UNMC
Biosafety Manual

University of Nebraska Medical Center
University of Nebraska at Omaha
Nebraska Medicine
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FOREWARD

This *Biosafety Manual* has been developed by the Office of Regulatory Affairs and the Institutional Biosafety Committee (IBC) at the University of Nebraska Medical Center (UNMC). The manual is part of the UNMC / University of Nebraska at Omaha (UNO) and Nebraska Medicine Biosafety Program instituted to accomplish the following goals to:

- protect personnel from exposure to infectious agents,
- prevent environmental contamination,
- provide an environment for high quality research while maintaining a safe work place,
- comply with applicable federal, state and local requirements, and
- create a secure laboratory environment to prevent unauthorized utilization of the biological agent.

The *Biosafety Manual* provides university-wide safety guidelines, policies and procedures for the use and manipulation of biohazards. Although the implementation of these procedures is the responsibility of the Principal Investigator (PI), its success depends largely on the combined efforts of laboratory supervisors and employees. Planning for and implementation of biological safety must be part of every laboratory activity in which biohazardous materials are used.

Recommendations in the Manual define a "standard of practice" that laboratories should follow.

In general, the handling and manipulation of biological agents and toxins, including recombinant DNA molecules, requires the use of various precautionary measures depending on the material(s) involved. This manual will provide assistance in the evaluation, containment and control of these biohazards. All parties involved or working with these materials should be familiar with the contents of this manual and must complete the required training. The IBC Chairperson and the Biosafety Officer (BSO) are available at UNMC to provide additional advice when requested.

SECTION 1: INTRODUCTION

A. BIOSAFETY PROGRAM ADMINISTRATION

The rules and procedures set forth in the *Biosafety Manual* have two major purposes: (1) to protect students, employees, and others against unnecessary and potentially harmful biohazardous materials exposure and (2) to provide for an atmosphere of biosecurity on campus. For these rules and procedures to be effective, a structured administrative format is important to have in place to define the roles and responsibilities of each person or administrative office.

1. Chancellor

The Chancellors are ultimately responsible for assuring that comprehensive campus-wide programs are in place for the safe handling of all biohazardous materials at UNMC/UNO/Nebraska Medicine campuses (hereafter referred to as "the Institution").

2. Institutional Biosafety Committee (IBC)

The IBC has been charged by Federal law with the planning and implementation of the campus Biosafety Program with a purpose to ensure the health and safety of all personnel working with biohazardous materials. At this Institution, membership on the IBC is appointed by the UNMC Associate Vice Chancellor for Academic Affairs and consists of the Chairperson, faculty, and community representatives. Two community members, with no Institutional affiliation other than membership on the IBC, are required and appointed to represent the interest of the surrounding community with respect to health and the protection of the environment. The Associate Vice Chancellor of Academic Affairs is an *ex officio* member. The IBC as a whole represents collective expertise and research experience in biohazardous materials and biosafety in experiments that may pose potential risks to health or the environment.

The IBC is responsible for ensuring that research conducted at the Institution is in compliance with the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) and the *Select Agent Rule*, drafting campus biosafety policies and procedures, and reviewing individual research proposals for biosafety concerns. Although the IBC does provide review for IBC protocols that originate with researchers at The Nebraska Medical Center, the IBC does not oversee specific patient care biosafety practices associated with the hospitals, clinics, or clinical laboratories. These are the responsibility of the Infection Control Committee.

Principal Investigators (PIs), who wish to perform research using biohazardous materials, must submit an application to the IBC (see IBC web site at http://www.unmc.edu/ibc for the application process and forms needed). The full Committee reviews all applications. Studies that involve work at Biological Safety Level (BSL) 2 or BSL3 containment are pre-reviewed by a primary and secondary reviewer. Research involving wild type BSL-1 organisms, as defined by the *CDC/NIH Guidelines on Biosafety in Microbiological and Biomedical Laboratories (BMBL)* manual, also requires an application to the IBC for approval. However, this approval may be granted by the full Committee following pre-review by the IBC Chairperson only. All reviews include an assessment of the a) containment levels required by the *NIH Guidelines* for the proposed research, b) laboratory facilities procedures and practices, and c) training and expertise of personnel involved in the research activity.

The IBC is authorized by the Chancellor to limit or suspend any research that does not comply with biosafety policies and procedures set forth in this *Biosafety Manual* and in the *NIH Guidelines*. Additionally, noncompliance with basic procedures as determined during the laboratory inspection may result in the halting of research until corrective action is taken. The BSO will consult with the IBC Chairperson and/or the Associate Dean for Research and Regulatory Affairs or designee to determine if non-compliance to established procedures poses a threat to public health.

