tool transfers can be arranged. This will assist in maintaining the containment capabilities of the laboratory and also to eliminate in any undue delays to the contractor while they are waiting for the decontamination of their tools and equipment.

#### 3.6.4 Buddy System:

New biomedical research laboratory staff members will be allocated a "**BUDDY**" for the first few days or weeks, until the appointee is familiar with the laboratory's layout and operating procedures. A new staff member should not work alone until this orientation is completed.

#### 3.6.5 After-hours Access:

Working during the silent hours of the building should be prearranged with the principle investigator or supervisor. It is recommended that no individual work alone in BSL2 or 3 labs without being in contact with, or having informed another employee outside the biomedical research laboratory.

## 4.0 Laboratory Operations and Safety Procedures

### 4.1 Introduction:

The procedures detailed in this Section **ARE APPLICABLE TO ALL STAFF AND ALL WORK PLACES WITHIN ANY BIOMEDICAL RESEARCH LABORATORY**. They are based on similar procedures used in other high security laboratories to minimize the risks of release of microorganisms to the outside environment, personnel exposure to infectious agents and injury or illness to staff as a result of accidents or mishandling of materials or equipment. The procedures are also consistent with the principles of good laboratory practice as described by Biosafety in Microbiological and Biomedical Laboratories, CDC-NIH.

In addition to procedures detailed here, staffs are expected to know and understand additional Standard Operating Procedures (SOP) that applies to their particular work.

### 4.2 Entry/Exit Procedures:

#### 4.2.1 Personnel:

1. Enter the lab ensuring that the door closes tightly behind you. Each employee upon entrance should verify that the doors have in fact closed.
2. Put on the designated protective equipment i.e. coverall suit/gowns, shoe covers, hair bonnet, respiratory protective gear and gloves as necessary.

When leaving the laboratory you should:

1. Remove your PPE. They should be put in a biohazard bag for decontamination.
2. Wash your hands in the designated hand wash sink in the main lab.
3. If you have experienced a spill or you believe that you may be contaminated, contact Safety & Health to report the spill.

#### 4.2.2 Equipment and Materials:

In general, materials should not be taken into the Biocontainment Area unless you wish them to remain inside. If you intend to remove materials at a later date, you **MUST** be prepared to decontaminate the materials upon removal.

Not all materials can be decontaminated easily: e.g. paper, foam rubber, leather, and Styrofoam should be avoided from taken into the lab.

When receiving incoming goods from a vendor every effort should be made to ensure that they are the correct item prior to entry to the Biocontainment area. Removal of incorrect items for return to supplier may not be guaranteed – e.g. open all cardboard boxes outside of containment to first verify contents and secondly to avoid unnecessary sterilization needs. (NOTE: Specimens are an exception.).

**NOTE: For safety reasons, pressurized liquid cylinders, compressed gas cylinders, pressure pack containers, open or closed liquid nitrogen vessels, and other hazardous chemicals must**

**not, under any circumstances, be treated in the autoclaves.**

Precautions must be taken to prevent the introduction of rodents, insects, parasites and other vermin into the Biocontainment Area. Incoming boxes, crates, and equipment are all possible sources of such pests. Wherever possible, all such items should be unpacked outside the Biocontainment Area before passing the goods through, but leave enough packing to prevent any damage to material in transit. Wooden pallets must not be brought into the Biocontainment area.

### **Removing Materials from the Biocontainment Area**

Once materials have entered the Biocontainment Area, they cannot be returned to the outside without decontamination or sterilization. This may be difficult for some materials, and it is wise to plan ahead.

Decontamination/removal is by one of the following methods:

**Autoclaving.** For materials which can withstand high temperature and steam. The standard barrier autoclave parameters should be 121 ° C for 45 minutes; variations may be allowed under certain circumstances (consult with the Biosafety Officer). Check with the Safety Department if you are uncertain about the heat/steam sensitivity of any material. Unwanted material can also be autoclaved out of the Biocontainment Area and discarded.

**Chemical disinfectants** containing 1% glutaraldehyde solution ("Wavicide"), 10% bleach and other EPA approved disinfectants can be used for disinfecting small articles which can stand immersion in liquid. MSDS's for biological agents in question should be consulted and all treated materials must be evaluated to the satisfaction of the Biosafety Officer as to their efficacy of disinfection. Containers must be immersed for a minimum period of 15 minutes. Alternate disinfectants can also be used for decontamination of hard surfaces, when suitable for the agents in use and validated. (See also Section 5.2.10 for more information)

#### **4.2.3 Specimens:**

External packaging should be removed and the safety pack should be transferred to a secondary container and transferred into the laboratory.

#### **4.2.4 Chemicals and Gases:**

It is the intention of the safety program to closely monitor and eliminate all employees' exposures to toxin, mutagenic and teratogenic chemicals and compounds. All procedures involving these chemicals and compounds will be reviewed by the Biosafety Officer and evaluated using personnel monitors to ensure procedures developed by the supervisor do eliminate these exposures.

Chemicals that have been deemed necessary and are to be used in the BSL-2 or 3 laboratories are to be transported in small quantities. The waste generated from these must be decontaminated prior to removal from the lab.

Gas cylinders can be used in the laboratory areas. They are normally used in an upright position and must be properly secured in a holder. Ensure that the correct reducing valve is used and that it is properly fitted. Cylinders **must be** transported on trolleys.

To operate, ensure that the regulator valve is turned off before turning on the main cylinder valve, and

then adjusted to the required pressure with the regulator valve. When finished, turn the cylinder off, let the remaining gas escape, then turn off the regulator valve.

The regulator must be removed before the cylinder is decontaminated by topical disinfection and removed from the containment area.

#### 4.2.5 Heat Sensitive Equipment:

**Decontamination with formaldehyde gas.** For materials which cannot be autoclaved satisfactorily. However, the gas has certain limitations, such as limited penetrating ability. It cannot readily get inside some equipment, rolls of tape, hoses, books etc. Normally such goods should not be submitted for formaldehyde fumigation. Materials such as foam rubber are difficult to fumigate and likewise it is difficult to remove the formaldehyde gas once trapped within the pores.

**Other Decontaminants** such as vaporized hydrogen peroxide can be considered with consultation with the Institutional Biosafety Officer for the treatment of equipment and areas of the BSL 2 or, 3 laboratories.

Dirty or greasy objects will not be accepted for fumigation.

Equipment from laboratories must be cleaned with a disinfectant/detergent solution. A 1:10 dilution of the hand washing liquid supplied at laboratory sinks is suitable for initial cleaning.

**A disinfectant** containing 1% glutaraldehyde solution ("Wavicide") and can be used for disinfecting small articles which can stand immersion in liquid. MSDS's for biological agents in question should be consulted and all treated materials must be evaluated to the satisfaction of the Biosafety Officer as to their efficacy of disinfection. Containers must be immersed for a minimum period of 15 minutes. The item is then rinsed in the shower and removed from the change area. Sporacide or bleach can also be used for decontamination of hard surfaces, when applicable and suitable for the agents in use and validated.

#### 4.2.6 Animals:

Animals are to be brought into the laboratory as needed. When the experiment is completed, the animals should be disposed of as contaminated organic waste and sterilized in the autoclave at 121 degrees Celsius for a minimum of 30 minutes or longer depending on the size of the animals.

### 4.3 Laboratory Work Practices:

In conjunction with the recommendations of the CDC/NIH concerning the operation of a standard BSL-2 or 3 facilities the following standard laboratory practices are required for the operations of biological research laboratories.

#### 4.3.1 Manipulations of Infectious Materials:

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
2. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas where there is reasonable likelihood of exposure to potentially infectious materials. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. All procedures are performed carefully to minimize the creation of splashes or aerosols.
6. Work surfaces are decontaminated at least once a day and after any spill of viable material and after the completion of work in the lab.
7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving or chemical treatment. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container and closed for transport from the laboratory. Materials to be transported from the laboratory to an off-site location for decontamination are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility. (See Safety and Health Policy HM-08-019)
8. An insect and rodent control program is in effect. All lab personnel are to monitor the lab for insects or stray rodents and are required to immediately report any unusual findings to the Biosafety Officers. An external exterminator will be utilized if the problem persists.
9. Laboratory doors are kept closed when experiments are in progress.
10. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. For example, persons who are immunocompromised or immuno-suppressed may be at risk of acquiring infections. Persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. The Institutional Biosafety Committee will also assist in performing risk assessments of any personnel who match these criteria.
11. The principle investigator is responsible for enforcing policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms.
12. When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.
13. Laboratory personnel receive the appropriate immunizations or tests for the agents handled or

potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

14. Baseline serum samples are collected and stored for all laboratory and other at-risk personnel. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory.
15. A Biosafety/Security Manual is prepared or adopted. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures.
16. Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural changes.
17. The laboratory director is responsible for insuring that, before working with organisms at Biosafety Level 2 or 3, all personnel demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in safe microbiological practices and techniques.
18. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plastic-ware should be substituted for glassware whenever possible.
  - a. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is into the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - b. Syringes which re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.
  - c. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles sharp equipment, and broken glass should be decontaminated before disposal, according to any local, state, or federal regulations.
19. All manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.
20. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials. Contaminated equipment

should also be decontaminated before it is sent for repair or maintenance or package for transport in accordance with applicable local, state, or federal regulations, before removal from the facility. Plastic-backed paper towelling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

21. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
22. All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories or animal rooms are decontaminated before disposal or reuse. When multiple agents are present in the BSL2 or 3 facility attempts should be made to separate the waste streams prior to decontamination.
23. Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material. Biohazard spill kits are to be available in all BSL2 and BSL3 labs.
24. Spills and accidents which result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained. All employee exposures are followed up in accordance with Infection Control Policy #31:EH-602.
25. Animals and plants not related to the work being conducted are not permitted in the laboratory.

#### **4.3.2 Handling of Hazardous (Non-infectious) Materials:**

##### **Storage of flammable chemicals:**

Serious dangers can arise because of the ease with which some common chemicals can be ignited. These include acetone, acetonitrile, benzene, diethyl ether, dioxane, ethanol, methanol, propanol, pyridine, toluene and xylene. Flammable chemicals must be stored in a flammable solvents cabinet when not being used. A small, fire-proof cabinet should be provided in each laboratory. Quantities of solvents to be kept in individual laboratories must be restricted to 2 litre containers of each solvent in regular use. Glass containers of solvents must be stored on properly designed shelving or in sectioned crates. They may NOT be kept on high shelving or loose on the floor.

Further information on the storage, labelling and safety hazards associated with flammable chemicals is available from the Safety and Health Department.

##### **Work with flammable and/or toxic chemicals:**

Flammable solvents should be used in fume hoods, whenever possible. The following precautions should be taken:

1. Heat solvents only with an electric heating mantle or with steam.
2. Use a safety screen if the material is highly chemically reactive.
3. If cloths or tissues are used to absorb small amounts of spilled liquid, allow drying in the hood

then dispose of in a plastic bag.

Quantities of < 100ml may be used in open laboratories PROVIDED THAT no ignition sources are nearby.

**THIS DOES NOT APPLY TO DIETHYL ETHER WHICH MAY ONLY BE OPENED AND USED IN A FUME HOOD.**

Flammable solvents **must not** be used in a Class II biological safety cabinet.

Users of toxic or carcinogenic materials must be familiar with any special precautions applicable. Purchases of chemicals should only be from suppliers who provide Material Safety Data Information. For further inquiries, contact the Biosafety Officer.

#### **Weighing chemicals:**

Particular care must be taken when weighing out chemicals known to be toxic or carcinogenic. As far as possible, addition or removal of the chemical should be carried out in a fume hood. Wear disposable gloves and a face mask. Plastic-backed absorbent paper may be placed on the work surface and carefully discarded after the task is complete.

#### **Disposal of waste solvents, flammable liquids and toxic compounds:**

The following procedure applies only to material NOT CONTAINING MICROORGANISMS:

Materials should be accumulated in appropriate labelled waste containers. The Biosafety Officer makes arrangements for removal from the facility.

#### **4.3.3 Use of Radioisotopes:**

Before commencing any work with these materials you must be aware of, and comply with all relevant statutory requirements and should refer to the **Ohio Department of Health**. In particular you are reminded that accurate records of movements, usage and storage of radioactive materials and radiation dose must be kept. If you are uncertain of the correct procedures for handling and working with radioactive materials you should consult the Radiation Safety Department (#383-4301-Ed Brentlinger), your supervisor or, in the case of work with infectious agents involving the use of radioisotopes, the Biosafety Officer.

#### **Precautions:**

In working with radioisotopes, the following precautions must be observed:

1. Use the minimum quantity of radioactivity compatible with the objectives of the experiment.
2. Always wear protective clothing, safety glasses and gloves when handling radioactive substances. You should also wear a personal radiation detection badge except when working with small quantities of low energy beta-emitting radio-nuclides in areas where no other radio-nuclides are used.
3. Never work with cuts and breaks in the skin unprotected, especially on the hands and forearms.

4. Use automatic pipettes with disposable tips for dispensing radioactive solutions.
5. Use clearly labeled disposable containers whenever possible.
6. Cover the work surface with plastic-backed paper to absorb any spilled material. If radioactive material is spilled on the bench, floor or on equipment, notify your supervisor or the Radiation Safety Department to determine the appropriate decontamination method.
7. Wash your hands with hot water and soap and monitor yourself with an appropriate radiation monitor (as specified by the Radiation Safety Department) before leaving the work area.
8. Work carefully and neatly to avoid accidents, and monitor the work area regularly to avoid contamination of yourself and others using the Laboratory area.

#### **Labeling of containers and work areas:**

Equipment containing radioactive material, or bench areas where the work is performed, must be labeled with radioactive warning labels or tape.

#### **Storage of radioisotopes:**

Label containers of radioactive material clearly, indicating the radio-nuclide type, total activity, compound, specific activity, date and level of radiation at the surface of the container so that products can be properly stored, handled and disposed of and are accessible only to competent people.

#### **4.3.4 Small animal Manipulation:**

##### **4.3.4.1 Institutional Animal Care and Use Committee:**

Before beginning research involving animals, laboratory personnel lead by the Principle Investigator are required to submit a document to the University's Institutional Animal Care and use Committee-IACUC.

##### **4.3.4.2 Uncontaminated Animal Handling:**

Animals being prepared for an experiment should be separated from those presently on test. Handling of the clean animals should be done prior to daily manipulations of infected animals and housed in a separate unit. Manipulations of the animals can be on an open bench.

##### **4.3.4.3 General Animal Inoculation:**

(IBC protocols will give agent specific information)

Animals being inoculated should be moved to the Biological Safety Cabinet. All manipulation should be carried out in the BSC as follows:

1. Line the cabinet with plastic backed adsorbent paper.

2. Work at least six inches inside the front grill.
3. Animals should be inoculated and then transferred to a new cage (preferably filter-top) that is appropriately labeled with the hazard, date of inoculation and responsible individual.
4. Infected animals are then treated with extreme care.

#### 4.3.4.4 Infected Animal handling/Necropsy:

1. Use extreme care when handling infected animals. Use leather gloves or forceps for handling infected animals.
2. All necropsies should be done in the BSC (depending on agent).
3. All dead animals and carcass remains must be placed in double leak proof bags and a rigid leak proof container and autoclaved daily. These are not stored in the lab.

#### 4.3.4.5 Disposal Procedures:

Cages are autoclaved or thoroughly decontaminated **before** bedding is removed or before they are cleaned and washed. Personnel wear gloves when handling infected animals. Gloves are removed aseptically and autoclaved with other animal room wastes before disposal.

### 4.3.5 Working Safely in a Biological safety Cabinet (BSC):

#### 4.3.5.1 Procedures for BSC work:

Lab coats that are knowingly contaminated with infectious material must be immediately removed and placed into an autoclave bag and into a tote box for autoclaving.

Lab coats that are **NOT** obviously contaminated with infectious material should be autoclaved and laundered weekly. They are bagged, brought into the lab and autoclaved, taken to laundry service and recycled.

Latex gloves (or suitable alternatives) should be worn whenever you are working with infectious materials, hazardous chemicals or radioisotopes. They should be put on and the tops secured over the cuffs of the gown. If they become contaminated, they should be removed and placed in a biohazard bag for decontamination and your hands washed immediately.

Some staff show allergic reactions to disposable gloves. A wide range of gloves are available from the lab supply stores. Inner cotton or silk liners are also available, which may help control the problem.

**Remember movement in and out of the BSC should always be minimized and materials should be accumulated in the hood until the end of the project or when they start to interfere with the airflow in the hood or the safe manipulation of equipment in the BSC.**

#### 4.3.5.2 Burner Alternatives:

Bunsen and other gas burners must be installed using flexible, reinforced, plastic tubing that is impermeable to natural gas. Clips should be used to fix the tubing firmly to the burner and to the gas cock. Extinguish burners when not in use. Do not site burners near areas where flammable solvents are in use.

#### **BURNERS SHOULD NOT BE USED IN BIOLOGICAL SAFETY CABINETS.**

If bacterial procedure requiring loops is being performed in the BSC then disposable loops or a Bac-T Incinerator should be used for the sterilization of the loops. Open flames can cause disturbance in the airflow and can also burn the filters.