3. Biosafety Officer (BSO)

The BSO duties include, but are not necessarily limited to, providing technical advice and training to the IBC and researchers on laboratory containment concerning biosafety and biosecurity procedures and for developing emergency plans for the handling of spills and personnel contamination. The BSO will oversee BSL-2 and BSL-3 laboratory inspections to ensure that established safety standards are rigorously maintained (see IBC web page for the laboratory inspection template) and the Institutional training program for biosafety. The BSO is also an Alternate Responsible Official (ARO) for the campus Select Agent Program and interacts with the Responsible Official (RO) to ensure that the requirements of 42 CFR Part 73 entitled, "Possession, use, and transfer of select agents and toxins rule" are met on behalf of the Institution.

4. Departmental Chairperson

The Departmental Chairperson is responsible for providing support to researchers which ensures that appropriate facilities are available to contain biohazardous materials and to enable the PI to comply with pertinent campus policies. The Chairperson is responsible for assuring that the PI has the training that is commensurate with the proposed project and that the project design and monitoring methods meet institutional safety standards.

4. Principal Investigator (PI)

The Principal Investigator (PI) is directly responsible for assuring that all laboratory personnel follow the institutional safety policies and procedures and that the laboratory is operated in a safe manner. His or her knowledge and judgement are critical in assessing risks and appropriately applying the biosafety guidelines. The PI shall have available in the laboratory a biosafety manual containing generalized and specific information for laboratory personnel.

B. BIOLOGICAL SAFETY AND BIOSAFETY LEVELS

Biological safety or biosafety is defined as **the development and implementation of administrative policies, work practices, facility design, and safety equipment to prevent transmission of biologic agents to workers, other persons, and the environment**. Biosafety defines the containment conditions under which infectious agents can be safely manipulated. The objective of containment is to confine biohazards and to reduce the potential exposure of the laboratory worker, persons outside of the laboratory, and the environment to potentially infectious agents. Containment can be accomplished through the following means:

Primary Barriers:

Protection of personnel and the immediate laboratory environment using good *microbiological technique* (*laboratory practice*) and using appropriate *safety equipment.*

Secondary Barriers:
Protection of the environment external to the laboratory from exposure to infectious materials through a combination of *facility design* and *operational practices.*

Combinations of laboratory practices, safety equipment, and special laboratory design can be made to achieve different levels of physical containment. Currently four Biosafety Levels (1-4) define the level of containment necessary to protect personnel and the environment. A Biosafety Level 1 (BSL-1) is the least restrictive, while Biosafety Level 4 (BSL-4) requires a special containment laboratory or facility, which is not available at the Institution. Most of the research at the Institution is conducted at Biosafety Levels 1 and 2 with a limited number of experiments at BSL-3. This manual will mainly focus on these three Biosafety Levels. For more information on BSL-4 requirements, refer to resources on the UNMC IBC web page or contact the BSO. A generalized summary of the different biosafety level requirements is shown below.

	Biosafety Level 1 (BSL-1)
Agents:	Not known to cause disease in healthy adult humans.
Practices:	Standard microbiological practices.
Safety Equipment: (Primary barriers)	None required.
Facilities: (Secondary barriers)	Open bench top with sink available.
	Biosafety Level 2 (BSL-2)
Agents:	Moderate risk agents that are present in the community and associated with human disease of mild to moderate severity.
Practices:	BSL-1 practice plus limited access, biohazard warning signs, "sharps" precautions, and a SOP defining any needed waste decontamination or medical surveillance policies.
Safety Equipment: (Primary barriers)	Primary barriers include a Class I or II Biological Safety Cabinet (BSC) or other physical containment devices used for the manipulation of agents that cause splashes or aerosols of infectious materials; Personal Protective Equipment (PPEs) including laboratory coats, gloves, face and eye protection as needed.
Facilities: (Secondary barriers)	BSL-1 plus the availability of an autoclave for decontamination.

	Biosafety Level 3 (BSL-3)
Agents:	Indigenous or exotic agents with a potential for aerosol transmission; and which may cause serious or potentially lethal infection.
Practices:	BSL-2 practice plus controlled access, decontamination of all waste, and decontamination of lab clothing before laundering.
Safety Equipment: (Primary barriers)	Primary barriers include a Class II BSC or other physical containment device used for the manipulation of agents, PPE to include protective lab clothing, gloves, face and eye protection, and respiratory protection as needed.
Facilities: (Secondary barriers)	BSL-2 plus physical separation from access corridors, self-closing and double door access, exhausted air not recirculated with negative airflow into laboratory

The most important element in maintaining a safe work environment is strict adherence to good microbiological and laboratory practices and techniques. Everybody working with infectious agents or potentially infected materials must be aware of the potential risks. In addition, they must be trained and proficient in the practices and techniques required for handling such material. T**he PI or designated person in charge of the laboratory is responsible to make sure that all people working in the laboratory have the proper training.** A web-based biosafety-training program is available and required for all individuals working within the research laboratory (refer to the UNMC IBC web site for access to the training program).

C. BIOSECURITY

Biosecurity is defined **as protection of high-consequence microbial agents and toxins, or critical relevant information against theft or diversion by those who intend to pursue intentional misuse.** The following biosecurity issues should be considered by all laboratories handling biohazardous agents:

- risk and threat assessment
- facility security plans
- physical security
- data and electronic technology systems
- security policies for personnel
- policies regarding accessing the laboratory and animal areas
- specimen accountability
- receipt of agents into the laboratory
- transfer or shipping of biohazardous agents from the laboratory
- emergency response plans
- reporting of incidents, unintentional injuries, and security breaches

As part of a biosecurity program and to comply with Federal legislation, special procedures are

required for the possession and transfer of specific biohazardous agents called Select Agents (see **Appendix A** for a listing of these agents).

As part of the Institutional biosecurity program, the PI should address the following issues in the conduct of research activities: (1) personnel suitability and reliability (including student access), (2) pathogen accountability (both on-site and through the transfer process), and (3) response to biosecurity incidences. Refer to **Appendix B**, USA PATRIOT Act for more information concerning biosecurity.

D. CLASSIFICATION OF INFECTIOUS AGENTS ON THE BASIS OF HAZARD

Worldwide there are several systems for classifying human and animal pathogens according to the hazard they present to an individual and the community. Although these classifications differ from each other, they all are based on the notion that some microorganisms are more hazardous than others. In general, the pathogenicity of the organism, mode of transmission, host range, availability of effective preventive measures and/or effective treatment are some of the criteria taken into consideration when classifying infectious agents. In the U.S., the most current classification is found in the *NIH Guidelines*. The human etiologic agents addressed in these guidelines are classified into four risk groups with **Risk Group 1** (**RG-1**) of low or no hazard and **Risk Group 4** (**RG-4**) representing highly infectious agents:

Basis for the Classification of Biohazardous Agents by Risk Group

Risk Group	Risk to individual and the community
RG-1	Agents that are not associated with diseases in healthy adult humans.
RG-2	Agents that are associated with human diseases which are rarely serious and for which preventive or therapeutic interventions are often available.
RG-3	Agents that are associated with serious or lethal human diseases for which preventative or therapeutic interventions may be available (high individual risk but low community risk)
RG-4	Agents that are likely to cause serious or lethal human diseases for which preventative or therapeutic interventions are not usually available (high individual risk and high community risk)

A comprehensive list of Risk Group 1, 2, 3, and 4 agents can be found in Appendix B of the *NIH Guidelines*. It is important to realize however that none of the lists is inclusive. In addition, those agents not listed in RG-2, RG-3, and RG-4 are not automatically classified in RG-1. Those unlisted agents need to be subjected to a risk assessment based on the known and potential properties of the agents and their relationship to agents that are listed.

Risk Groups = Biosafety Level?
Determining the risk group of a biological agent is part of the biosafety risk assessment and helps in assigning the correct biosafety level for containment. In general, RG-2 agents are handled at BSL-2, and RG-3 agents at BSL-3 containment. However, the use of certain RG-2 agents in large quantities might require BSL-3 conditions, while some RG-3 agents may be safely manipulated at a BSL-2 under certain conditions. For more information, contact the BSO or the IBC Chairperson.

E. BIOHAZARDOUS MATERIALS

Biohazardous materials are defined as materials of biological origin that have the capacity to produce deleterious effects on humans or animals including:

i) recombinant DNA molecules
ii) micro-organisms containing recombinant DNA molecules
iii) micro-organisms classified as risk group 1 (RG-1) non-exempt, RG-2, RG-3, or RG-4
iv) biological products derived from RG-1 (non-exempt), RG-2, RG-3, or RG-4 microorganisms (Note: Use of commercially produced non-infectious reagents other than toxins from non-exempt microorganisms does not require review and/or registration to the IBC.)
v) diagnostic specimens used in research known or reasonably expected to contain pathogens in RG-1 (non-exempt), RG-2, RG-3, or RG-4
vi) clinical/medical waste used in research derived from the medical treatment of humans.

All studies using RG-2 and RG-3 biohazardous materials must undergo IBC review. These experiments must be reviewed and approved by the full IBC prior to the initiation of experiments. Some experiments using RG-1 organisms are required to be registered with the IBC. Refer to the **Appendix C** of this manual for examples of recombinant DNA research that is exempt. Some experiments using RG-1 organisms are exempt and thus full board review by the IBC is not required. Refer to the UNMC policy #IBC35, Registration Process of Use of Exempt Recombinant DNA, on how to register those experiments that are exempt i.e., they do not require full board review with the IBC.

A sub-category of biohazardous agents referred to as Select Agents are defined as biological select agents and toxins (BSAT) as regulated by the Department of Health and Human Services and/or the USDA. These agents require special procedures for transfer and possession. Contact the UNMC BSO or campus RO for further information concerning these biohazardous agents.