#### 4.3.5.3 Material and Equipment Distributions

**NOTE: Training in the function and use of Biological Safety Cabinets is available for new laboratory staff through the Safety and Health Department. All are expected to be fully familiar with the features and operation of BSC's before working with them in the laboratory. See Annex "A".**

Biological safety cabinets are primary containment devices that provide immediate containment of infectious material, separating it from the laboratory worker.

**Class II cabinets** are not absolute devices, but offer a degree of protection to the workers. **Class II** cabinets provide a laminar flow of HEPA filtered air over the working surface, and provide good product protection, as well as protection of the worker.

**The Class IIA/B3 Cabinet** provides both personnel protection and product protection. The cabinets are free-standing, have their own fans and HEPA filters and discharge filtered exhaust air into the room. Air is re-circulated in the cabinet through a HEPA filter mounted horizontally, providing a down-flow of clean air that continually flushes the work surface.

About 30% of the total air is exhausted to the room through a second HEPA filter, and a corresponding amount is drawn through the front of the cabinet as a directed curtain to protect the operator

**The Class IIB2 Cabinet** provides a higher level of personal protection because these are ducted to a ventilation unit and none of the air is re-circulated into the work space or into the work environment.

Class II BSCs may be used for any work involving micro-organisms.

A power outlet is provided within the Class II cabinet for small items of electrical equipment. It is recommended that rechargeable battery-operated pipette aids are used, to prevent trailing leads.

**Flammable or toxic solvents must not be used in Class IIA/B3 cabinets. This includes alcohol-based disinfectants, except for small amounts applied by cotton wool swab. In**

**some instances, very small quantities can be used in the IIB2 cabinets but must be approved in advance by the Biosafety Officer.**

Class II cabinets must be checked for efficacy and safety before initial use and annually thereafter, or following relocation.

All Class II cabinets are to remain operating while the personnel are in the lab and being utilized. Make sure the work surface is clean. Assemble all materials required in the cabinet. Do not overload the work surface or obstruct the front grille, as protective air flows will be distorted. **DO NOT USE GAS BURNERS.** Do not allow electrical leads or tubes to enter through the front opening. Cabinet should always have the work surface covered by a plastic backed adsorbent paper.

**A record of all infectious work with Class II cabinets should be recorded in a log book. Record the date, time and agent used, disinfection procedure and operator's name and signature.**

After completion of your work, transfer infectious material to an appropriate container for incubation or storage and discard disposable material into a BIOHAZARD BAG or SHARPS CONTAINER within the cabinet. Disinfect any equipment to be removed with the appropriate disinfectant, pick up the adsorbent paper and discard as infectious/biological waste and wipe down the work surface with disinfectant. Disinfect your gloves before removal, and wash your hands at the sink. With the cabinet operating, lift the work floor and remove any debris. Clean the work tray, edges and undersurface and the sump floor with disinfectant solution.

**Do not use hypochlorite solutions unless specifically directed. Hypochlorite causes corrosion of any stainless steel surfaces if you are required to use bleach on your stainless surfaces rinse with plain water.** Clean front grill and cabinet windows prior to re-assembly.

#### 4.3.5.4 Recommended Best Practices in BSC's:

Preparing a written checklist of materials necessary for a particular activity and placing necessary materials in the BSC before beginning work serves to minimize the number of arm-movement disruptions across the fragile air barrier of the cabinet. The rapid movement of a worker's arms in a sweeping motion into and out of the cabinet will disrupt the air curtain and may compromise the partial barrier containment provided by the BSC. Moving arms in and out slowly, perpendicular to the face opening of the cabinet will reduce this risk. Other personnel activities in the room (e.g., rapid movement, open/closing room doors, etc.) may also disrupt the cabinet air barrier.<sup>5</sup>

Laboratory coats should be worn buttoned over street clothing; latex gloves are worn to provide hand protection. A solid front, back-closing lab gown provides better protection of personal clothing than a traditional lab coat. Gloves should be pulled over the knitted wrists of the gown, rather than worn inside. Elasticized sleeves can also be worn to protect the investigator's wrists.

Before beginning work, the investigator should adjust the stool height so that his/her face is above the front opening. Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the cabinet. This allows the cabinet to stabilize and

to "air sweep" the hands and arms to remove surface microbial contaminants. When the user's arms rest flatly across the front grille, room air may flow directly into the work area, rather than being drawn through the front grille. Raising the arms slightly will alleviate this problem. The front grille must not be blocked with research notes, discarded plastic wrappers, pipetting devices, etc. All operations should be performed on the work surface at least four (4) inches from the inside edge of the front grille.

Closure of the drain valve under the work surface should be done prior to beginning work so that all contaminated materials are contained within the cabinet should a large spill occur.

Materials or equipment placed inside the cabinet may cause disruption to the airflow, resulting in turbulence, possible cross-contamination, and/or breach of containment. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet. Only the materials and equipment required for the immediate work should be placed in the BSC.

BSCs are designed to be operated 24 hours per day, and some investigators find that continuous operation helps to control the laboratory's level of dust and other airborne particulates. Although energy conservation may suggest BSC operation only when needed, especially if the cabinet is not used routinely, room air balance is an overriding consideration. In some instances, room exhaust is balanced to include air discharged through ducted BSCs.

Cabinet blowers should be operated at least three to five minutes before beginning work to allow the cabinet to "purge". This purge will remove any particulates in the cabinet. The work surface, the interior walls (not including the supply filter diffuser), and the interior surface of the window should be wiped with 70% ethanol (EtOH), a 1:100 dilution of household bleach (i.e., 0.05% sodium hypochlorite), or other disinfectant as determined by the investigator to meet the requirements of the particular activity. When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine, which may eventually corrode stainless steel surfaces. Wiping with non-sterile water may recontaminate cabinet surfaces, a critical issue when sterility is essential (e.g., maintenance of cell cultures).

Similarly, the surfaces of all materials and containers placed into the cabinet should be wiped with 70% EtOH to reduce the introduction of contaminants to the cabinet environment. This simple step will reduce introduction of mold spores and thereby minimize contamination of cultures. Further reduction of microbial load on materials to be placed or used in BSCs may be achieved by periodic decontamination of incubators and refrigerators. **Do not use hypochlorite solutions unless specifically directed. Hypochlorite causes corrosion of any stainless steel surfaces if you are required to use bleach on your stainless surfaces rinse with plain water.**

Plastic-backed absorbent toweling can be placed on the work surface (but not on the front or rear grille openings). This toweling facilitates routine cleanup and reduces splatter and aerosol formation<sup>3</sup> during an overt spill. It can be folded and placed in an autoclavable biohazard bag when work is completed.

All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet (Figure 11). Similarly, aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward the rear of the cabinet to take advantage of the air split described in Section III. Active work should flow from the clean to contaminated area across the work surface. Bulky items such as

biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the interior of the cabinet.

Certain common practices interfere with the operation of the BSC. The autoclavable biohazard collection bag should not be taped to the outside of the cabinet. Upright pipette collection containers should not be used in BSCs nor placed on the floor outside the cabinet. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. Only horizontal pipette discard trays containing an appropriate chemical disinfectant should be used within the cabinet. Furthermore, potentially contaminated materials should not be brought out of the cabinet until they have been surface decontaminated. Alternatively, contaminated materials can be placed into a closable container for transfer to an incubator, autoclave or for other decontamination treatment.

Many procedures conducted in BSCs may create splatter or aerosols. Good microbiological techniques should always be used when working in a biological safety cabinet. For example, techniques to reduce splatter and aerosol generation will minimize the potential for personnel exposure to infectious materials manipulated within the cabinet. Class II cabinets are designed so that horizontally nebulized spores introduced into the cabinet will be captured by the downward flowing cabinet air within fourteen inches<sup>24</sup> of travel. Therefore, as a general rule of thumb, keeping clean materials at least one foot away from aerosol-generating activities will minimize the potential for cross-contamination.

The work flow should be from "clean to contaminated (dirty)" (see Figure 11). Materials and supplies should be placed in such a way as to limit the movement of "dirty" items over "clean" ones.

Several measures can be taken to reduce the chance for cross-contamination when working in a BSC. Opened tubes or bottles should not be held in a vertical position. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impaction of downward air. Bottle or tube caps should not be placed on the towel. Items should be recapped or covered as soon as possible.

Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence which disrupts the pattern of HEPA-filtered air supplied to the work surface. When deemed absolutely necessary, touch-plate microburners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops can also be used.

Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter (see Figure 12). This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to kill the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste.

Investigators must determine the appropriate method of decontaminating materials that will be removed from the BSC at the conclusion of the work. When chemical means are appropriate, suitable liquid disinfectant should be placed into the discard pan before work begins. Items should be introduced into the pan with minimum splatter, and allowed appropriate contact time as per manufacturer's instructions. Alternatively, liquids can be autoclaved prior to disposal. Contaminated items should be placed into a biohazard bag or discard tray inside the BSC. Water should be added to the bag or tray prior to autoclaving. **Materials and wastes should not be allowed to accumulate and should be discarded immediately after completion of BSL2/3 manipulations.**

When a steam autoclave is to be used, contaminated materials should be placed into a biohazard bag or discard pan containing enough water to ensure steam generation during the autoclave cycle. The bag should be taped shut or the discard pan should be covered in the BSC prior to removal to the autoclave. The bag should be transported and autoclaved in a leakproof tray or pan. It is a prudent practice to decontaminate the exterior surface of bags and pans just prior to removal from the cabinet.

#### *Surface Decontamination*

All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. At the end of the work day, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back, and the interior of the glass. If necessary, the cabinet should also be monitored for radioactivity and decontaminated when necessary. Investigators should remove their gloves and gowns in a manner to prevent contamination of unprotected skin and aerosol generation and wash their hands as the final step in safe microbiological practices. **Do not use hypochlorite solutions unless specifically directed. Hypochlorite causes corrosion of any stainless steel surfaces if you are required to use bleach on your stainless surfaces rinse with plain water.**

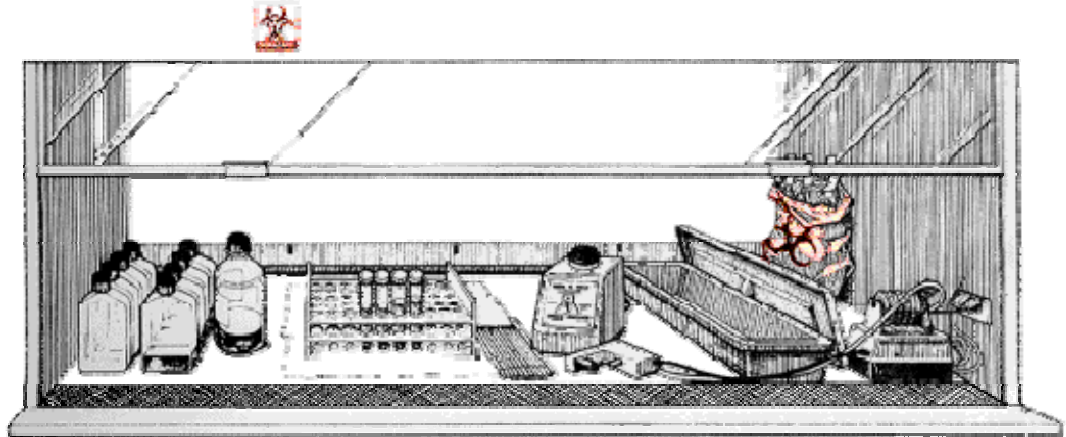
Small spills within the BSC can be handled immediately by removing the contaminated absorbent paper toweling and placing it into the biohazard bag. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately wiped with a towel dampened with decontaminating solution. Gloves should be changed after the work surface is decontaminated and before placing clean absorbent toweling in the cabinet. Hands should be washed whenever gloves are changed or removed.

Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination. All items within the cabinet should be surface decontaminated and removed. Decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan.

Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. Manufacturer's directions should be followed. The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag. The drain pan should be emptied into a collection vessel containing disinfectant. A flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. This procedure serves to minimize aerosol generation. The drain pan should be flushed with water and the drain tube removed.

See the following link for addition information: <http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm>

## Recommended Set up of a Typical BSC



### 4.3.6 Safety Precautions for Laboratory Equipment:

#### 4.3.6.1 Centrifuges:

Before using any centrifuge, you must be trained with its operation (read the manufacturer's instructions) and the appropriate safety precautions provided. Large refrigerated centrifuges and ultracentrifuges must not be used by any staff member until a training course has been completed, and authorization given to allow safe, unsupervised use. All staff, whether trained or not should seek assistance from their supervisor or colleagues if unfamiliar or inexperienced with ultracentrifuge use. Not only will accidents create microbiological hazards but may require very expensive repairs or replacement of equipment. Centrifuges can be hazardous because of mechanical failure, such as tube breakage, incorrect fitting of trunnions on rotors or even rotor failure. Always make sure that rotors are loaded symmetrically and that tubes, adaptors and cups are properly balanced. Centrifuge rotors, tube compartments and buckets must be cleaned regularly and checked for damage or corrosion. If any is detected, report it to the Laboratory supervisor.

Aerosol covers must be used **whenever infectious material** is being centrifuged.

Training in, and authorization for the use of preparative centrifuges and ultracentrifuges will be arranged by your supervisor. The Safety and Health Department has a training video tape on the proper use of centrifuges.

#### 4.3.6.2 Internal Transfer and Storage of Etiologic Agents:

In general, infectious materials can only be moved within the laboratory in approved transport containers. **See Section 10.0.** This requirement is designed to minimize hazards in the case of an accident and to preserve the non-contaminated status of the environment. Storage usually includes ultra-low temperature freezers and in some cases with liquid nitrogen back-up.

#### **4.3.6.3 Vacuum Apparatus:**

Pumps and vacuums are necessary for the functioning of specialized research equipment. They are also a source for concentrating contaminated fluids, solids and air. All vacuum lines should be protected with 0.2 micron in-line HEPA filters and regularly inspected for holes, connections and blockage. Vacuum pumps and motors, when they fail should be totally immersed in a liquid disinfectant (or gassed if all of the parts can be opened). Unfortunately these pumps and motors should not be repaired unless the problem is known and of relatively minor consequence for exposure to a biological hazard. All such work decontamination or repairs must be coordinated with the Biosafety Officer.

#### **4.3.6.4 Autoclaves:**

Autoclaves are instruments which maintain saturated steam at high temperatures and under pressure. They are used to sterilize laboratory equipment and materials by destroying potentially infectious agents. A typical autoclave cycle of 15 minutes at 121°C and 15 psi is usually sufficient to kill the most heat resistant microbiological agents, i.e. bacterial spores. Longer cycles are needed for larger loads, larger volumes of liquids and denser materials. Proper loading and packing procedures include the following precautions:

- Read and receive training in operation of the autoclave
- Ensure that operating instructions are kept close to the autoclave
- Wrap packages to allow for steam penetration. Aluminum foil prevents steam from penetrating. Avoid use.
- Do not overload the chamber
- Avoid over-packing of autoclave bags
- Do not seal bags or close bottles
- Do not stack containers
- Never autoclave hazardous chemicals or solvents
- Add some moisture to dry loads

#### **Safety precautions:**

1. Wear protective clothing (i.e. autoclave gloves and apron) when removing the contents from the autoclave.
2. Autoclaves and contents present severe burn hazards. Standing away from the door minimizes the risk of burns due to evacuation of steam or fluids from the autoclave. Open the doors slowly after a cycle and let the humid air dissipate.
3. Autoclaves operate under pressure. To prevent a burn and physical shock hazard never attempt to force autoclave doors open before the end of a cycle or when the jacket pressure is greater than zero.

#### **Biological Indicators:**

Biological indicators are used to develop the processing times for typical loads and monitor the efficacy of the decontamination process.

The basic procedure for **efficacy monitoring using biological indicators** is the following:

1. Place biological indicator (Getinge/Castle Biosign Biological indicators (spores/indicator:  $10^4$  *B. stearothermophilus* and  $10^6$  *B. subtilis var. niger*) in the centre of a typical load.
2. The load is processed according to standard operating procedures, taking into account the lag time necessary for the internal temperature in the centre of the load to reach the sterilization temperature (this time will vary depending on the nature of the load being sterilized); even though the spores of *B. stearothermophilus* are killed when exposed to 121°C for 15 minutes, the total cycle time depends on the load
3. After completion of the cycle, the autoclave is opened and the biological indicator is removed.
4. Indicators are incubated at 55°C, along with a positive control that did not go through the autoclave process; they are examined at 24- and 48 h. for growth; a color change from red to yellow indicates growth and sterilization failure i.e. parameters of time and/or temperature have not been met in the test indicator.
5. Absence of growth in the test indicators signifies that sterilization of the load was achieved; representing a 4-6  $\log_{10}$  reduction in *B. stearothermophilus* spores.
6. Failure to achieve sterilization may be due to improper loading or overloading of the autoclave (i.e. the centre of the load failed to achieve sterilization temperature), or insufficient sterilization time. The process should be repeated until the necessary loading configuration and sterilizing time have been determined. This time and load configuration should be used for all subsequent cycles for that type of load.

#### 4.3.6.5 Lypholiser:

Freeze drying apparatus work under extreme negative pressure and can be the cause of serious laboratory accidents. It is imperative that familiarization of the correct operation instructions be followed at all times. Any malfunctioning of the equipment should be immediately reported to the Biosafety Officer.

#### 4.3.7 Pipetting:

Mechanical pipetting aids must be used for all pipetting tasks. Pipetting by mouth is **NOT** permitted. The preferred pipette-aid preferred is the battery-operated type, especially in biological safety cabinets. Improper handling of pipettes can lead to contamination of the user and/or to generation of hazardous aerosols. Selection of a pipetting device should be based upon:

- Intended use
- Ease of handling
- Delivery accuracy
- User preference
- Quality of seal formed with pipettes to be used; liquid should not leak from the pipette tip.
- Whether the pipetting aid can be sterilized.

If infectious aerosols are likely to be generated, perform pipetting operations in a biological safety cabinet. Methods for handling pipettes are described below:

- Plug pipettes with cotton
- Check pipettes before using; cracked or chipped suction ends may damage the seals of the pipetting aid.
- Keep pipettes upright while in use and between steps of a procedure to prevent contamination of the mechanical aid.
- Gently expel contents close to the surface of the liquid or allow to flow down the side of the container.
- Avoid mixing fluids by alternate suction and blowing, or by bubbling air from the pipette.
- Avoid forceful ejection of the contents from the pipette.
- Use easier to handle shorter pipettes when working inside a BSC
- Submerge used non-disposable pipettes horizontally in disinfectant solution; dropping them vertically may force out any liquid remaining in the pipette.

#### 4.3.8 Needles and Syringes:

Serious laboratory incidents can occur from the improper use of needles and syringes. Wherever possible in normal laboratory work, use a pipette if a needle and syringe is not essential.

##### Choosing needles and syringes:

If possible, select a "Luerlock" or equivalent type syringe. These allow a positive attachment of the needle via a screw thread.

##### Using needles and syringes:

If you use friction-fit needles, make sure that they are firmly pushed onto the syringe before use.

Care should be taken whenever syringes and needles are used, especially if infectious material is involved.

NO ATTEMPT should be made to part the needle and syringe, whether Luerlock or friction-fit. Removing the needle increases the chance of injury and can result in spilling the material and creating aerosols.

#### **Discarding used syringes:**

Used syringe and needle combinations are recognized as presenting a real hazard in laboratories and health care organizations. **Most accidents result from careless disposal, attempts to separate needle and syringe or during re-capping.**

Discard used combinations directly into an appropriate sharps container WITHOUT taking the needle off and WITHOUT putting the cap back on.

Vacutainer-needles (Becton Dickinson) may be removed more safely and easily by using the Automatic Needle Disconnect (A.N.D.) disposal unit. The one-hand operation automatically unscrews the vacutainer needle from the holder and discards it into the container.

#### **4.3.9 Containers Labeling:**

All containers in the BSL-2 or 3 laboratories should be labeled with their contents, the date and the responsible individual. **A complete list of all hazards present in the laboratory, biological, chemical and radiological should be regularly updated and kept in the lab.**

## **5.0 Laboratory Decontamination Methods/ Procedures:**

### **5.1 Introduction:**

It is important to distinguish between sterilization and disinfection. Whereas sterilization results in destruction of all forms of microbial life, disinfection results in destruction of specific pathogenic microorganisms. There are specific procedures and equipment necessary for accommodating both sterilization and disinfection for BSL3 Laboratories. Other internal laboratories may adopt similar practices however this section is primarily dedicated to BSL3 labs. Procedures identified are to be followed by all laboratory workers and any exceptions should be agreed to with the Biosafety Officer.

### **5.2 Specific Procedures:**

#### **5.2.1 Sharps Disposal:**

Approved sharps containers should be made available for the disposal of needles, syringes, blades broken glass and other laboratory items considered as sharps by local legislation. The containers selected must be such to eliminate the potential for injury during handling after use.

Containers should be decontaminated by autoclaving and placed in the University's approved leak-

proof disposal bins for off campus disposal.

## 5.2.2 General Laboratory Waste from BSL2/3 Facilities:

General waste from the BSL-2 or 3 labs will all be treated as potentially Infectious/Biohazardous contaminated waste. Waste of this type generated in BSL2/3 laboratories must be autoclaved or chemically treated to render them non-infectious and suitable for packaging in final waste receptacles for shipping. **Waste generated from BSL2/3 manipulations should be immediately autoclaved and disposed of after all experiments are completed.** The typical autoclave cycle would be 121 degrees C for 30 minutes or through the use of an approved chemical disinfectant. As a precautionary step these material are then removed from the autoclave, re-packed into leak-proof infectious waste bins and then transported for off-site treatment. Chemically treated liquids can be disposed of to the sanitary sewer systems as this is acceptable in the State of Ohio.

Waste generated from BSL2/3 procedures and manipulations such as pipettes and other experimental materials should be held in the BSC until the experiment is complete or until the materials start to interfere with proper technique, or disrupt airflow in the BSC. This will minimized movement in an out of the hood. (See [4.3.5 Working Safely in a Biological safety Cabinet \(BSC\):](#) ) and ([4.3.5.4 Recommended Best Practices in BSC's:](#)) The infectious waste materials should then be transferred to a leak-proof biohazard container labeled on at least two sides that is lined with a red autoclave bag that will allow for autoclaving prior to disposal of material to large lined 28-gallon infectious waste bins typically located in the research area's autoclave rooms.

## 5.2.3 Chemical Waste

Chemical waste needs to be collected in containers that can be sealed. Each chemical waste is treated differently. If the waste has disinfectant capabilities and the potential for sustained biological activity then:

1. Top off the container with the waste minimizing the amount of air in the container.
2. Seal the lid tight and agitate to insure contact with the entire surface.
3. Surface decontaminate the container and remove through the change room.

If the waste still could be biologically contaminated then:

1. Fill waste containers only half full.
2. Fill the rest of the container with appropriate disinfectant. (Avoiding chemical reactions)
3. Seal container and allow 24 hour contact time.
4. Surface decontaminate the container and remove and wash down through the change room.

## 5.2.4 Organic Waste:

Organic waste is difficult to decontaminate and will need to be decontaminated in the autoclave. Specific load validation tests must be done according to the type of load and density of the material. To achieve sterilization all waste must be:

1. Double bagged and appropriately labeled.
2. Autoclave in a secondary container for the time and temperature determined by the load testing study. Typically this sterilization time could last up to 90 minutes and is dependant on the temperature and time achieved at the core of the material.

#### 5.2.5 Laboratory Clothing:

Laboratory clothing should be decontaminated at least once a week unless contaminated. If the clothing has been contaminated it should be double bagged and autoclaved immediately.

#### 5.2.6 Glassware:

BSL-2 and 3 operational requirements recommend that disposable laboratory products be used in place of glassware. In some cases, this is not possible. If glassware is used then it should be disinfected and decontaminated prior to washing.

#### 5.2.7 Gas Cylinder:

Gas cylinders should be taken offline before they are completely discharged. The tanks should be moved to the shower on a trolley. The valve should be opened momentarily to clear the valve fittings of potentially contaminated air. The surface of the tank should be disinfected and allowed 15 min contact time and then rinsed.

#### 5.2.8 Effluent Traps:

Dry effluent traps can be a source of sewer gas and also an entrance point for air movement. It is necessary to fill all drain traps weekly with water and place 50 ml of mineral oil in them to slow down the evaporation process. **Sink traps must be treated after each use with an appropriate disinfectant i.e. bleach.**

#### 5.2.9 Laboratory Housekeeping and Cleaning:

Laboratories should be kept clean and tidy if accidents are to be minimized. Limit bulk storage within laboratory of items readily available from the store. Avoid storage of materials on top of wall cabinets and at floor level. Decontaminate and remove any unwanted materials from your laboratory, refrigerators and freezers. **Work surfaces should be cleaned daily after use.** A disinfectant such as bleach should be used in areas where infectious agents have been handled. **Do not use hypochlorite solutions unless specifically directed. Hypochlorite causes corrosion of any stainless steel surfaces if you are required to use bleach on your stainless surfaces rinse with plain water.** Extraneous clutter should be avoided in the lab on work surfaces that could potentially interfere with application of disinfectants and routine cleaning.

#### 5.2.10 Disinfection

An operational protocol must be in place in your SOP and include "how to use" instructions for an appropriate disinfectant.

Effective disinfectants are recommended on each MSDS.

## **DISINFECTING WITH BLEACH**

Household Bleach (5.25% concentration of Sodium Hypochlorite, NaOCl) is widely recommended as a disinfectant to inactivate viruses and bacteria.

The Centers for Disease Control (CDC) recommends the following dilutions of bleach or concentrations of Sodium Hypochlorite for the:

### Inactivation of HIV and Hepatitis B

- Bleach diluted 1:10; 0.50% NaOCl (5,000 ppm)

**DO NOT USE HYPOCHLORITE SOLUTIONS UNLESS SPECIFICALLY DIRECTED. HYPOCHLORITE CAUSES CORROSION OF ANY STAINLESS STEEL SURFACES IF YOU ARE REQUIRED TO USE BLEACH ON YOUR STAINLESS SURFACES RINSE WITH PLAIN WATER.**

Routine wipe down of surfaces:

- Bleach diluted 1:100 E\_\_\_0.05% Sodium Hypochlorite (NaOCl) (500 ppm)

Health Canada recommends for Biohazard Spills:

- Bleach diluted 1:5; 1.0% Sodium Hypochlorite NaOCl (10,000 ppm)

Biohazard Spill - Gently cover the spill with adsorbent paper towel and apply 1% sodium hypochlorite, starting at the perimeter and working towards the center. Full strength household bleach loses most of its activity in six months at room temperature. The rate of breakdown accelerates rapidly at low dilutions. Therefore, working dilutions should be prepared daily.

NOTE: SODIUM HYPOCHLORITE IS CORROSIVE TO METALS AND PROLONGED CONTACT WITH METALS PARTICULARLY AT HIGH CONCENTRATIONS, SHOULD BE AVOIDED.

Alcohol: 70% Ethyl or 70% Isopropyl alcohol are effective against many bacteria and viruses. Alcohols are non-corrosive, flammable and have a high evaporation rate.

Phenolics: (Lysol, Fullphene)

Iodophores: (Wescodyne)

Gluteraldehydes: Cidex

Quaternary Ammonium Compounds: Not recommended for Biohazards

For any disinfectant:

1. Ask for independent laboratory test results which may show antimicrobial activities against:  
Staphylococcus aureus                      Pseudomonas aeruginosa  
Mycobacterium bovis                        Polio virus  
Salmonella typhimurium                    Rota virus
2. Use as per manufacturer's directions.
3. Obtain Material Safety Data Sheet (MSDDS)
4. Perform your "in use" test if possible, using your "target" organism with the disinfectant which is prepared under

Disinfectant

Uses

Alcohols	Ethyl or isopropyl alcohol at 70-80% concentration is a good general purpose disinfectant; not effective against bacterial spores.
Phenols	Effective against vegetative bacteria, fungi, and viruses containing lipids; unpleasant odor.
Formaldehyde	Concentration of 5-8% formalin is a good disinfectant against vegetative bacteria, spores, and viruses; known carcinogen; irritation odor.
Quaternary Ammonium Compounds	Cationic detergents are strongly surface active; extremely effective against lipoviruses; ineffective against bacterial spores; may be neutralized by anionic detergents (i.e., soaps).

Chlorine	Low concentrations (50-500 ppm) are active against vegetative bacteria and most viruses; higher concentrations (2,500 ppm) are required for bacterial spores; corrosive to metal surfaces; must be prepared fresh; laundry bleach (5.25% chlorine) may be diluted and used as a disinfectant.
Iodine	Recommended for general use; effective against vegetative bacteria and viruses; less effective against bacterial spores; Wescodyne diluted 1 to 10 is a popular disinfectant for washing hands.

### 5.3 Physical/ Mechanical Systems:

#### 5.3.1 HEPA Housing/Duct Work:

HEPA housings and the contaminated duct work must be decontaminated with formaldehyde gas, vaporized hydrogen peroxide or other decontamination agent and approved by the Biosafety Officer prior to being opened or penetrated for maintenance.

#### 5.3.2 Biological Safety Cabinets:

Prior to the annual certification of the biological safety cabinet (BSC) it should be decontaminated with formaldehyde vapor or other means by a qualified and NSF certified technician.

#### 5.3.3 Cage Rack:

This piece of equipment houses the potentially infectious live mice and provides filtered and directional airflow that is extracted upwards to the ceiling exhaust vent. Every 3 months or during experiment turnover the rack shall be verified for operational performance. Before this and after an experiment is finished the unit shall be washed with an appropriate disinfectant, filters removed and bagged for sterilization and smoke tested for proper and directional airflow.

#### 5.3.4 Renovation Waste (e.g. pipes, valves, ceiling tiles):

Prior to major construction of the area, the entire lab need to be shut down. This would include any renovations requiring the critical system for maintaining the containment capabilities were being taken out of service.

Small renovations can occur and the waste generated must be decontaminated. Porous waste cannot be formaldehyde decontaminated or disinfected. It must be autoclaved.

Pipes, valves, solid plastic can either be cleaned and disinfected or decontaminated with formaldehyde vapors. Small sections can be autoclaved.

#### 5.3.5 Laboratory Equipment:

Laboratory equipment must be decontaminated prior to leaving the laboratory. The surfaces should be wiped with disinfectant, and if there are areas which cannot be disinfected then it will require formaldehyde decontamination. The change room area would be sealed to be used as a temporary decontamination chamber. This should be coordinated by the Biosafety Officer and performed only by qualified experience personnel.

### 5.3.6 Suite Decontamination:

To decontaminate the entire suite would require formaldehyde, or other forms of treatment. This should be coordinated by the Biosafety Officer and performed by qualified experienced personnel. Typically all doors, vents and opening would be sealed using 8 mil poly and duct tape, frying pans with paraformaldehyde flakes would be actively boiled, temporary fans would circulate the gas for a period up to 24 hrs, afterwards ammonium carbonate would be used as a neutralizer up to another 24 hours and finally the lab would be vented, washed down if necessary and brought back on line.

## 6.0 Emergency Procedures

### 6.1 Introduction:

Contingency planning is a necessary part of laboratory operations. Preparedness to react to specific and known emergency situations helps to minimize damage, personal and environmental threats and program disruptions. This section identifies specific procedures to follow when emergency situations arise. Knowledge about these procedures is not all that is necessary. Practice and training for emergency situations is also needed, and is mandatory for all laboratory workers.

### 6.2 Spill Response:

#### 6.2.1 Biological

The following procedures apply to the BSL-3 Laboratory.

**Note: Report all BSL2&3 incidents to the Biosafety Officer, Michael Valigosky (ext 383-4521)**

**Pager: 292-6321 / home phone: 861-1050/ cell 419-266-5491**

#### 1. Spills inside a Class II biological safety cabinet:

**Small** spills should be immediately wiped up using *Disinfectant*-soaked paper towel or other absorbent material while the cabinet is still running. Allow the disinfectant to act (for about 15 minutes) before discarding waste into an autoclave bag for disposal. Do not let the material dry onto the BSC work surface. **Report the incident to the Biosafety Officer if you believe that it occurred as the result of poor practice or equipment failure.**

**Significant** spills (i.e. greater than about 5 ml, or any material with a titer more than  $10^6$  per ml) which are contained within the cabinet are treated as follows: With the cabinet running, immediately cover the liquid with disinfectant-soaked paper towel or other absorbent material.

The cabinet operator must use their discretion when deciding how to remove and discard protective equipment. For example, surface disinfection of gloves may be required and an assistant with clean gloves assist in the removal of a soiled gown, into the cabinet, whilst the cabinet operator withdraws their arms and hands. Wash hands and arms thoroughly.

**Notify the Biosafety Officer** of the spill as soon as possible. Inform the Biosafety Officer of the agent in use, the volume and approximate titer. The Biosafety Officer will arrange inspection and

consider further action. If no further action is specified, clean up the area with absorbent material and disinfectant, ensuring that all surfaces of the BSC are disinfected. Lift the cabinet work floor and clean its undersurface and the sump floor with Disinfectant. Do not use hypochlorite-based disinfectants.

## 2. Spills outside a biological safety cabinet:

**Small** spills of infectious material (less than 5 ml with a titer of less than  $10^6$  per ml, and where there has been **NO** significant splashing or contamination of personnel) are treated as follows - Avoid breathing any aerosols released and immediately cover the spill with disinfectant-soaked paper towel or other absorbent material.

**Notify the Biosafety Officer** of the spill as soon as possible. Inform the Biosafety Officer of the agent in use, the volume and approximate titer.

If no further action is specified by the Biosafety Officer, clean up the spill and dispose of waste materials by autoclaving and disposal to leak-proof infectious waste container. Otherwise, leave the spill until the Biosafety Officer is able to inspect it.