F. HUMAN GENE TRANSFER EXPERIMENTATION

Human gene transfer continues to raise safety, ethical, and scientific issues in need of public discussion and analysis. *NIH Guidelines* Section III-C describes "Experiments that Require Institutional Biosafety Committee (IBC) and Institutional Review Board Approvals and Recombinant DNA Advisory Committee (RAC) Review Before Research Participant Enrollment". In this section is listed one subsection entitled, "Experiments Involving the Deliberate Transfer of Recombinant DNA, or DNA or RNA Derived from Recombinant DNA, into One or More Human Research Participants" (Section III-C-1). The general process to obtain approval for human gene transfer experimentation is to submit for review the following: (1) RAC [adhere to the guidelines for submission outlined in Appendix M, "Points to Consider in the Design and Submission of Protocols

for the Transfer of Recombinant DNA Molecules into One or More Human Research Participants"], (2) IBC, and (3) IRB.

For human gene transfer experimentation, it is the responsibility of the IBC to ensure that:
1. a RAC review has been conducted.
2. all issues raised by the RAC in a summarization letter to the PI and the sponsor have been considered.
3. no participant is enrolled until RAC review has been completed and IBC and IRB approval have been obtained.

(Refer to the IBC web page for additional information concerning human gene transfer research.)

G. POSSESSION, USE, AND TRANSFER OF SELECT AGENTS AND TOXINS

All laboratories possessing, using, and/or transferring biological select agents and toxins (BSATs) must adhere to the guidelines as established in the 42 CFR Part 73 federal regulations (refer to the IBC web page for a link to these regulations). These regulations contain detailed information pertaining to laboratory registration, personnel security risk assessment, personnel suitability assessments, safety plans, security plans, emergency response plans, training, record keeping, inspections, and notifications. Any laboratory possessing, using, and/or transferring a select agent and/or toxin must contact the BSO or the Institution RO before research activities are considered. The RO is ultimately responsible to ensure that the requirements of the 42 CFR Part 73 regulations are met on behalf of the Institution.

H. RULES, REGULATIONS, AND GUIDELINES

The following is a brief summary of the regulatory authorities that either regulate or provide guidelines for the use of biological materials, infectious agents and recombinant DNA molecules. Copies of these documents are available by access to the appropriate website.

1. *National Institute of Health (NIH): Guidelines for Research Involving Recombinant DNA Molecules*, (http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines) These guidelines address the safe conduct of research that involves construction and handling of recombinant DNA (rDNA) molecules and organisms containing them. In 1974, a recombinant DNA Advisory Committee (RAC) was established to determine appropriate biological and physical containment practices and procedures for experiments that potentially posed risks to human health and the environment. Because of the committee's activity, the initial version of the *NIH Guidelines* was published in 1976. This guideline has been amended and revised many times since then. Included in the *NIH Guidelines* is a requirement for the institution to establish an Institutional Biosafety Committee (IBC) with authority to approve or disapprove proposed rDNA research using the *NIH Guidelines* as a minimum standard.

2. *Centers for Disease Control and Prevention (CDC) and the National Institute of Health (NIH) Guidelines on Biosafety in Microbiological and Biomedical Laboratories,* 5th Edition, 2009 (BMBL manual) (http://www.cdc.gov/biosafety/publications/bmbl5/index.htm). In 1984, the CDC/NIH published the first edition of the BMBL Manual. This document describes combinations of standard and special microbiological practices, safety equipment, and

facilities that constitute Biosafety Levels 1-4, which are recommended for working with a variety of infectious agents in various laboratory settings. This manual has been revised several times and is commonly seen as the standard for biosafety.

3. *Occupational Safety and Health Administration: Bloodborne Pathogens Standard* (http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051). In 1992, the Occupational Safety and Health Administration (OSHA) promulgated a rule to deal with the occupational health risk caused by exposure to human blood and other potentially infectious materials. OSHA's rule includes a combination of engineering and work practice controls, personal protective clothing and equipment, training and medical follow-up of exposure incidents, vaccination, and other provisions.

4. *Department of Health and Human Services (HHS): Select Agent Rule* (http://www.selectagents.gov/Regulations.html). In 1996, HHS published a set of rules that require facilities and institutions to be registered and approved in order to transfer or receive certain biological agents and toxins. HHS requires UNMC to comply with the BMBL manual (see above), OSHA's Laboratory Safety Standard 29 CFR 1910.1450, and 42 CFR Part 73. A copy of the most current list of restricted agents and toxins covered under this rule is included in **Appendix A**. Also, refer to the USA PATRIOT Act of 2001 for additional information concerning the possession of BSATs **(Appendix B).**

5. Packaging, Shipping, and Transportation
Packaging, shipment and transportation requirements for infectious substances, diagnostic specimens and biological products are addressed in the following rules and guidelines:

United Nations
Recommendations of the Committee of Experts on the Transportation of Dangerous Goods

International Civil Aviation Organization (ICAO)
Technical Instructions for the Safe Transport of Dangerous Goods by Air

International Air Transport Association (IATA)
Dangerous Goods Regulations
http://www.iata.org

U.S. Department of Transportation
49 CFR Part 72
http://www.access.gpo.gov/cgi-bin/cfrassemble.cgi?title=200649

U.S. Public Health Service
42 CFR Part 72

U.S. Postal Service
39 CFR Part 111

U.S. Department of Labor, OSHA
29 FR 1910.1030

6. Importation and Transportation Permits

Importation permits are required for infectious agents, biological materials and animals as outlined in U.S. Public Health Service, 42 CFR Part 71, *Foreign Quarantine*. In addition, the Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) requires permits for importation and transportation of controlled materials, certain organisms or vectors. This includes animal and plant pathogens, certain tissue cultures and live animals. APHIS also regulates the importation, interstate movement, or environmental release of genetically engineered organisms as regulated under 7 CFR Part 340.