**Significant** spills (greater than 5 ml or where the titer is greater than  $10^6$  per ml), or spills which have splashed extensively or have contaminated personnel, are treated as follows -

Avoid breathing any aerosols, and evacuate the immediate area. Try to cover the spill with a drape/bag if possible. **DO NOT ATTEMPT TO DECONTAMINATE BEFORE CONTACTING THE BIOSAFETY OFFICER!**

**EVACUATE ALL PERSONNEL FROM THE IMMEDIATE LABORATORY** as quickly as possible. Remove gowns and shoes if contaminated and move to ante-room.

Discard remaining clothing and shower out of Laboratory. Put on emergency clothing.

Place the "**STOP NO ENTRY**" sign over the door and contact the Biosafety Officer to report the details of the incident. The Biosafety Officer will arrange inspection and clean-up of the spill, and will advise any further action required.

## 3. Spills in laboratory centrifuges

If a failure is suspected **during** a centrifuge run:

Immediately switch the machine off and allow the rotor to come to rest.

**DO NOT OPEN THE CENTRIFUGE.**

Avoid breathing any aerosols and evacuate the Laboratory. (Follow instructions listed above in 2)

Place a "**STOP NO ENTRY**" sign on the Laboratory door handle and contact the Biosafety Officer.

Advise the Biosafety Officer of the nature of the incident, the agent involved and its volume and titer. The Biosafety Officer will arrange inspection, clean-up of the spill and decontamination of the centrifuge.

If a spill is discovered **after** opening the centrifuge or is visible through a transparent lid,

- Avoid breathing any aerosols and immediately close the centrifuge if open. (Follow instructions listed above in 2)
- Evacuate the Laboratory, place a "**STOP NO ENTRY**" sign on the Laboratory door handle and contact the Biosafety Officer. Advise the Biosafety Officer of the nature of the incident, the agent involved and its volume and titer.
- The Biosafety Officer will arrange exposure assessment; refer for treatment, inspection, clean-up of the spill and decontamination of the centrifuge.

#### 4. Accidental Inoculations

These are most commonly caused by broken glass and needle-stick. If you or someone else in the Laboratory is injured, proceed as follows:

Attend to any immediate medical needs. If possible, wash the affected area with copious quantities of water. Use disinfectants cautiously.

Report the incident to the Biosafety Officer and advise of the agent involved and extent of the injury. **IT IS MOST IMPORTANT TO REPORT INCIDENCES INVOLVING INOCULATION WITH LEVEL 3 AGENTS AS QUICKLY AS POSSIBLE.** Immediately fill out an occurrence report.

The Biosafety Officer will arrange exposure assessment, refer for treatment, please wait any further instructions. All exposures require follow-up with University Health Services or in the Emergency Department after hours as arranged by the Biosafety Officer.

#### 6.2.2 Chemical:

This general procedure should be adopted for each area specific to the design of the laboratory, type of work and the quantities and types of chemicals and other hazards present within the specified area. Consult Safety and Health Policy # HM-08-013.

1. Attend to any persons who may have been contaminated.
2. Notify persons in the immediate area about the spill.
3. Evacuate all nonessential personnel for the spill area.
4. If the spill material is flammable, turn off ignition and heat sources.

5. Avoid breathing vapors of the spilled material. If required use respiratory protection if available; if not leave area to a safe location until equipment required is available. Contact the Biosafety Officer by calling X 77 on the HSC, or 2600 on the MC.
6. Leave on all available exhaust ventilation systems, this may require increasing air exchanges for the specific area. Contact the Safety and Health Officer immediately by calling X77 (HSC), or X2600 (Main Campus).
7. Use available material to begin neutralization of the spilled material as soon as it is identified. If insufficient material is available for the complete cleanup, identify what is required and contact the spill response team.
8. During the cleanup all procedures to ensure that additional contamination of the site and personnel does not occur must be followed.
9. Once the spilled substance is identified, a Material Safety Data Sheet should be made available for additional information.

### **6.2.3 Radiological:**

Remember you may also have other hazards involved when cleaning up these types of spills. DO NOT FORGET.

#### **Minor Spills** (include only diluted radioactive material)

1. Wearing disposable gloves and a lab coat or disposable coveralls, clean up the spill using absorbent paper or pads and place it in a plastic bag for transfer to a radioactive waste container.
2. Avoid spreading contamination, work from the outside of the spill toward the centre.
3. Wipe test or survey for residual contamination as appropriate. Repeat decontamination, if necessary, until contamination monitoring results are background levels or less than 2 times background levels. These results must be sent to the Radiation Safety Department for confirmation prior to the area being used for any operational work. Reference your Radiation Safety Manual for procedures on wipe testing.
4. Check hands and clothing and shoes for contamination.
5. Report the spill and cleanup to the supervisor provide the results for confirmation and send these to the Radiation Safety Department- Ed Brentlinger ext 4301 pager 249-9514 along with a report on the spill occurrence.
6. Adjust the inventory and waste records appropriately.

**Major Spills** (where any release of volatile material occurs, including any release of radioactive gases or spillage of stock solutions occur)

1. Clear the area. Persons not involved in the spill should leave the immediate area. Limit the movement of all personnel who may be contaminated until they are monitored.
2. If the spill occurs in a laboratory, leave the fume hood running to minimize the release of volatile radioactive materials to adjacent rooms and hallways.
3. Close off and secure the spill area to prevent entry. Post warning sign(s).
4. Notify the Radiation Safety Department through the campus police Department at X77, or 2600.
5. The Radiation Safety Department will direct the personnel decontamination and will decide about decay or cleanup operations.
6. Follow the procedures for minor spills (if appropriate).
7. Record the names of all persons involved in the spill. Note the details of any personnel contamination.
8. The Radiation Safety Department will arrange for any necessary bioassay measurements.
9. A written report **MUST** be submitted to the Radiation Safety Department detailing the spill actions taken who is involved and any other information on the incident is required within 24 hours of the completion of the cleanup.
10. A report will be submitted to the Ohio Department Health (ODH) within 5 working days of the incident if no overexposure has occurred. If an overexposure has occurred the ODH will be contacted within 24 hours of the occurrence.

### 6.3 Fire Management (CODE RED):

Fire can have devastating effects on life and program operations. It is important that all people associated with the laboratory receive the appropriate training and implement regular inspections, drills and testing.

#### 6.3.1 Authorities/Contacts:

**All laboratory workers have primary responsibility to react to a fire emergency. Under no circumstance shall any person put themselves or others at risk. If a fire should occur the worker must assess the situation and react accordingly. He/she may attempt to provide a first response by using the local fire suppression equipment. If the fire is out of control the worker must immediately leave the area, release local fire alarms and immediately contact fire and emergency extension "77" (HSC), or "2600" (Main Campus) and the Biosafety Officer, Michael Valigosky (419-383-4521), pager 419-292-6321, home 419-861-1050 and cell 419-266-5490.**

#### 6.3.2 Fire Prevention:

Fire prevention is necessary to minimize damage and personal injury. There are different stages of fire prevention and combines personal and physical solutions. In order to prevent a fire or to minimize the propagation of a fire apply the following:

- Train all personnel on the appropriate use of equipment to minimize the initiation of a fire
- Apply procedures that minimize the hazard of fire initiation. i.e. turn computers off during off-hours.
- Minimize the amount of flammable substances stored within the laboratory and store them in appropriate containers and flammable rated cabinetry.
- Minimize spark producing equipment and substances in a laboratory.
- Minimize open flames or select alternative solutions.
- Select the correct fire suppression equipment and agent.
- Avoid storing reactive substances with opposing agents and conditions.
- Train all personnel on the use of fire extinguishers.
- Do not impede access to portable fire extinguishers or the egress corridors.
- Train all personnel on fire management procedures including response to authorities.

### 6.3.3 Fire Extinguisher Training and Use:

Portable fire extinguishers are intended as a first aid measure to cope and respond to fires of a limited size. In the event of a small fire follow as required:

- Evaluate the fire to see if it can be smothered. Proceed if appropriate.
- Lift extinguisher and approach fire.
- Remove pin at top of extinguisher.
- Direct arm and nozzle at base of fire. Do not spray too close to fire as this may spread the fire.
- Squeeze trigger
- Move nozzle around fire until extinguished.
- If fire becomes larger and out of control, move back and **LEAVE AREA IMMEDIATELY.**
- Call fire authorities immediately or as soon as possible. Contact "77 (HSC) or 2600 (MC)"
- Do not put partially spent extinguisher back-Call authorities for full replenishment and inspection..

### 6.3.4 Fire Reporting:

After a fire has occurred the person involved in first response is required to submit a brief fire report explaining the nature and source of the fire, identifying possible causes, time, extent, response methods, and personal actions. During off-hours, first response personnel may be from the maintenance support team. The report shall be submitted directly to the Safety and Health Department.

### 6.3.5 Evacuation Procedures:

There are designated routes for emergency exiting in case of fire and it is necessary that all personnel be trained and familiarize themselves with the routes for safe egress. All exit doors are appropriately marked and upon egress proceed rapidly without panic. Staff members must ensure that visitors are accompanied and accounted for throughout the evacuation process. All personnel must obey instructions from fire official designates.

## **KEEP CALM - PANIC IS INFECTIOUS**

- Upon the breakout of an uncontrolled fire in the immediate laboratory zone, all personnel must leave the premises as soon as possible.
- In the event of a fire in adjacent areas to the laboratory, and alarm will sound for you to begin

evacuation.

- Personnel shall stop current operations and secure biological specimens in containers or locate within the BSC. React carefully and quickly as possible.
- People should remove their outer Laboratory coat.
- Once out of the lab follow instructions from fire control designates or if none visible proceed through the corridor to the nearest building exit
- **Do not use the ELEVATORS**
- Once departed from the building await instructions from fire officials before re-entering the building.

### 6.3.6 Suggested Maintenance Requirements of Fire Systems:

Maintenance and Inspection Schedules of Suppression and Detection Systems will be maintained jointly by Facilities Maintenance and Campus Police.

#### Portable Fire Extinguishers

<u>Task</u>	<u>Inspection Frequency</u>
Inspect all portable fire extinguishers	Monthly
Service all portable extinguishers	Annually
Hydrostatically test Carbon Dioxide extinguishers	Every Five years
Empty and service pressure type extinguishers	Every Six Years
Hydrostatically test dry chemical extinguishers	Every 12 years

#### Alarm Systems

<u>Test</u>	<u>Frequency</u>
Test Fire Alarm System	Monthly
Test Voice communications	Monthly
Inspect Stand-by Batteries	Monthly
Records all testing of these systems	

<u>Maintenance</u>	<u>Frequency</u>
Inspect and service all alarm components	Annually
Inspect and service all components of voice communications system	Annually
Inspect and service monitoring agencies transmission device	Annually

### 6.4 Bomb Threat (CODE BLACK):

**All security issues must be reported to Campus Police - x77 (HSC) or x2600 (Main Campus).** If a bomb threat has been received or a suspicious package found, the building bomb threat emergency procedure must be put into effect unless there is strong evidence to the contrary. Initiate an emergency call using 77 and then activate the University's **CODE BLACK** bomb threat emergency procedure which should be familiar to all the buildings' occupants and will be exercised at least once annually.

After the bomb threat has been received the receiver will contact **Campus Police Extension x77 (HSC) or x2600 (Main Campus)**. After the group has reviewed the threat, a decision will be made onto which steps should be taken to continue to discontinue the emergency response procedures. If an evacuation is necessary personnel must leave their Departments open, but secure all documents if time permitting.

Upon arrival, the police should be given recent plans to the building. One or all of the members of the emergency response team listed above has the authority to, with the advice of the police, give the order to re-enter the premises, once the threat has been dealt with.

### **6.5 First Aid:**

All staff members working in the BSL-2 or 3 laboratories are encouraged to have First Aid CPR training annually.

### **6.6 Accident Reporting (OSHA requirements):**

All accidents are to be reported to the Biosafety Officer within one day of the occurrence. All accidents **must** be reported utilizing the occurrence report form and near miss reporting. Accidents which involve etiologic agent will be reported also to the Biosafety Officer so that appropriate biomedical screening can be arranged.

### **6.7 Natural Disasters (CODE YELLOW):**

A natural or man made disaster can occur at any time or place with or without warning. The disaster may be coordinated with the local disaster response plans or organizations or it may be isolated to the Laboratory site. The response required will vary depending on the magnitude of the situation and the types of emergency response required. The personnel within this site will be expected to respond to the emergency situation and ensure that they prevent further injury to fellow employees, preserve life and not endanger themselves or other by their actions.

#### **Actions to be taken:**

1. On-scene personnel will complete whatever initial emergency actions are called for. This can include such procedures as: evacuating the area, reporting the fire, only fighting the fire if and only if it can be put out or to aid someone in evacuation, providing First Aid/CPR, and informing management of the situation.
2. If the situation is such that homes and family could also be affected, personnel will be released as soon as possible to check on the welfare and location of family or pets.
3. Remaining personnel will operate as directed by the Campus Police Department.
4. The Responsible Building Coordinator will continue to control and coordinate actions until the building is returned to normal operations.
5. Once the Laboratory requirements have been met, a policy of maximum assistance to others in the community will be followed.

## **7.0 Procedures for Repair and Renovation**

This section defines various methods to help make modifications to the laboratory. Rarely, unless major mechanical revisions are underway, will the laboratory have to be shut down. Specific procedures for lab shut down are necessary when this occurs. Personnel involved with the repairs must be knowledgeable in containment principles and given training accordingly. Personnel must also take the necessary precautions to

prevent personal injury and extended damage when repairs are underway. It is highly recommended that all repair work and work orders be issued with an attached cautionary document outlining the various hazards and laboratory operating principles (security, ventilation, entrance procedures and decontamination methods). Any repairs of the containment laboratory must be identified to the Biosafety Officer.

### **7.1 Wall Repairs:**

All laboratory walls require regular maintenance to maintain a washable, durable and sealed surface. Regular inspections and re-painting is necessary on a routine basis. The following procedures should apply:

- All delaminated, peeled and scarred surfaces need to be noted on profiled drawings indicating type of problem, estimated area and location
- A work request should be immediately issued to maintenance support and a work order should commence with cooperative timing with the researchers. Typically, week-end or off-hour work is ideal for this type of repair.

### **7.2 Ceiling Tile and Surface Repairs:**

The current design recommends FRP (fiber reinforced plastic) tiles laid on a 2'X 4' metal suspended grid. Replacement is typically the solution when one or more of these tiles are damaged. When tiles are damaged they are carefully taken off the grid and cut into pieces that can be washed down with a disinfectant and placed into a bag, removed out of the lab and disposed as regular waste.

### **7.3 Floor Repairs:**

The floor in biocontainment laboratories should be a solid sealed surface preferably epoxy coated and coved up the walls of the room. The floors may also be of a resilient seamless vinyl 0.087" sheet that is installed floor to floor with a 4" rubber cove base. These vinyl sheets are seamed with a heated welded rod that fuses the two sides of the sheets together to create the "seamless" feature hence the sealed floor barrier finish. Repairs are common when large equipment is constantly rolling or being moved from one located to another causing scars or tears in the floor. The seams fail occasionally and should be carefully monitored for tightness. Moving of equipment should be done with caution and concern for floor damage. The cove base can sometimes delaminate from the wall to floor joint and should also be monitored for separation. Re-applying adhesive after careful removal, cleaning and replacement of the cove is acceptable. Applying a layer of silicone sealant between the cove's floor and wall connection improves the concept of a contiguous barrier.

Should a tear be noticed or incurred, the laboratory personnel should apply a suitable disinfectant and initiate a procedure for repair.

- Apply and flood a suitable disinfectant in the damaged area
- Create an immediate work request with the (trained) maintenance department
- The maintenance staff should initiate repairs within 7 working days
- The disinfectant should be left in place and re-applied until repairs are initiated.
- Once repairs commence the disinfectant should be mopped up and a square hole should be cut around the scar (if large) and a replacement patch with compatible characteristics should be laid with adhesive and seamed as per the initial installation. If the hole is small enough a silicone or epoxy putty should be immediately applied after cleansing.
- No holes in floors, walls or ceilings should be left unattended without initiating repairs.

## **7.4 Plumbing Re-fits:**

Occasionally, plumbing pipes fail, new lines are added or modifications are needed to support different research work. When this occurs, scheduling of the work is key to avoid major program disruptions. Piping or equipment brought into the laboratory should be verified to be "pest-free". Since many pipe materials come from storage yards the inside of the pipe is suspect for pests. Once in, repairs can begin requiring all workers (in-house employees or contractors) to be trained in containment principles and emergency procedures.

**NO GAS LINES MAY BE RUN TO THE INTERIOR OF A BIOLOGICAL SAFETY CABINET AT ANY TIME FOR PURPOSES OF FLAMING LOOPS OR FOR THE STERILIZING OF OTHER EQUIPMENT WITHIN THE CABINET AS THIS PRESENTS A SERIOUS FIRE SAFETY RISK TO USERS. CABINETS ARE NOT RATED FOR USE OF NATURAL GAS USE AND HEPA FILTER COMPONENTS ARE ALSO FLAMMABLE.**

## **7.5 Electrical Repairs:**

All electrical repairs should be done by a licensed electrician with trained knowledge on the containment principles for the laboratory. Working with electricity is extremely dangerous and the buddy-rule system should apply in the lab when repairs occur. All materials as per the plumbing refits should be cut down and sterilized before being sent to waste. Heat sensitive material should be thoroughly disinfected and removed from the lab.