7. Dual Use Research of Concern

Dual Use Research of Concern (DURC) is research that, based on current understanding, can be reasonable anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health and safety, agricultural crops and other plants, animals, the environment, or materiel. Scientists have a professional responsibility to: understand dual use research issues and concerns, be aware of the implications of their work and the various ways in which information and products from their work could be misused, and take steps to minimize misuse of their work. The campus IBC has been tasked to review all biological research for the potential to be DURC and will consult with the PI when the potential arises that DURC is possible. Additional information can be found on the NIH website at:
http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/dual-use-research-concern

SECTION 2: PRACTICES AND PROCEDURES

A. ROUTES OF INFECTION

Working in a biological research environment, it is reasonable to expect that a laboratory person working with infectious materials is more likely to become infected than members of the general community. An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route and overcome the body's defense system. The following routes of infection have been reported for laboratory-acquired infections:

1. Through the mouth by:
   -eating, drinking and smoking in the laboratory
   -mouth pipetting
   -transfer of microorganisms to the mouth by contaminated fingers or articles
2. Through the skin by:
   -accidental inoculation with a hypodermic needle, other sharps instrument or glass
   -cuts or scratches
3. Through the eye by:
   -splashes of infectious material into the eye
   -transfer of microorganisms to the eye by contaminated fingers
4. Through the lungs by:
   -inhalation of airborne microorganisms

The general laboratory procedures outlined in this manual provide for guidance in handling infectious or potentially infectious materials.

B. ADMINISTRATIVE CONTROLS

Biohazard Warning Sign
A biohazard label is required for all areas or equipment in which RG-2 or RG-3 agents are handled or stored or where BSL-2 or BSL-3 procedures are required. The appropriate place for posting the label is at the main entrance door(s) to laboratories and animal rooms, and on equipment such as refrigerators, incubators, transport containers, and/or lab benches.

Training
Good microbiological and laboratory practices are essential for a safe work environment. Training and education on these practices and procedure needs to start at the undergraduate level. All personnel working with RG-1, RG-2 or RG-3 agents are required to receive laboratory specific training from the PI or laboratory supervisor. In addition, all personnel listed on active IBC protocols must complete the web-based General Biosafety training provided by the Institution. Specific training in BSL-3 practices and/or the utilization of BSATs may also be assigned based on the protocol needs. Training should include at a minimum:

- good laboratory and animal use practices as applicable
- site specific information on risks, hazards and procedures
- laboratory or environment specific BSL-2 or BSL-3 procedures as applicable

Bloodborne Pathogen (BBP) Program
In accordance with OSHA requirements, UNMC has established an Exposure Control Plan covering the potential exposure to bloodborne pathogens (e.g., HIV, Hepatitis B virus) found in human blood, serum and tissue, as well as in other potentially infectious materials. BBP training is required on an annual basis and available through a University sponsored web-based training program.

Recombinant DNA Program
All research at the Institution involving recombinant DNA, independent of the funding source, needs to be in compliance with the requirements of the *NIH Guidelines* and is subject to IBC review and approval.

CDC Select Agents Requirements
The Centers for Disease Control and Prevention (CDC) mandates specific requirements for facilities transferring or receiving certain infectious BSATs (*HHS: Additional Requirements for Facilities Transferring or Receiving Select Agents*). A list of these restricted agents is included in **Appendix A**. The RO will function as the Responsible Facility Official for the transfer of these BSATs from different institutions by the PI. . Please contact the RO for more information. In addition to these requirements for the possession and handling of BSATs , all researchers on campus must adhere to the requirements of the federal law, Uniting and Strengthening America by Providing Appropriate Tools Required to Intercept and Obstruct Terrorism Act of 2001 (USA PATRIOT Act). The requirements of this law are summarized in **Appendix B**

Institutional Biosafety Committee (IBC)
The IBC gives oversight on all projects involving biohazardous agents (RG-1 non-exempt, RG-2, and RG-3) and certain toxins at the campuses.

C. ENGINEERING CONTROLS

Biological Safety Cabinets (BSCs)
BSCs are designated to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets (BSCs), designated as Class I, II and III have been developed to meet various research and clinical needs. BSCs use high efficiency particulate air (HE PA) filters in their exhaust and/or supply systems. BSCs must not be confused with other Laminar flow devices or "clean benches": in particular, horizontal flow cabinets that direct air towards the operator. Clean benches should <u>never</u> be used for handling infectious, toxic or sensitizing materials.

Laboratory personnel must be trained in the correct use and maintenance of BSCs to ensure that personnel and product protection (where applicable) are maintained. Before selecting any biosafety cabinet for purchase, contact the Biosafety Officer for guidance. The Senior Mechanical Engineer (9-3679) should be contacted for issues pertaining to certification and maintenance.

1. Class I Biological Safety Cabinet
This is a ventilated cabinet for personnel protection with an unrecirculated inward airflow away from the operator. This unit is fitted with a HEPA filter to protect the environment from discharged agents. The Class I BSC is suitable for work involving low to moderate risk

agents, where there is a need for containment, but not for product protection (e.g., sterility).

2. Class II Biological Safety Cabinet

This is a ventilated cabinet for personnel, product and environmental protection that provide inward airflow and HEPA-filtered supply and exhaust air. The Class II BSC has four designs depending on how much air is recirculated and/or exhausted and if the BSC is hard-ducted to the ventilation system or not. Most laboratories need only a Class II, A1 (not exhausted) or a Class II, A2 (thimble connected exhaust) for the work they perform. A Class II, B2 hard ducted hood is rarely needed and should only be considered under unique circumstances. Contact the BSO for additional guidance for selecting a BSC.

3. Class III Biological Safety Cabinet

The Class III BSC is a totally enclosed ventilated cabinet which is gas-tight, and maintained under negative air pressure (0.5 inches water gauge). The supply air is HEPA-filtered and the exhaust air has two HEPA filters in series. Work is performed in the cabinet by the use of attached rubber gloves.

Negative Pressure Rooms

All laboratories where a ducted exhaust air ventilation system is provided and where the directional airflow draws air into the laboratory i.e. negative pressure, must have a method to monitor whether or not the direction of airflow is proper. It is recommended that a visual and audio monitoring device that indicates and confirms directional inward airflow be provided at the lab entry.

In special containment laboratory areas (BSL-3 labs and autopsy suites), a quantitative electronic monitoring of the air flow should be conducted at least annually to test for proper operation.

Exhaust systems with HEPA filters require a mechanism to monitor proper functioning of the filter to determine when replacement is needed.

Other Safety Equipment

1. Safety Showers

Safety showers provide an immediate water drench of an affected person. Standards for location, design and maintenance of safety showers are available from Facilities Maintenance.

2. Eyewash Stations

Eyewash stations are required in all laboratories where injurious or corrosive chemicals are used or stored and where employees perform tasks that might result in splashes of potentially infectious materials.

3. Ventilation Controls

Ventilation controls are those controls intended to minimize employee exposure to hazardous chemicals and infectious or toxic substances by removing air contaminants from the work site.

D. PERSONAL PROTECTIVE EQUIPMENT (PPE)

PPE is used to protect personnel from contact with hazardous materials and infectious agents.

Appropriate clothing may also protect the experiment from contamination. Personal protective devices and safety equipment must be provided to all employees under the appropriate circumstances and employees have the responsibility of properly using the equipment. The following PPE is recommended for regular use:

1. Face Protection

Splash goggles or safety glasses with solid side shields in combination with masks, or chin length face shields or other splatter guards are required for anticipated splashes, sprays or splatters of infectious or other hazardous materials to the face

2. Laboratory Clothing

This category includes laboratory coats, smocks, scrub suits, and gowns. Long-sleeved garments should be used to minimize the contamination of skin or street clothes. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect clothing from contamination. If the garment is not disposable, it must be capable of withstanding sterilization in the event it becomes contaminated. At a minimum, a laboratory coat should be worn in all laboratories working at a BSL-2. Additional criteria for selecting clothing are: comfort, appearance, closure types and location, antistatic properties and durability. Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas. Disposables should be available for visitors, maintenance and service workers in the event it is required. All protective clothing should be either discarded in the laboratory or laundered by the Institution. Personnel must not take laboratory clothing home.

3. Gloves

Gloves must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with biohazards, toxic substances, hazardous chemicals and other physically hazardous agents. Temperature resistant gloves must be worn when handling hot material or dry ice. Delicate work requiring a high degree of precision dictates the use of thin walled gloves. Protection from contact with toxic or corrosive chemicals may also be required; appropriate gloves must be used for the chemical(s) in question Powdered and non-powdered latex gloves should not be used at the Institution.

4. Respirators

For certain protocols and projects, additional PPE such as respiratory protection may be required. Respirator selection is based on the hazard and the protection factor required. Fit testing for N-95 HEPA-filtered respirators is available through Employee Health (552-3563) located in the south Doctors Building, 6th Floor Suite 600. All personnel utilizing a BSL-3 containment facility must have a passing "Fit Test Report" on file; annual fit-testing is required.

E. RECOMMENDED WORK PRACTICES

Pipettes and Pipetting Aids

Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used. Confine pipetting of biohazardous or toxic fluids inside a BSC if possible. If pipetting is done on the open bench, use absorbent pads or paper on the bench. Use the following precautions should be followed.

- Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.
- Biohazardous materials should not be forcibly discharged from pipettes. Use "to deliver pipettes rather than those requiring "blowout."
- Do not discharge biohazardous material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
- Place contaminated reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them.
- Autoclave the pan and pipettes as a unit before processing them for reuse.
- Discard contaminated Pasteur pipettes in an appropriate size sharps container.
- When work is performed inside a BSC, all pans or sharps containers for contaminated glassware should be placed inside the cabinet as well while in use.
- Disposable pipettes and pipette tips are placed into a hard-walled container and disposed as biohazardous waste (whether contaminated with a biohazardous agent or not).

Syringes and Needles

Syringes and hypodermic needles are dangerous objects that need to be handled with extreme caution to avoid accidental injection and aerosol generation. Generally, the use of syringes and needles should be restricted to procedures for which there is no alternative. Do not use a syringe and needle as a substitute for a pipette.

Use needle locking syringes or disposable syringe-needle units in which the needle is an integral part of the syringe. When using syringes and needles with biohazardous or potentially infectious agents:

- Work in a BSC whenever possible.
- Wear gloves.
- Fill the syringe carefully to minimize air bubbles.
- Expel air, liquid and bubbles from the syringe vertically into a cotton pad moistened with a disinfectant.

Needles should not be bent, sheared, replaced in the sheath or guard (capped), or removed from the syringe following use. If it is essential that a contaminated needle be recapped or removed from a syringe, the use of a mechanical device or the one-handed scoop method must be used. Always dispose of needle and syringe unit promptly into an approved sharps container. Do not overfill sharps containers (2/3 filled = full) before discarding.

Cryostats

Frozen sections of unfixed human or animal tissue infected with an etiologic agent pose a risk because accidents can occur. Freezing tissue does not necessarily inactivate infectious agents. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material. Gloves should be worn during preparation of frozen sections. When working with biohazardous material in a cryostat, the following is recommended:

- Consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% ethanol or any other disinfectant suitable for the agent(s) in use.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination,
- Defrost and decontaminate the cryostat with a tuberculocidal hospital type disinfectant once a week and immediately after tissue known to contain bloodborne pathogens, *M. tuberculosis*

or other infectious agents is cut.

- Handle microtone knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
- Consider solutions for staining potentially infected frozen sections to be contaminated.

Centrifuge Equipment

Hazards associated with centrifuging include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users should be properly trained and operating instructions including safety precautions should be prominently posted on the unit. Aerosols are created by practices such as filling centrifuge tubes, removing supernatant and re-suspending sediment pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging biohazardous material, the following procedures should be followed:

- Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, 0- rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Fill and open centrifuge tubes, rotors and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Add disinfectant to the space between the tube and the bucket to disinfect material in case of breakage during centrifugation.
- Always balance buckets, tubes and rotors properly before centrifugation.
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters.
- Work in a BSC when resuspending sediment material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Small low - speed centrifuges may be placed in a BSC during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatigue resulting in disintegration of rotors and to use proper cleaning techniques and centrifuge components. Manufacturer's recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.
- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, appropriate chemical disinfectants are necessary for decontamination.

Blenders, Ultrasonic Disrupters, Grinders and Lyophilizes

The use of any of these devices results in considerable aerosol production. Blending, cell-disrupting and grinding equipment should be used in a BSC when working with biohazardous materials.

Safety Blenders

Safety blenders, although expensive, are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation, and to with stand sterilization by

autoclaving. If blender containers are not leak-proof, they should be tested with sterile saline or dye solution prior to use with biohazardous material. The use of glass blender jars is not recommended because of the breakage potential. If they must be used, glass jars should be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. A towel moistened with disinfectant should be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle. The device should be decontaminated promptly after use.

Lyophilizer and Ampoules

Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible.

Opening ampoules containing liquid or lyophilized infectious culture material should be performed in a BSC to control the aerosol produced. Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant soaked towel, hold the ampoule upright and snap it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the container. Discard the towel and ampoule top and bottom as biohazardous waste.

Ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries and exposure to the infectious agent. The use of polypropylene tubes eliminates this hazard. These tubes are available dust free or pre-sterilized and fitted with polyethylene caps with silicone washers.

Loop Sterilizers and Bunsen Burners

Sterilization of inoculating loops or needles in an open flame generates small particle aerosols which may contain viable microorganisms. The use of a shielded electric incinerator or hot bead sterilizers minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available or recommended.

Continuous flame gas burners should not be used in BSCs. These burners can produce turbulence that disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter.

Laundry

All personal protective clothing must be cleaned, laundered and disposed of by the employer at no cost to employees. Apparel contaminated with human blood or other potentially infectious materials should be handled as little as possible and needs to be collected in special hamper (labeled or color coded) or in biohazard bags. Materials containing a biohazardous agent that could drip or those contaminated with a RG-3 agent should be decontaminated by steam sterilization. The decontaminated materials should subsequently be sent to Linen Services for cleaning. All other materials can be placed in biohazard bags and given to the laundry for cleaning prior to decontamination.