## **7.6 Ventilation Repairs:**

The ventilation system is one of the most important systems for primary control of containment. It provides directional airflow from non-contained to contained areas and ensures a proper amount of air is required for human and animal comfort and equipment support. Repairs usually require shut down of ventilators, dampers, exhaust cabinets and electrical support. To avoid repairs on an unplanned basis it is imperative that an aggressive preventive maintenance program is applied. Maintenance programs can usually forecast repairs before failure and these forecasts can be scheduled around experiment turn-over or off-hours during non-critical times. The maintenance department should also be prepared to react on an immediate basis and should have in stock (or access with quick response times) all critical pieces of equipment to be installed on a quick turn around basis. Otherwise, valuable research may be at stake and containment control may be compromised.

Should any repairs be required as a result of ventilation performance or failure please call:

Biosafety Officer: Michael Valigosky-383-4521 pager -292-6321 cell -266-5491

## **7.7 Casework and Benchtop Repairs:**

All labs have laboratory furniture that on occasion requires be refitting, remodelling, removing or repairing. All work should be done under to coordination of the maintenance and safety Department. Laboratory personnel should not attempt to do any repairs to the furniture on their own. All furniture material inside the lab should be considered contaminated and would require thorough disinfection upon removal. It is impractical to think that laboratory furniture should be sterilized through the autoclave. Existing bench-work should be considered for relocation and re-installation within the laboratory before it is unnecessarily removed.

Proper protection against personal injury should be applied when installing and removing leg frames, cabinetry and epoxy surfaces.

### **7.8 Service Penetration Seals:**

Work requiring penetrating the barrier of containment requires specific approval of the Safety Department in advance of work commencement. All services entering the lab comes through a conduit or whole in the walls, floors or ceilings. Normally, upon initial construction for this type of laboratory, these penetrations are sealed around the service in the hole or around the conduit and service itself. Effectively, no air shall be able to pass from adjacent zones through the penetration. New services, water pipes, telecom lines, wiring require the same tightness as per the initial construction. Therefore for new services penetrating the surface barrier apply the following principles:

### **7.9 Laboratory Equipment Repairs:**

There are many types of equipment associated with a containment laboratory. Biological safety cabinets, autoclaves, incubators, freezers, pumps, centrifuges should all be regularly tested and maintained for effective operation. When repairs are necessary the following procedures apply:

- The laboratory worker issues a work request to the maintenance department (with copies to the Safety and Health Department) which then reviews it for consideration as to who best will provide the maintenance. The criticality and the timing of the repairs must be mentioned in the work request.
- The maintenance department will coordinate with the safety Department for all necessary repairs.
- Repair workers must adopt the entrance and egress principles of containment, as well all of the decontamination principles on removing waste and material.
- Repair workers will record the repair work, test and certify that the equipment is performing at the expected level of performance.
- All work orders and repair work will be saved per equipment for history logging and future trouble shooting.

Laboratory workers must be reminded to follow the above process for all major equipment repairs. For minor work on special equipment some repairs can be accommodated by the lab personnel themselves. Discretion applied by the laboratory worker will be monitored by the maintenance and safety Department. To avoid the problems of decontaminating regularly used tools, it is required that a fully equipped tool box be assigned within the lab zone and also be accounted for on a regular basis.

## 8.0 Building Plans / System Performance Criteria

### 8.1 Principles of Containment:

Containment laboratories have unique design solutions and operating practices that make it possible for laboratory workers to handle hazardous and infectious materials without endangering themselves, the community or the environment. Terminology of containment laboratories includes **primary containment, secondary containment and tertiary containment**. Briefly explained these are as follows:

**Primary Containment** devices are provided to contain the principle sources and activities of infectious materials and the consequences generated by the program manipulations. Effectively, BIOLOGICAL SAFETY CABINETS and other task venting devices are primary containment devices to capture and direct air flows to filters or external to the environment. **BSC use is mandatory for all infectious micro-organism manipulations.**

**Secondary Containment** devices include the general design of the laboratory to react to a spill situation. The room must be secure, have access control, have sealed durable surfaces capable for liquid and gas decontamination, have negative air pressure, have directional air flows within the lab, have sturdy and chemically resistant casework., have appropriate room filtration (as required) and have properly located BSCs (best at the rear of a lab- away from traffic and turbulent air currents. These devices are all designed to support the programmers to avoid a problem or to react to a problem if it exists.

If properly designed, problems such as spills, malfunctioning equipment can still control the spread of biohazardous material outside of the laboratory.

**Tertiary Containment** is less easily defined as it is many times misunderstood, sometimes misinterpreted and often varied in application. The constant of tertiary containment is the operational practices that avoid generating problems. Another example of physical tertiary containment is the way the containment laboratory is designed within an overall facility. Labs located on inner warm walls are better controlled from external environmental influences than those adjacent to an external wall. Tertiary containment combines operational practices with overall facility design and supports the program by first avoiding problems and second, if they exist how the facility helps to react to them.

## 9.0 Containment Laboratory Certification Requirements

### 9.1 Biological Safety Cabinets (BSC):

Testing of Biological Safety Cabinets are required to meet the build and testing specifications of the National Sanitation Foundation NSF-49 Standard - See Annex "A" which describes biosafety cabinets and the test procedures required.

### 9.2 Directional Airflow and Failure Tests:

Annually the laboratory should be tested for directional airflow demonstrated by field tests with visual smoke. Installation of spot indicators recommended for spot verification. This can be done by smoke pencils and possibly theatrical fog machines.

In order to understand the impact of ventilation failure modes it is recommended that the laboratories be smoke tested during a simulated failure. Testing damper closings, shutdowns, system reaction times and ultimate effect on the directional airflow should be validated with visual smoke.

### 9.3 HEPA Filtration:

The installed HEPA filter requires means for testing, decontamination and changing on a periodic basis. The current installation requires modification or extensive measures in order to achieve the desired testing program.

All HEPA-filters tested to meet NSF 49 requirements after installation. HEPA filters are normally clean (when first installed) with a rated static pressure of approximately 0.75" under normal airflow. The filter should be changed out and replaced when it reaches 2"SP. Follow procedures for isolating the housing, decontaminating the housing volume, protecting the worker, and removal and replacement of a similar filter. After re-installation the in-situ filter should be field tested for rated efficiency 99.97% at 0.3 micron particle size.

### 9.4 Autoclaves:

Autoclaves require regular and frequent inspections. Some autoclaves have up to 30 different types of isolating and modulating valves and are highly sensitive. Every three months each autoclave should be regularly serviced. Every year the autoclave should be re-certified using biological indicators as per the initial performance expectations. Loads will require regular bio-indicators to demonstrate proof of sterilization.

### 9.5 Review of Operating Procedures:

Operational protocols should be reviewed and updated annually. Also training on the changes should be carried out annually.

## 10.0 Transport of Biohazardous Material

There are several national and international regulations that dictate how biohazardous material is packaged and transported in the United States. See below - 10.3. Recent interest in preventing terrorist attacks has also influenced regulations for shipping, facilities support and registration purposes. **It is the policy of the University of Toledo to strictly adhere to these packaging and shipping regulations. All personnel involved in the packaging or shipping of biohazardous material will require specific training to be arranged through the Biosafety Officer.** In addition to the required packaging and transport requirements apply the following principles:

- Limit the number of moves,
- Reduce the possibility of breakage,
- Contain the material in the event of a leak or spill.

### 10.1 Transport within or Between Labs:

In accordance with Basic leakproof packaging methodology II as specified by regulations, the following precautions are recommended when transporting within or between laboratories:

- Place specimens in leakproof and breakage-resistant containers: close with screw-caps rather than snap caps or rubber stoppers.
- Use unbreakable leakproof secondary containers. For light loads which can be carried, ensure the secondary containers have solid and secure handgrips.
- Use a cart for heavier items. Load the cart so the contents will not dislodge.

## 10.2 Transport between Buildings:

The following precautions are recommended when moving biohazardous substances from one building to another:

- Ensure that the biohazardous material is enclosed and sealed within a primary receptacle such as a test tube, vial or flask
- Place cushioning and absorbent material around the primary container
- Use a secondary leak-proof container which can withstand dropping or crushing while in transit,
- If necessary, place coolant (e.g. Dry ice, crushed ice) inside an insulated tertiary vessel.
- Take spill supplies and disinfectant in case of spill.

## 10.3 Transport by Public Carrier:

The need to support research on a local, national and international basis to combat disease and public health issues makes transport of infectious materials necessary. The regulations that govern this transport are as follows:

- US Public Health Service, 42 CFR Part 73, Interstate Shipment of Etiologic Agents - there have been some changes to this regulation to identify "Additional Requirements for Facilities Transferring or Receiving Select Agents" -42 CFR Part 73
- US Postal Service, 39 CFR Part 111, Mailability of Etiologic Agents, Mailability of Sharps and other Medical Devices, and Publication 52, Acceptance of hazardous, Restricted, or Perishable Matter.
- US Department of Transportation, 49 CFR Parts 171-180 and amendments
- United Nations, Recommendations of the Committee of Experts on the Transport of Dangerous Goods.
- International Civil Aviation Organization, Technical Instructions for the Safe Transport of Dangerous Goods by Air.
- International Air Transport Association, Dangerous Goods Regulations
- US Department of Labour, Occupational safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne Pathogens
- See Safety and Health Policy #HM-08-031 "Shipping of Hazardous Materials"

Individuals involved in the packaging and shipping of biohazardous material must familiarize themselves with the above regulations and also be aware of pending changes.

## 11.0 Contacts and Inquiries:

Any inquiries regarding this manual, its contents or its references should be directed to the Biosafety Officer. The following people, Departments and contact numbers are as follows:

<b>Biosafety Officer:</b>	<b>Phone – 419-383-4521</b>
<b>Responsible Official (RO)</b>	<b>Pager – 419-292-6321</b>

Mike Valigosky Home – 419-861-1050  
Cell – 419-266-5491

Assistant Biosafety Officer: Phone – 419-530-3603  
Asst. Responsible Official (ARO) Pager – 419-245-9600  
Heather Lorenz Cell – 419-206-0896

Safety and Health Department 419-383-5069

Radiation Safety Department: Phone- 419-383-4301  
Ed Brentlinger Pager- 419-640-3217  
Home-419-693-7682

**CAMPUS POLICE:** 419-383-3770 (Health Science Campus)  
419-530-2600 (Main Campus)

**FIRE/EMERGENCY:** x77 (HSC) or x2600 (Main Campus)

## 12.0 Recommended Reading

The following reading material is recommended for people associated with the use of containment laboratories at the University of Toledo, Toledo, Ohio:

- Laboratory Safety (Second Edition)-Principles and Practices Fleming, Richardson, Tulis, Vesley
- Laboratory Biosafety Manual-Current Edition-World Health Organization -Geneva
- Biosafety in Microbiological and Biomedical Laboratories- Current Edition CDC-NIH Publication-US Department of Health and Human Services. This guideline is by far the most critical, important and pertinent.
- Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets- CDC-NIH-Publication of US Department of Health and Human Services.
- Health Canada- Laboratory Biosafety Guidelines-Current Edition.
- Biohazards Management Handbook-Current Edition, Revised and Expanded- Edited by Daniel F. Liberman.
- Containment Standards for Veterinary Facilities- Agriculture and Agri-Food Canada-Current Edition.
- Agriculture Research Service Manual- ARS 242.1 -US Department of Agriculture
- Institutional Animal care and Use Committee Guidebook-US Department of Health and Human Services-National Institutes of Health. NIH Publication No. 92-3415.
- Canadian Council on Animal Care-Guide to the Care and Use of Experimental Animals- Volumes 1&2. 1980-84
- Biosafety Reference Manual, Second Edition. Heinsohn PA, R Jacobs and BA Concoy, eds American Industrial Hygiene Association. AIHA Publications, Fairfax VA, 1995
- Hausler WJ Jr, KL Herrmann, HD Isengerg and HJ Shadomy, Sterilization, disinfection and antisepsis in the hospital. Manual of Clinical Microbiology, Current Edition, A. Balows editor. American Society for Microbiology, Washington, DC.
- Nicklas W, V Kraft and B Meyer. Contamination of Transplantable Tumors, cell Lines, and Monoclonal Antibodies with Rodent Viruses. Lab Animal Science 43(4): 296-300, 1993

- **Department of Health and Human Services- 42 CFR Part 73 -Additional Requirements for Facilities Transferring or Receiving Select Agents**- This publication is also extremely important as, the current primary agent within the Level 3 lab, is a listed select agent.

## 13.0 Bio-security Issues

### Purpose

This plan is design to protect people, the environment and the University of Toledo from those adverse outcomes that might arise because of activities that involve the use “select” biological agents by persons with an association with the University of Toledo.

### Introduction

The USA Patriot Act of 2001 established criminal penalties for possession of certain biological agents and toxins if used as a weapon or for any reason not reasonably justified for prophylactic, protective, bona fide research or other peaceful purposes (select agents <http://www.cdc.gov/od/sap/resource.htm>). The Act established certain controls over select agents to ensure that no “restricted person” transports, ships or possesses select agents.

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (PHSBPRA) greatly expanded the control of possession, transport and use of select agents in the United States of America. PHSBPRA mandates the following:

- The formation of lists of biological agents and toxins that have the potential to pose severe threats to the public’s health and safety by the U.S. Department of Health and Human Services (HHS) and the Department of Agriculture (USDA)
- The promulgation of regulations by HHS and USDA establishing the following: safety measures for select agents including proper training and appropriate skills to handle select agents and proper laboratory facilities to contain and dispose of the agents; the security of select agents to prevent their use in domestic and international terrorism; procedures to protect the public safety in the event of the transfer of such materials in violation of the act; and ensure the availability of biological agents and toxins for research, education and other legitimate purposes
- The promulgation of regulations by HHS and USDA for the possession, use and transfer of select agents, registration of individuals including provisions to ensure that persons registering have a lawful purpose to possess, use and transport the agents; and procedures to identify and characterize the agents held at a facility
- Prompt notification of the release of a select agent outside the biocontainment area
- The promulgation of regulations by HHS and USDA to ensure that appropriate safeguards and security arrangements for persons possessing, using or transferring the agents exist at a facility. Registered persons shall have their names and other identifying information submitted to the Department of Justice (DOJ). Access shall be denied to those identified as restricted persons; access shall be granted to only those individuals identified by the Secretaries of HHS and USDA and DOJ; the DOJ shall use criminal, immigration, national security and other electronic data bases to determine if a person is a restricted person or otherwise suspected of committing a crime, being involved in an organization that engages in domestic or international terrorism, or being an agent of a foreign power.
- Establishes penalties for violation of the Act.

Federal Regulation, 42CFR73 "Possession, Use & Transfer of Select agents & Toxins, Interim Final Rule," mandates that an entity develop and implement a security plan establishing policy and procedures that ensure the security of areas containing select agents and toxins.

The security plan must be based on a systematic evaluation in which threats are defined, vulnerabilities are examined and risks associated with those vulnerabilities are mitigated with a security systems approach. (See Hazard Vulnerability Analysis (HVA) located in Annex "C")

The University of Toledo (UT) Biosecurity Plan specifies security requirements for laboratories using select agents. The Institutional Biosafety Committee recommends that all users of biological agents consider adopting the requirements outlined in this Biosecurity Plan. (See also Policy #HM-08-030 Select Agent and Toxins - Handling, Storage & Access.

## Responsibilities

### *Responsible Official (RO)*

- Develop and implement safety, security and emergency response plans
- Allowing only approved individuals to have access to select agents or toxins
- Providing appropriate training for safety, security and emergency response
- Assures that transfer of select agents or toxins is done according to the rules of the Act
- Provide timely notice of any theft, loss or release of a select agent or toxin
- Maintain detailed records of information necessary to give a complete accounting of all activities related to select agents or toxins
- Escort or arrange for the escort of individuals who do not possess keys, proper identification or clearance to enter controlled biocontainment areas on the campus. This may include but is not limited to the following persons: Facilities Maintenance Personnel, outside contractors or special visitors/inspectors.
- Report the identification of a select agent or toxin as a result of diagnosis, verification or proficiency testing
- Conduct regular inspections, at least annually, of the laboratory where select agents or toxins are stored or used to ensure compliance with all procedures and protocols of the safety plan. The results of these inspections must be documented and any deficiencies must be corrected.
- Provide oversight and control regarding the transfer, acquisition and destruction of select agents on/off campus.

### *Alternate Responsible Official (ARO)*

- The ARO must meet all the qualifications for the RO and must be able to conduct all the activities of the RO in the absence of the RO

### *Select Agent User*

- Maintain a log of select agent stock quantities stored – select agent identification; storage location; amount stored; date; initial of person doing entry.
- Maintain a use log of select agent and reconciliation – amount used; date, initials of user.
- Will report to the Responsible Official or Alternate Responsible Official:
  - Any loss or compromise of their keys, passwords, combinations
  - Any suspicious persons or activities
  - Any loss of theft of select agents or toxins
  - Any release of select agents or toxins
  - Any sign that inventory and use records of select agents or toxins have been altered or otherwise compromised.
- Allow unescorted access only to approved individuals who are performing a specifically authorized function during hours required to perform the defined job.

- Allow individuals to conduct routine non-laboratory functions only when escorted and continually monitored by approved individuals
- Will report to the RO/ARO all select agents used
- Will notify the RO/ARO of all authorized personnel to use select agents or toxins.

### **Inventory Control Procedures**

The select agent user must keep a record of the agents location, how it is used, inventory, transfers – external/internal and destruction and access.

Copies of logs will be provided to the RO/ARO during inspections. Researcher will keep record of logs indefinitely.

The following logs are required:

- Name, characteristic and source date of agent
- The quantity acquired, the source and date of acquisition
- Quantity held on the date of the first inventory (toxin only)
- Current quantity held (toxin only)
- The quantity used and dates of the use (toxin only)
- The name of each person who has accessed any select agent or toxin
  - The select agent or toxin used
  - Date removed
  - The amount of toxin used
  - The date agent was returned to storage
  - Quantity of toxin returned
- The quantity transferred, transfer date, recipient's name

### **Shipping Procedures:**

Shipping must conform to applicable regulations. Prior to shipment, contact the Biosafety Officer/RO for instructions. The Biosafety Officer will provide regulatory paperwork and oversight. The following protocols must be adhered to:

- A CDC/APHIS Form #2 must be filled out and submitted to RO prior to transferring the agent. No select can be shipped unless the CDC/APHIS Form #2 is complete to be in compliance with Federal regulations.
- The sender should fax CDC/APHIS Form #2 to the governing agency to gain an approval number to be placed on Form #2.
- Recipient and shipper must have a registration number
- Shipper must receive confirmation in writing that shipment has been received. A copy of receipt will be sent to the RO/ARO.
- The RO/ARO of the recipient provides a completed paper copy or FAX of the CDC/APHIS Form #2 to the sender and to the HHS within 2 business days of receipt of the select agent or toxin.
- The recipient immediately reports to the HHS if the select agent or toxin has not been received within 48 hours after the expected delivery time or if the package received containing select agents or toxins has been leaking or was otherwise damaged.
- All shipments of biological agents will meet the packaging requirements defined by DOT and IATA Policy #HM-08-031 "Shipping Infectious and Biological Substances."
- All persons who wish package and ship select agents or other infectious substances must attend the Hazardous Material Shipping class sponsored by the Safety and Health Department every two years.
- The quantity, volume or mass destroyed or otherwise disposed and the date of each action.

- Destruction/disposal of select agents requires specific documentation and submission of information to CDC or USDA. Please use the form found in Appendix A when agents are disposed or destroyed. Submit the form to the Biosafety Department for processing with the appropriate regulatory agency.
  - RO/ARO must be informed 7 days prior to destruction or disposal of select agent.
  - Destruction/disposal of select agents/toxins must adhere to Federal protocols.
  - A CDC/APHIS Form #2 form must be filled out and submitted to the RO/ARO.
  - The CDC/APHIS Form #2 must be submitted within 5 business days to HHS. The RO/ARO will send the form to HHS.
  - Destruction must be witnessed by another individual.
  - The Biosafety Officer can offer assistance in the destruction of select agents on the campus of the University of Toledo.

## Risk Assessment

Based on crime statistics provided by Campus Police and past occurrence of weather and fire events, the probability of occurrence and severity of impact has been evaluated for the following events: (See HVA Annex "C")

Freezer power loss	medium probability	high severity
Lab fire	low probability	high severity
Theft of agent	low probability	high severity
Hurricane damage	low probability	high severity
Tornado	medium probability	high severity
Animal Activists	low probability	moderate severity

The Physical Security Section of the plan will address cost effective strategies to protect critical facility assets where select agents are used.

## Physical Security Systems

Based on the risk assessment the following precautions are required for select agent laboratories and recommended for other laboratories using biological agents or toxins:

- A graded level of security protection will be implemented based on site-specific risk and threat analysis (See Policy # SM-08-003)
  - Threat levels will be matched to the Department of Homeland Security and UT specific procedures will be implemented and monitored in all laboratories
- Laboratories must be locked when unoccupied, with the BSL-3 facility locked at all times
- Keys or other security devices will be used to permit entry into these areas; key/card distribution will be controlled
  - Only those individuals having a background check and fingerprints taken can be issued keys/cards as approved by the Biosafety Officer.
- Lock all freezers, refrigerators, cabinets, incubators and other containers where select agents are stored when they are not in direct view of a laboratory worker
- Only authorized personnel will have access to select agents
- Unauthorized personnel entering select agent areas must be escorted and monitored by authorized personnel
- Visitor access to the area where select agents are used or stored must be controlled. A log will be kept with the following information:
  - The name of each visitor accessing area
  - Date and time of entry

- Date and time of leaving
- Access logs will be maintained by the lab personnel and made available to the RO/ARO and other authorized individuals.
- An up-to-date list of persons who possess door keys and programmed card access number activated for the security system.
- Personnel using select agents or toxins must report immediately the following to the RO.
  - Any loss or compromise of their keys, passwords, I.D. Cards
  - Any suspicious persons or activities
  - Any loss or theft of select agents or toxins
  - Any release/exposure of select agents or toxins
  - Any sign that inventory and use records of select agents or toxins have been altered or otherwise compromised.
- Routine cleaning, maintenance, repairs will only be done only when escorted and can be continually monitored by approved individuals.

### **Personnel Security and Reliability**

Every new employee will be informed about the Biosecurity plan at new employee orientation. The training will provide information about restricted access areas, how they are identified and what unauthorized personnel must do to access these areas.

Unauthorized personnel entering into a restricted area will need to log in/out. All visitors will be escorted to the restricted area by an authorized employee and be required to log in/out.

Authorized personnel will not share their means of accessing the area where select agents or toxins are stored/used. This includes passwords, keys, keycards, or combinations.

Importation permits are also required for certain biologicals coming into the United States, and shipped to other states.

### **Obtaining a Security Risk Assessment (BSL3 Specific)**

The Institutional Biosafety Committee will not authorize the use of a select agent or toxin until the individual is approved by the HSS Secretary or the USDA Secretary based on a security risk assessment by the Attorney General.

- Individuals seeking access to the BSL3 facility must contact the Biosafety Officer so that the facilities Table 4b of the registration can be altered to include the new person.
- After Table 4b has been submitted to the CDC they will respond by issuing a unique identifying number for that specific individual.
- Each individual seeking clearance must submit a "Bioterrorism Preparedness and Response Act FBI Information" form (FD-961) to the RO (RO/ARO will supply the form and fingerprint package upon request)
- Individuals seeking clearance also will need to submit fingerprints to the FBI. Finger print cards can be obtained from the RO.
- Individuals will then report to Campus Police for fingerprinting. After fingerprinting ask the person from Campus Police to give you the cards or have them mail them to Michael Valigosky in the Safety and Health Department.
- The RO/ARO will submit the FD-961 and fingerprints to the FBI.
- The following individuals are prohibited from accessing and using select agents or toxins as published by PHSBPRA

- Is under indictment for a crime punishable by imprisonment for a term exceeding 1 year
- Has been convicted in any court of a crime punishable by imprisonment for a term exceeding 1 year
- Is a fugitive from justice
- Is an unlawful user of any controlled substance
- Is an alien illegally or unlawfully in the U.S.
- Has been adjudicated as a mental defective or has been committed to any mental institution
- Is an alien who is a national of a country as to which the Secretary of State has made a determination that such country has repeatedly provided support for acts of international terrorism –Cuba, Iran, Iraq, Libya, North Korea, Syria and Sudan
- Has been discharged from the Armed services of the U.S. under dishonorable conditions
- Has involvement with an organization that engages in domestic or international terrorism or with any other organization that engages in intentional crimes of violence
- Clearances will be valid for five years from date of approval.

### **Obtaining a Key and I.D. Access Card (BSL3 Specific)**

Upon successful completion of the SRA process the RO will make a request to key control for a PS-4. Additionally, the RO will make a request to IS and Campus Police to obtain an access card and have it activate for access to the BSL3 DLAM facilities.

### **Biosecurity Incident Response**

Any security breach of a select agent use area must be immediately reported to the RO and Campus Police. By State Statute, Campus Police have jurisdiction over campus crimes. Any security breach will be investigated and prosecuted according to Ohio State law.

### **Data, IT, and Cyber Security**

The most likely risk to information security is inadvertent disclosure by employees. The following measures will be taken to minimize these risks:

- *Hiring Permanent Employees*  
Hiring supervisors are to review applications, carry out interviews and check references before making their final selection.
- *Work-Study Students and Temporary Employees*  
Confidentiality and safeguarding of information should be covered by each work-study employer as students are hired to work in each department. Work-Study and other temporary employees shall have unique email addresses instead of sharing an address with past, present, or future temporary employees.
- *Existing Employees*  
All current employees who have access to the select agent information must be informed of information security requirements.
- *Ongoing Training*  
As part of the annual review process, the supervisor will review information security requirements with their respective employees or any employee who has access to select agent information.
- *Access to Select Agent Information*  
Only employees whose job duties require them to access select agent information shall have access.
- *Computer vaults for network lines*  
Will be locked and monitored by a card access system at all times additionally special care will be taken to limit the recording of select agent information to portable storage devices. Computers with select agent

information should be password, screen saver and kept behind locked doors when not attended.

## Information Systems

Information systems include network and software design, and information processing, storage, transmission, retrieval, and disposal. Lack of adequate procedures and technical controls creates a risk of unauthorized access to the information systems, and of accidental loss in the event of disasters or system failures. The following measures will be taken to minimize this risk.

- *Paper Storage Systems*  
Supervisors should instruct employees on the need to keep confidential files out of public view, and also provide for storage of confidential files in locked cabinets wherever feasible.
- *Computer Information Systems*  
The University has data ownership and access procedures that apply to all centrally managed data supported by administrative information systems under the jurisdiction of the Chief Information Officer. These procedures also apply to all user-developed systems that may access common data.

The rules, procedures and guidelines govern the management and accessibility of central UT data regardless of the environment where the data resides. This includes departmental mini-computers, data servers, individual personal computers, and data as it resides in any other media (printouts, microfiche, etc.).

The Data Management Procedures may be found at UT's IT website.

- *Customer Information Disposal*  
Information Systems erases all tapes and disks prior to disposal or surplus and adheres to the Department's procedure for cleaning storage medium.
- *Centralized Protection from E-Invasion*  
The University's central administrative data is located behind two load balance and redundant firewalls and safe-guarded by an Intrusion Prevention System in accordance with the Administrative Computing Services Data Network Procedures. Patches and maintenance for the systems in support of the administrative data are applied in accordance with the Information Systems Maintenance Standards. UT has a site license for virus software, which is installed on applicable servers with central administrative data located in Information Systems.
- *Detecting, Preventing, and Responding to Security Breaches*  
Prevention measures are addressed in prior sections. The risk to information security is compounded when breaches are not detected and remedied promptly. The following measures will be taken to minimize this risk. All information security breaches should be reported and rectified as soon as possible. Employees will be notified to report to their supervisors any suspected breaches of privacy or information security. The unit responsible for the information that has been compromised will report serious breaches to the Officers, who will keep a record of all such breaches and the remedies. Primary responsibility for detecting and remedying breaches lies with the supervisors in each unit. In addition, the Officers will look at the aggregate data for patterns among breaches to enable more preventive action. Employees and students who are at fault for breaches may be disciplined. (See also Information System Policies located in Annex "D")

## Final destruction of your Select Agent stocks

Before destroying select agents please contact Safety and Health to verify the procedure being used for destruction. Destruction of Select Agents MUST be reported to CDC/USDA. Please call the Biosafety Department at X419-383-5069 you have questions.

## ANNEX "A" – Biological Safety Cabinets

### INTRODUCTION

#### Biological Safety Cabinets - Utilization

- When properly installed and certified, these cabinets protect your work area, the environment and the worker from infectious aerosols.
- To maximize this protection, the worker must understand the workings of the cabinet and follow a strict working protocol.
- Annual certification is required to recalibrate the cabinet to CSA-Z316.3.95 or NSF-49 performance standards.

#### Do's and Don'ts of Biological Safety Cabinets Use

- ALWAYS wipe the working surface and interior with an appropriate disinfectant before and after work. Pay close attention to the front intake grille.
- NEVER rely on UV light for disinfecting the cabinet.
- ALWAYS turn the cabinet on at least 5 minutes prior to working and turn off 5 minutes after finishing.
- ALWAYS make periodic checks of gauges or warning signals.
- NEVER change the air speed in a cabinet without the use of an air velocity meter.
- ALWAYS use aseptic techniques.
- NEVER use a burner other than a touch-o-matic or electric burner. (A Bunsen Burner is rarely needed inside a cabinet, as the need to flame necks of bottles is removed in a sterile environment.)
- NEVER block air intake grilles.
- NEVER use Class I or II cabinets for work with Level 4 agents.

#### TYPES OF BIOLOGICAL SAFETY CABINETS:

The similarities and differences in protection offered by the various classes of biosafety cabinets are reflected in Table 1. Please also refer to Table 2 and Section IV for further considerations pertinent to BSC selection and risk assessment.

##### 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 (Figure 2). In the Class I BSC, unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow as long as a minimum velocity of 75 linear feet per minute (lfpm) is maintained (5) through the front opening. 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.

The Class I BSC is hard-ducted to the building exhaust system, and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter as it enters the exhaust plenum. A second HEPA filter may be installed at the terminal end of the exhaust.

Some Class I BSCs are equipped with an integral exhaust blower; the cabinet blower must be interlocked with the building exhaust fan. In the event that the building exhaust fan fails, the cabinet exhaust blower must turn off so that the exhaust ducts are not pressurized. Filters should be installed on the intake side of the fan. Also note that use of two filters increases the static pressure on the fan. If the ducts are pressurized and the HEPA filter develops a leak, contaminated air could be discharged into other parts of the building or the environment.

A steel panel with arm holes to allow access to the work surface can be added to the Class I cabinet. The restricted opening results in increased inward air velocity, thereby increasing worker protection. For added safety, arm-length gloves can be attached to the panel. Makeup air is then drawn through an auxiliary air supply opening (which may contain a filter) and/or around a loose-fitting front panel.

## Class II BSC

As biomedical researchers began to use sterile animal tissue and cell culture systems, particularly for the propagation of viruses, cabinets were needed that also provided product protection. In the early 1960's, a principle evolved stating that unidirectional air moving at a steady velocity along parallel lines (i.e., "laminar flow") would aid in the capture and removal of airborne contaminants (31). Biocontainment technology incorporated this laminar flow principle with the use of the HEPA filter to develop a workstation that provided a particulate-free work environment. This combination protected the laboratorian from the potentially infectious microorganisms being manipulated and provided necessary product protection.

The Class II (Types A, B1, B2, and B3)(24) biological safety cabinets provide personnel, environmental and product protection. Air flow 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. Because cabinet air has passed through the exhaust HEPA filter, it is contaminant-free (environmental protection), and may be re-circulated back into the laboratory (Type A BSC) or ducted out of the building (Type B BSC).

HEPA filters are effective at trapping particulates and infectious agents, but not at capturing volatile chemicals or gases. Only BSCs that are ducted to the outside should be used when working with volatile toxic chemicals (see Table 2).

All Class II cabinets are designed for work involving microorganisms assigned to biosafety levels 1, 2 and 3 (6). Class II cabinets provide the microbe-free work environment necessary for cell culture propagation, and also may be used for the formulation of non-volatile antineoplastic or chemotherapeutic drugs (30).

1. The Class II, Type A BSC - An internal blower (Figure 3) draws sufficient room air through the front grille to maintain a minimum calculated or measured average inflow velocity of at least 75 lfpm at the face opening of the cabinet. The supply air flows through a HEPA filter and provides particulate-free air to the work surface. Laminar airflow reduces turbulence in the work zone and minimizes the potential for cross-contamination.

The downward moving air "splits" as it approaches the work surface; the blower draws part of the air to the front grille and the remainder to the rear grille. Although there are variations among different cabinets, this split generally occurs about half way between the front and rear grilles, and two to six inches above the work surface.

The air is then discharged through the rear plenum into the space between the supply and exhaust filters located at the top of the cabinet. Due to the relative size of these two filters, approximately 30% of the air passes through the exhaust HEPA filter and 70% re-circulates through the supply HEPA filter back into the

work zone. Most Class II, Type A cabinets have dampers to modulate this 30/70 division of airflow.

An un-ducted Class II Type A BSC is not to be used for work involving volatile or toxic chemicals. The build-up of chemical vapors in the cabinet (by re-circulated air) and in the laboratory (from exhaust air) could create health and safety hazards (see Section IV).

It is possible to duct the exhaust from a Type A cabinet out of the building. However, it must be done in a manner that does not alter the balance of the cabinet exhaust system, thereby disturbing the internal cabinet air flow. The typical method of ducting a Type A cabinet is to use a "thimble", (13) or canopy hood, which maintains a small opening (usually 1 inch) around the cabinet exhaust filter housing (Figure 4). The volume of the exhaust must be sufficient to maintain the flow of room air into the space between the thimble unit and the filter housing (contact manufacturers for any additional specifications). The thimble must be removable or be designed to allow for operational testing of the cabinet (see Section VI). The performance of a cabinet with this exhaust configuration is unaffected by fluctuations in the building exhaust system.

"Hard-ducting" (i.e., direct connection) of Class II Type A cabinets to the building exhaust system is not recommended. The building exhaust system must be precisely matched to the airflow from the cabinet in both volume and static pressure. However, fluctuations in air volume and pressure that are common to all building exhaust systems make it difficult, if not impossible, to match the airflow requirements of the cabinet.

2. The Class II, Type B1 BSC - Some biomedical research requires the use of small quantities of certain hazardous chemicals, such as carcinogens. The powdered form of these carcinogens should be weighed or manipulated in a chemical fume hood or a static-air glove box. Carcinogens used in cell culture or microbial systems require both biological and chemical containment (19).

The Class II, Type B cabinet originated with the National Cancer Institute (NCI)-designed Type 2 (later called Type B) biological safety cabinet (Figure 5A), which was designed for manipulations of minute quantities of these hazardous chemicals with in vitro biological systems. The National Sanitation Foundation (NSF) Standard 49 definition of Type B1 cabinets (24) includes this classic NCI design Type B, as well as cabinets without supply HEPA filters located immediately below the work surface (Figure 5B), and/or those with exhaust/recirculation downflow splits other than 70/30%.

The cabinet supply blowers draw room air (plus a portion of the cabinet's re-circulated air) through the front grille and then through the supply HEPA filters located immediately below the work surface. This particulate-free air flows upward through a plenum at each side of the cabinet and then downward to the work area through a back-pressure plate. In some cabinets there is an additional supply HEPA filter to remove particulates that may be generated by the blower/motor system.

Room air is drawn through the face opening of the cabinet at a minimum inflow velocity of 100 lfpm. As with the Type A cabinet, there is a split in the down-flowing air stream just above the work surface. In the Type B cabinet, approximately 70 percent of the down flow air exits through the rear grille, passes through the exhaust HEPA filter, and is discharged from the building. The remaining 30 percent of the down flow air is drawn through the front grille. Since the air which flows to the rear grille is discharged into the exhaust system, activities that may generate hazardous chemical vapours or particulates should be conducted towards the rear of the cabinet (27).

Type B1 cabinets must be hard-ducted, preferably to their own dedicated exhaust system, or to a properly designed laboratory building exhaust. As indicated earlier, blowers on laboratory exhaust systems should

be located at the terminal end of the ductwork. A failure in the building exhaust system may not be apparent to the user, as the supply blowers in the cabinet will continue to operate. A pressure-independent monitor should be installed to sound an alarm and shut off the BSC supply fan, should failure in exhaust air flow occur. Since this feature is not supplied by all cabinet manufacturers, it is prudent to install a sensor in the exhaust system as necessary. To maintain critical operations, laboratories using Type B BSCs should connect the exhaust blower to the emergency power supply.

3. The Class II, Type B2 BSC - This BSC is a total exhaust cabinet; no air is re-circulated within it (Figure 6). This cabinet provides simultaneous primary biological and chemical containment. The supply blower draws in room air or outside air at the top of the cabinet, passes it through a HEPA filter and down into the work area of the cabinet.

The building or cabinet exhaust system draws air through both the rear and front grilles, capturing the supply air plus the additional amount of room air needed to produce a minimum calculated or measured inflow face velocity of 100 lfpm. All air entering this cabinet is exhausted, and passes through a HEPA filter (and perhaps some other air cleaning device such as a carbon filter) prior to discharge to the outside. Exhausting as much as 1200 cubic feet per minute of conditioned room air makes this cabinet expensive to operate.

Should the building or cabinet exhaust fail, the cabinet will be pressurized, resulting in a flow of air from the work area back into the laboratory. Cabinets built since the early 1980's usually have an interlock system installed by the manufacturer to prevent the supply blower from operating whenever the exhaust flow is insufficient. Presence of such an interlock system should be verified; systems can be retrofitted if necessary. Exhaust air movement should be monitored by a pressure-independent device.

4. The Class II, Type B3 BSC - This biological safety cabinet (Figure 7) is a ducted Type A cabinet having a minimum inward air flow of 100 lfpm. All positive pressure contaminated plenums within the cabinet are surrounded by a negative air pressure plenum. Thus, leakage in a contaminated plenum will be into the cabinet and not into the environment.
5. Special applications - Class II BSCs can be modified to accommodate special tasks. For example, the front sash can be modified by the manufacturer to accommodate the eye pieces of a microscope, or the work surface can be designed to accept a carboy, a centrifuge, or other equipment that requires containment. A rigid plate with arm holes can be added if needed. Good cabinet design, microbiological aerosol tracer testing of the modification, and appropriate certification (see Section VII) are required to ensure that the basic systems operate properly after modification. Maximum containment potential is achieved only through strict adherence to proper practices and procedures (see Section V).

### Class III BSC

The Class III biological safety cabinet (Figure 8) was 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 Class III biosafety 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).

Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow for manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials. The trade off is clearly on the side of maximizing personal safety. Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment.

Several Class III cabinets can be joined together in a "line" to provide a larger work area. Such cabinet lines are custom built; the equipment installed within the cabinet line (e.g., refrigerators, small elevators, shelves to hold small animal cage racks, microscopes, centrifuges, incubators, etc.) is generally custom-built as well. Furthermore, Class III cabinets are usually only installed in maximum containment laboratories that have controlled access and require special ventilation or other support systems (such as steam for autoclaves). The reader should consult more definitive literature on these systems (16,21,23).

### Horizontal Laminar Flow "Clean Bench"

Horizontal laminar flow clean air benches (Figure 9A) are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user. These devices only provide product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices. These benches should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials. The worker can be exposed to materials (including protein aqueous antigens) being manipulated on the clean bench, which may cause hypersensitivity. Horizontal clean air benches should never be used as a substitute for a biological safety cabinet in research, biomedical or veterinary laboratories and/or applications.

### Vertical Laminar Flow "Clean Bench"

Vertical laminar flow clean benches (Figure 9B) also are not BSCs. They may be useful, for example, in hospital pharmacies when a clean area is needed for preparation of intravenous drugs. While these units generally have a sash, the air is usually discharged into the room under the sash, resulting in the same potential problems as the horizontal laminar flow clean benches.

## **SECTION VII- Certification of Biological Safety Cabinets**

### Development of Containment Standards:

The evolution of containment equipment for varied research and diagnostic applications created the need for consistency in construction, certification and performance. A Federal standard was developed (b) to establish classes of air cleanliness and methods for monitoring clean work stations and clean rooms where HEPA filters are used to control airborne particulates.

The first "standard" to be developed specifically for BSCs (17) served as a Federal procurement specification for the NIH Class II, Type 1 (now called Type A) biological safety cabinets, which had a fixed or hinged front window or a vertical sliding sash, vertical downward laminar airflow and HEPA-filtered air supply and exhaust. This guideline specified design criteria and defined prototype tests for microbiological aerosol challenge, velocity profiles, and leak testing of the HEPA filters. A similar procurement specification was generated (20) when the Class II Type 2 (now called Type B1) cabinet was developed to control both airborne particulates and some gaseous contaminants.

The National Sanitation Foundation (NSF International) Standard No. 49 for Class II (Laminar Flow) Biohazard Cabinetry (24) was first published in 1976, providing the first independent standard for design, manufacture and

testing. This standard "replaced" the NIH specifications which had been used by other institutions and organizations when purchasing BSCs. NSF Standard 49 incorporates specifications regarding design, materials and construction. This Standard for biological safety cabinets establishes performance criteria for biological safety cabinets and provides the minimum requirements that are accepted in the United States. Cabinets which meet the standard and were certified by the NSF bear an NSF49 Seal.

Standard No. 49 pertains to all models of Class II cabinets (Type A, BI, B2, and B3) and lists a series of specifications regarding:

- design/construction,
- performance,
- installation recommendations,
- recommended microbiological decontamination
- procedure, and
- references and specifications pertinent to Class II
- Biohazard Cabinetry.

While the NSF standard does not cover field testing of BSCs, it is common for many of its test methods and parameters to be applied in the field, and these are included in Annex "F" of the document. Most recently revised in 1992 (24), this Standard is reviewed every five years by a steering committee to ensure that it remains consistent with developing technologies. (c)

The operational integrity of a new BSC must be validated by certification before it is put into service or after a cabinet has been repaired or relocated. Relocating a BSC may break the HEPA filter seals or otherwise damage the filters or the cabinet. Each BSC should be tested and certified at least annually to ensure continued proper operation.

On-site testing following the recommendations for field testing (NSF Standard 49) must be performed by experienced, qualified personnel. Some basic information is included here to assist in understanding the frequency and kinds of tests to be performed. In 1993, NSF began a program for accreditation of certifiers based on written and practical examinations. Education and training programs for candidate accredited field certifiers are offered through the Eagleson institute, Sanford, ME; the Harvard School of Public Health, Cambridge, MA; NuAire Inc., Plymouth, MN; Forma Scientific Inc., Marietta, OH; and Lab Conco Corporation, Kansas City, MO. Other training, education and certification programs may be developed in the future. Selecting competent individuals to perform testing and certification is important, and it is suggested that the institutional biosafety Department be consulted in identifying companies qualified to conduct the necessary field performance tests.

It is strongly recommended that accredited field certifiers be used to test and certify BSCs whenever possible. If in-house personnel are performing the certifications, then these individuals should become accredited. The importance of proper recertification cannot be emphasized enough, since persons who manipulate infectious micro-organisms are at increased risk of acquiring an occupational illness when their BSCs are functioning improperly.

The annual tests applicable to each of the three classes of biological safety cabinets are listed in Table 3. Table 4 indicates where to find information regarding the conduct of selected tests. BSCs perform consistently well when proper annual certification procedures are followed; cabinet or filter failures tend to occur infrequently.

Performance Testing BSCs in the Field:

BSCs are the primary containment device that protect the worker, product and environment from exposure to microbiological agents, and their performance as specified by Standard No. 49 needs to be verified at the time of installation and annually thereafter. The purpose and acceptance level of the performance tests (Table 3) are to ensure the balance of inflow and exhaust air, the distribution of air onto the work surface, and the integrity of the cabinet. Other tests check electrical and physical features of the BSC.

- A. Downflow Velocity and Volume Test: This test is performed to measure the velocity of air moving through the cabinet work space, and is to be performed on all Class I and II biosafety cabinets.
- B. Inflow Velocity Test: This test is performed to determine the calculated or directly measured velocity through the work access opening, to verify the nominal set point average inflow velocity and to calculate the exhaust airflow volume rate.
- C. Airflow Smoke Patterns Tests: This test is performed to determine if the airflow along the entire perimeter of the work access opening is inward, if airflow within the work area is downward with no dead spots or refluxing, if ambient air passes onto or over the work surface, and if there is refluxing to the outside at the window wiper gasket and side seals. The smoke test is an indicator of airflow direction, not velocity.
- D. HEPA Filter Leak Test: This test is performed to determine the integrity of supply and exhaust HEPA filters, filter housing, and filter mounting frames while the cabinet is operated at the nominal set point velocities. An aerosol in the form of generated particulates of dioctylphthalate (DOP) or an accepted alternative is required for leak-testing HEPA filters and their seals. Although DOP has been identified as a potential carcinogen, competent service personnel are trained to use this chemical in a safe manner. The aerosol is generated on the intake side of the filter, and particles passing through the filter or around the seal are measured with a photometer on the discharge side. This test is suitable for ascertaining the integrity of all HEPA filters.
- E. Cabinet Leak Tests: The pressure holding test is performed to determine if exterior surfaces of all plenums, welds, gaskets, and plenum penetrations or seals are free of leaks. It need only be performed just prior to initial installation when the BSC is in a free-standing position (all four sides are easily accessible) in the room in which it will be used, after a cabinet has been relocated to a new location, and again after removal of access panels to plenums for repairs or a filter change. This test may also be performed on fully installed cabinets.
- F. Electrical Leakage and Ground Circuit Resistance and Polarity Tests: These safety tests are performed to determine if a potential shock hazard exists by measuring the electrical leakage, polarity ground fault interrupter function, and ground circuit resistance to the cabinet connection. They may be performed by an electrical technician other than the field certification personnel at the same time the other field certification tests are conducted. The polarity of electrical outlets is checked (see Table 3, E) using a polarity tester. The ground fault circuit interrupter should trip when approximately 5 milliamperes (ma) is applied.
- G. Lighting Intensity Test: This test is performed to measure the light intensity on the work surface of the cabinet as an aid in minimizing cabinet operator's fatigue.
- H. Vibration Test: This test is performed to determine the amount of vibration in an operating cabinet as a guide to satisfactory mechanical performance, as an aid in minimizing cabinet operator's fatigue, and to prevent damage to delicate tissue culture specimens.
- I. Noise Level Test: This test is performed to measure the noise levels produced by the cabinets, as a guide

to satisfactory mechanical performance and an aid in minimizing cabinet operator's fatigue.

- J. UV Lamp Test: A few BSCs have UV lamps. When used, they must be tested periodically to ensure that their energy output is sufficient to kill micro-organisms. After having been turned off and allowed to cool, the surface on the bulb should be cleaned with 70% ethanol prior to performing this test. Five minute after the lamp has been turned on, the sensor of the UV metre is placed in the centre of the work surface. The radiation output should not be less than 40 microwatts per square centimetre at 254 nanometres (nm).

Finally, accurate test results can only be assured when the testing equipment is properly maintained and calibrated. It is appropriate to request the calibration information for the test equipment being used by the certifier.

(b) Federal Standard No. 209B, Clean Room and Work Station Requirements (7) has evolved into Federal Standard No. 209E, Airborne Particulate Cleanliness Classes in Clean rooms and Clean Zones (8). This standard does not apply to BSCs and should not be considered a basis for their performance or integrity certification. However, the methodology of 209E can be used to quantify the particulate count within the work area of a BSC. 209E defines how to classify a clean room/clean zone. Performance tests and procedures needed to achieve a specified cleanliness classification are outlined by the Institute of Environmental Sciences - Testing Clean Rooms (IES-RP-CC-006-84-T) and Laminar Flow Clean Air Devices

(c) The standard can be ordered from NSF for a nominal charge at

NSF International,  
3475 Plymouth Road,  
P.O. Box 130140,  
Ann Arbor, Michigan, 48113-0140  
USA.  
Telephone 313-769-8010;  
Fax 313-769-0109;  
Telex 753215 NSF INTL

Department of Health and Safety,  
Centers for Disease Control and Prevention,  
1600 Clifton Road N.E., Mail Stop F05  
Atlanta, Georgia 30333, USA  
Last Modified: 1/2/97

## **Annex "B" – IACUC Protocol Document**

**IACUC Protocols (INSERT AS NECESSARY)**

## **Annex "C" – Hazard Vulnerability Analysis (HVA)**

**Hazard Vulnerability Analysis (HVA)** (CONSULT SAFETY AND HEALTH 419-383-5069)

## **Annex "D" – Information System Policies**

**Information System Policies (INSERT AS NECESSARY)**

## **Annex "E" – MSDS's for Biological Agents**

**MSDS's for Biologic Agents (INSERT AS NECESSARY)**

## Annex “F” – Use of Viral Vectors

### Use of Viral Vectors

#### University of Toledo Institutional Biosafety Committee (IBC) Viral Vector Policy

May, 2005

All research involving viral vector expression systems must be registered by completing the IBC form “University of Toledo Biosafety Forms, RGA Form #s RGA600-604”. This includes those vectors that are not normally believed to pose a risk to human health. It is especially critical and important that the information included on the registration form be detailed and complete. This information will be used by the Institutional Biosafety Committee and the Safety & Health Department to assign the appropriate handling precautions.

The following table shows the prescribed precautions for working with three commonly used vectors. Understand that the precautions outlined below are the minimal requirements for working with each agent. Those systems, which express genes that expand the host range of the vector, or increase the hazard of the virus beyond wild-type, may necessitate higher containment practices and facilities.

Viral Vector	Hazard(s)	Biosafety Level; Animal Biosafety Level	Additional Precautions	Animal Handling (IACUC/DLAM approval required)	Transport/Waste Issues	Spills (Wearing PPE) see Emergency Response Guide
Adenovirus	Droplet, aerosol, injection	BSL-2; ABSL-2  Special considerations for transgenes, ex., toxins, oncogenes, elements that alter host range, immune suppressors, etc.	All work done in BSC; Negative pressure lab preferred.  Cuffed lab gown/gloves.  Safety & Health approved lab audit.  SOPs developed and available.  BSL-2 Biohazard sign on door.  Disinfect surfaces with 1-10% bleach	Viruses must be screened for replication competence before injection of animals.  SOPs developed and available for animal handling.  Special handling of bedding and cages for 48 hours post injection of animals.  Incineration or autoclaving of bedding.  Incineration of carcasses.  DLAM assigned signage on cages and doors.  Housing approved by DLAM.	Transport specimens in a secondary, sealed container.  All waste initially autoclaved and then disposed to infectious waste bins by laboratory staff familiar with precautions.	Alert people in immediate area of spill.  Allow aerosols to settle.  Absorb spill with paper towel/lab diaper.  Expose to 10% bleach for 20-30 minutes.  Use paper towels to wipe up spill.  Clean spill area with fresh towels soaked in 10% bleach.  Place towels in autoclave bag and decontaminate in autoclave.  Notify Safety & Health x5069.

<p><b>Viral Vector Vaccinia</b></p>	<p><b>Hazard(s)</b> Droplet, aerosol, injection</p>	<p><b>Biosafety Level;</b> <b>Animal Biosafety Level</b> BSL-2; ABSL-2</p> <p>Special considerations for transgenes, ex., toxins, oncogenes, elements that alter host range, immune suppressors, etc.</p> <p>MVA strains encouraged for use.</p>	<p><b>Additional Precautions</b> All work done in BSC; Negative pressure lab preferred.</p> <p>Cuffed lab gown/gloves.</p> <p>Safety &amp; Health approved lab audit.</p> <p>SOPs developed and available.</p> <p>BSL-2 Biohazard sign on door.</p> <p>Consult with Occupational Medicine physician for a medical evaluation regarding vaccination. (Note: use of MVA strains does not require vaccination)</p>	<p><b>Animal Handling (IACUC/DLAM approval required)</b> Special handling of bedding and cages post injection of animals.</p> <p>SOPs developed and available for animal handling.</p> <p>Workers handling or decontaminating bedding or cages must consult with Occupational Medicine physician for a medical evaluation regarding vaccination.</p> <p>Incineration or autoclaving of bedding.</p> <p>Incineration of carcasses.</p> <p>DLAM assigned signage on cages and doors.</p> <p>Housing approved by DLAM.</p>	<p><b>Transport/Waste Issues</b> Transport specimens in a secondary, sealed container.</p> <p>All waste initially autoclaved and then disposed to infectious waste bins by laboratory staff familiar with precautions and who have been evaluated by Occupational Medicine physician for vaccination.</p>	<p><b>Spills (Wearing PPE)</b> see Emergency Response Guide</p> <p>Alert people in immediate area of spill.</p> <p>Allow aerosols to settle.</p> <p>Absorb spill with paper towel/lab diaper.</p> <p>Expose to 10% bleach for 20-30 minutes.</p> <p>Use paper towels to wipe up spill.</p> <p>Clean spill area with fresh towels soaked in 10% bleach.</p> <p>Place towels in autoclave bag and decontaminate in autoclave.</p> <p>Notify Safety &amp; Health x5069.</p>
<p><b>Retrovirus</b></p>	<p>Injection, splash to face</p>	<p>BSL-2; ABSL-2</p> <p>Special considerations for transgenes, ex., toxins, oncogenes, elements that alter host range, immune suppressors, etc.</p>	<p>Sharps precautions emphasized.</p> <p>Full face protection if working outside BSC.</p> <p>Safety &amp; Health approved lab audit.</p> <p>SOPs developed and available.</p> <p>BSL-2 Biohazard sign on door with agent specified.</p>	<p>Work with amphotrophic viruses must be screened for replication competence before injection of animals.</p> <p>Incineration or autoclaving of bedding.</p> <p>Incineration of carcasses.</p> <p>DLAM assigned signage on cages and doors.</p> <p>Housing approved by DLAM.</p>	<p>Transport specimens in a secondary, sealed container.</p> <p>All waste initially autoclaved and then disposed to infectious waste bins by laboratory staff familiar with precautions.</p>	<p>Alert people in immediate area of spill.</p> <p>Allow aerosols to settle.</p> <p>Absorb spill with paper towel/lab diaper.</p> <p>Expose to 10% bleach for 20-30 minutes.</p> <p>Use paper towels to wipe up spill.</p> <p>Clean spill area with fresh towels soaked in 10% bleach.</p> <p>Place towels in autoclave bag and decontaminate in autoclave.</p> <p>Notify Safety &amp; Health x5069.</p>
<p><b>Lentiviruses</b></p>		<p><i>BSL-2 facilities, BSL-3 work practices;</i></p> <p><i>ABSL-2 facilities, ABSL-3 work practices.</i></p> <p><b>Biosafety Level;</b></p>	<p><i>All work with lentivirus must be done in a BSC.</i></p>	<p><b>Animal Handling</b></p>		<p><b>Spills (Wearing PPE)</b></p>

<b>Viral Vector</b>	<b>Hazard(s)</b>	<b>Animal Biosafety Level</b>	<b>Additional Precautions</b>	<b>(IACUC/DLAM approval required)</b>	<b>Transport/Waste Issues</b>	<b>see Emergency Response Guide</b>
Other Viral Vectors (Ex., Baculovirus, Adeno-associated virus, Simliki		Use minimum requirements for Adenovirus	Use minimum requirements for Adenovirus	Use minimum requirements for Adenovirus	Use minimum requirements for Adenovirus	

## UT Institutional Biosafety Committee

### Policy for Safety Testing of Lentiviral Vector Stocks for Replication Competent Particles

#### **Containment for Pseudotyped Lentiviral Vector systems: BL2 but BL2+ if transgene/insert is involved in oncogenesis:**

The IBC recognizes the enhanced safety features embedded in the latest lentiviral vector systems. These safety features, the use of multiple plasmids for vector packaging, the deletion of overlapping sequences between the packaging plasmids plus the minimalist design of the vector backbone itself, are all aimed at reducing the risk of generation of replication competent virus during vector stock preparation. According to published data, certain lentiviral vector systems generate less than 1 replication competent particle in  $10^8$  vector particles to 1 in  $10^{12}$  depending on the vector-packaging system. Publications, along with data and personal communication from various investigators developing and using these plasmid systems indicate that detection of replication competent virus is difficult to none detected (personal communication, Prof. Inder Verma, Salk Institute).

Even though there is published data to indicate that these vectors may indeed be safer, less rep+ virus, they are also more efficient and effective. This latter property may increase the risk to researchers in case of accidental exposure to the vector. Thus the IBC decided to retain the same containment paradigm for lentiviral systems as for the murine retroviral vector systems. BL2 containment is acceptable for lentiviral vectors with non-oncogenic gene products. BL2+ procedures and practices provide additional physical barriers to further minimize the chance of possible accidental exposures.

#### **Characterization of Lentiviral Vector Stocks: Use of one assay for rep+ particles, reduced testing frequency and submission of data for IBC review:**

The IBC has decided that investigators should select a use one assay for replication competent viral contaminants and show that the assay works and is sensitive. This assay should be one that the PI is familiar with and able to do on a relatively routine basis. Investigators must assay at least two consecutive independently generated lentiviral vector preps to show that the lentiviral vector system works as expected from publications, in their hands, in their laboratory. The investigator must send the results of the assay on these two preps to the IBC. Once the lentiviral vector system is working as safely as expected, investigators are not required to test every lentiviral vector preparation. The frequency of testing of vector stocks is dependent upon the frequency of generation of vector preps (see below). The information submitted by researchers to the IBC will help the committee assess whether these requirements provide the appropriate level of oversight for this research or whether more frequent testing is needed.

- All vector preps should be tested until at least two consecutive preps give the expected negative (undetected) results in the selected assay. The rep+ assay needs to be done in such a way that a measure of the sensitivity of the assay can be assessed as well.
- Once researchers have gained enough experience with these vectors, and these vectors work in their lab as expected (based on the results from two vector preps), researchers will NOT be required to test every vector preparation. Depending on the frequency that vector preps are generated within a group, vector preps should be tested on a periodic basis. Some groups will generate and use multiple preps a week, others will be making far fewer. Laboratories that generate multiple vector preps a month should test 3 representative preps every 2-3 months. Low frequency (low number of preps generated) labs should test 2 vector preps every 4-6 months. A low frequency lab generates and uses up a prep approximately every month or so. This is difficult to specify but the committee is asking that about 1 in every 3 preps be tested. This will provide "ongoing" testing but will not require that every prep be tested as long as the investigators

are able to show that they are able to generate at least two consecutive independent preps with no detectable rep+ virus.

- The IBC requires that the data on detection of rep+ viral particles be submitted in a timely manner. The data must also indicate and show the sensitivity of the assay. Receipt of this data is necessary for continued IBC and Safety & Health (biosafety) approval of the research project.

## Annex "G" – Recombinant DNA Technology

### Recombinant DNA Technology

- INTRODUCTION
- BIOLOGICAL EXPRESSION SYSTEMS
- PROPERTIES OF THE DONOR ORGANISM AND CLONED DNA
- VIRAL VECTORS FOR GENE TRANSFER
- TRANSGENIC AND KNOCK-OUT ANIMALS
- TRANSGENIC PLANTS
- CONCLUSION
- BIOLOGICAL USE AUTHORIZATION AND REGISTRATION

#### INTRODUCTION

Recombinant DNA (R-DNA) technology involves combining genetic information from different sources thereby creating genetically modified organisms (GMOs) that may have never existed in nature before. Initially there was concern among molecular biologists that such organisms might have unpredictable and undesirable properties and would represent a biohazard if they escaped from the laboratory. This concern resulted in the famous Asilomar conference held in 1975. At that meeting safety issues were discussed and the first guidelines for R-DNA technology were proposed. More than 25 years have now passed and no adverse incidents associated with this technology have been revealed. This demonstrates that genetic engineering is safe, provided that appropriate safety measures are observed.

R-DNA technology or genetic engineering was first used to clone DNA segments of interest in bacterial hosts in order to produce enough sufficiently pure materials for further studies. More recently, R-DNA molecules have also been used to create genetically modified higher organisms such as transgenic and "knock-out" animals and transgenic plants (see the relevant sections below).

R-DNA technology has already had an enormous impact on biology and medicine and will have an even greater influence in the near future. Now that the nucleotide sequence of the entire human genome is available, tens of thousands of genes of unknown functions will be studied, and R-DNA technology will be one of the means of doing so. Furthermore, gene therapy is expected to become an accepted treatment for certain diseases in the future, and many new vectors for gene transfer will be devised using genetic engineering techniques. Finally, transgenic plants produced by R-DNA technology may play an increasingly important role in modern agriculture.

When considering the use or construction of GMOs, the risk assessment process for work in the laboratory is perhaps even more important than that for work with genetically normal (non-modified) organisms. Whereas the latter are likely to be well characterized with respect to pathogenic properties, the former will be novel, and evaluation of the potential hazards associated with working with such organisms cannot build on experience only.

The risk assessment will identify the biological containment system to be used. The properties of the donor organism, the nature of the DNA sequences that will be transferred, the properties of the recipient organism and the properties of the environment must be evaluated. All of these factors will determine the BSL that is required for the safe handling of the resulting GMO. The following paragraphs provide some background information with respect to these criteria.

## BIOLOGICAL EXPRESSION SYSTEMS

Biological expression systems are vectors and host cells that fulfil a number of criteria that make them safe to use. A good example of a biological expression system is plasmid pUC18 (or derivatives thereof), which is frequently used as a cloning vector in combination with *Escherichia coli* K12 cells. The pUC18 plasmid and its derivatives have been entirely sequenced. More importantly, all genes required for efficient transfer to another bacterium have been deleted from the precursor plasmid pBR322 providing significant containment. *E. coli* K12 is a strain that lacks the genes known to render some *E. coli* strains pathogenic. Furthermore, *E. coli* K12 cannot permanently colonize the gut of healthy humans or animals. Thus, most routine genetic engineering experiments can be performed safely in *E. coli* K12/pUC18 at BSL1 provided the inserted foreign DNA sequences do not require a higher BSL (see below).

## PROPERTIES OF THE DONOR ORGANISM AND CLONED DNA

Risk assessment must consider not only the vector/host system used but also the properties of the DNA to be cloned. In most cases the risk assessment will show that the inserted DNA sequences are unlikely to alter the biological properties of the host organism, but in some cases they may do so, for example, if they are derived from a pathogenic organism. Obviously not all genes of a pathogenic organism contribute to the virulence of the agent. Therefore, insertion of well-characterized DNA sequences that are unlikely to be involved in pathogenicity may not require additional safety measures. However, in cases where these sequences are not characterized, a situation that is typically encountered when a library of genomic DNA of an organism is being established, a higher BSL will be required.

An important consideration is whether the gene product has potential pharmacological activity. Cloning of genes coding for proteins such as toxins may therefore require higher BSLs. Overexpression of gene products from eukaryotic viral vectors can have unexpected consequences when these proteins have pharmacological activity.

## VIRAL VECTORS FOR GENE TRANSFER

Viral vectors are used not only for gene therapy but also for efficient transfer of genes to other cells. Adenovirus vectors have become popular for gene therapy. Such vectors lack certain genes that are required for virus replication and therefore have to be propagated in cell lines that complement the defect. Although such vectors are replication-defective, they should be handled at the same BSL as the parent adenovirus from which they are derived. The reason for this is that the virus stocks may be contaminated with replication-competent viruses, which are generated by rare spontaneous recombination events in the complementing cell line.

## TRANSGENIC AND "KNOCK-OUT" ANIMALS

Animals carrying foreign genetic information (transgenic animals) should be handled in the containment levels appropriate to the characteristics of the products of the foreign genes. Animals with targeted deletions of specific genes ("knock-out" animals) do not generally present particular biological hazards.

Examples of transgenic animals include animals expressing receptors for viruses normally unable to infect that species. If such animals escaped from the laboratory and transmitted the transgene to the wild animal population, an animal reservoir for that particular virus could theoretically be generated.

This possibility has been discussed for poliovirus and is particularly relevant in the context of poliomyelitis

eradication.

Transgenic mice expressing the human poliovirus receptor generated in different laboratories were susceptible to poliovirus infection by various inoculation routes and the resulting disease was clinically and histopathologically similar to human poliomyelitis. However, the mouse model differs from humans in that alimentary tract replication of orally administered poliovirus is either inefficient or does not occur. It is therefore very unlikely that escape of such transgenic mice to the wild would result in the establishment of a new animal reservoir for poliovirus. Nevertheless, this example indicated that for each new line of transgenic animal, detailed studies should be conducted to determine the routes by which the animals can be infected, the inoculum size required for infection and the extent of virus shedding by the infected animals. In addition, all measures should be taken to assure strict containment of receptor transgenic mice.

## TRANSGENIC PLANTS

Transgenic plants expressing genes that confer tolerance to herbicides or resistance to insects are currently a matter of considerable controversy in large parts of the world. The discussions mainly focus on the safety of such plants as food and on the long-term ecological consequences of growing such plants on a large scale, which are not the subjects of this chapter.

Transgenic plants expressing genes of animal or human origin should remain strictly contained within the facility. Such transgenic plants should be handled at BSLs appropriate to the characteristics of the products of the expressed genes.

## CONCLUSION

When creating or handling recombinant organisms, it is essential to perform a detailed risk assessment, which must take into account the nature of the donor, the recipient organism and the environment. In many cases, the risk assessment will show that the recombinant organism can be handled at the same BSL as the wild-type recipient. In some instances, however, higher BSLs will be required. This is the case, for example, when ill-defined DNA sequences from a donor organism are transferred, which could potentially increase the virulence of the recipient organism. This situation is typically encountered in random ("shot-gun") cloning experiments in which genomic DNA libraries are established. Risk assessment is particularly important when creating GMOs expressing proteins with pharmacological activity, such as toxins. It is obvious that such organisms must be handled with caution. Some pharmacologically active proteins are only toxic when expressed at high levels. In this case, the risk assessment becomes very demanding and requires an estimation of the expected expression levels of the protein by a particular recombinant organism and the levels at which a given protein becomes toxic in an organism accidentally exposed to it. The NIH, which established guidelines for work with GMOs, helps scientists classify their work at the appropriate BSL. Risk assessment is thus a dynamic process and has to take into account new developments and the progress of science. It is the responsibility of the scientists involved in genetic engineering to keep up to date on these developments and to respect the guidelines established by the NIH.

## BIOLOGICAL USE AUTHORIZATION AND REGISTRATION

### Recombinant DNA

Experiments involving the generation of R-DNA require registration and approval by the IBC. NIH Guidelines for Research Involving Recombinant DNA Molecules, published by NIH, is the definitive reference for R-DNA research in the U.S., and has been adopted by UT's IBC. If the experimental protocol is not covered by the guidelines, contact the Biosafety Officer at 419-383-4521 for determination of further review. If you have any specific questions about a particular vector/host system not covered by the guidelines, please call the BSO to

request review by the IBC.

## **Transgenics**

### **Transgenic Animals**

Investigators who create transgenic animals must complete an R-DNA registration document and submit it to Research and Sponsored Programs, at <http://hsc.utoledo.edu/research/> for IBC approval prior to initiation of experimentation. In addition, IACUC requires that these protocols be approved by its committee prior to initiation of work.

### **Transgenic Plants**

Experiments to genetically engineered plants by R-DNA methods also require registration with the IBC. To prevent release of transgenic plant materials to the environment, the NIH Guidelines for Research Involving Recombinant DNA Molecules published by NIH, provides specific plant biosafety containment recommendations for experiments involving the creation and/or use of genetically engineered plants.