Housekeeping

Good housekeeping in laboratories is essential to reduce risks and protect the integrity of biological experiments. Routine housekeeping must be relied upon to provide work areas free of significant sources of contamination. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.

Laboratory personnel are responsible for cleaning laboratory benches, equipment and areas that require specialized technical knowledge. Additional laboratory housekeeping concerns include:

- Keep the laboratory neat and free of clutter. Surfaces should be clean and free of infrequently used chemicals, glassware and equipment. Access to sinks, eyewash stations, emergency showers and exits, and fire extinguishers must not be blocked.
-
- Proper disposal of chemicals and wastes. Old and unused chemicals should be disposed of promptly and properly.
- Providing a workplace that is free of physical hazards. Aisles and corridors should be free of tripping hazards. Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment and avoidance of the creation of electrical hazards in wet areas.
- All laboratory equipment needs to be cleaned and certified of being free of hazards before being released for repair or maintenance.

F. BIOLOGICAL RISK ASSESSMENT

The assessment of risk is an essential element of safety in the laboratory. For most situations, guidelines and regulations have clearly defined the procedures and practices to be followed in order to achieve safety in the work place. However, a newly isolated agent or toxin, or a new procedure never before employed needs further evaluation. Questions concerning the appropriate safety equipment, training and waste disposal need to be addressed, as well as safe procedures and practices. Something is considered safe if the risk associated with it is judged acceptable. However, since individual judgement involves both personal and social values, opinions on what is safe or not can vary significantly. In order to find a common ground for an acceptable *risk* assessment, the "rule of reason" needs to be applied. Refer to **Appendix D** for additional information on performing a risk assessment. Some general factors to consider include:

1. **Custom of usage (or prevailing professional practice)**
   Many laboratory procedures involve the maintenance of sterility and cleanliness. These procedures are commonly considered safe, since adverse effects would have been obvious over time. However, because a procedure has been used for many years does not necessarily imply that it is safe. The best example is mouth pipetting, which was used for centuries and finally considered a very dangerous procedure and habit.

2. **Best available practice, highest practicable protection, and lowest practicable exposure**
   It should be common practice in the laboratory to use the best available procedures with the highest level of protection. This not only provides for a safe work environment but also fosters excellence in scientific conduct.

3. **Degree of necessity or benefit**
   The common question to ask is, are the benefits worth the risk? There is no need to use a human pathogen causing severe gastroenteritis in a teaching laboratory when principal microbiological practices can be taught with an organism that is not considered to be infectious.

4. **No detectable adverse effects**
   This can be a very weak criterion since it involves uncertainty or even ignorance.

5. **Principal knowledge**
   Many times, existing procedures are modified, which involve the same or similar biological agents. For that reason, similar safety procedures should be applied. If new agents are isolated, an assessment of what is known about the close relatives is done. Many agents of known etiologic character are already categorized in risk groups allowing for the selection of the appropriate biosafety level. New isolates from infected animals or humans with known infectious relatives warrant at a minimum the same level of protection.

Taking the above-mentioned factors, as well others into consideration will allow for a reasonable approach to a new challenge. The IBC is available to assist in this process and should be contacted for questions concerning biological safety. Once a risk assessment is completed, the results should be communicated to everyone involved in the process. Written standard operating procedures (SOPs) should be established and communicated with all personnel within the laboratory.

H. WORKING WITH TISSUE CULTURES

When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line can be classified at the same RG level as that recommended for the agent. The CDC and OSHA recommend that all cell lines of human origin be handled at BSL-2 level.

Cell lines which are non-primate or are of normal primate origin, which do not harbor a primate virus, which are not contaminated with bacteria or fungi and which are well established, may be considered Class I cell lines and handled at a BSL-1. Appropriate tests should confirm this assessment.

Primate cell lines derived from lymphoid or tumor tissue, all cell lines exposed to or transformed by a primate oncogenic virus, all clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy), all primate tissue, all cell lines new to the laboratory (until shown to be free of all adventitious agents) and all virus and *Mycoplasma*-containing primate cell lines are classified as RG-2 and should be handled at a BSL-2.

I. PREVENTING THE TRANSMISSION OF TUBERCULOSIS

Since 1985, the incidence of tuberculosis in the United States has been increasing steadily, reversing a 30-year downward trend. Recently, drug resistant strains of M*ycobacterium tuberculosis* have become a serious concern. Outbreaks of tuberculosis, including drug resistant strains, have occurred in healthcare environments. Several hundred employees have become infected after workplace exposure to tuberculosis, requiring medical treatment. A number of healthcare workers have died.

In December 2005, the CDC published *Guidelines for Preventing the Transmission of Tuberculosis in Health-Care Setting, 2005 (*MMWR., 54[No.RR-17]). The guidelines contain specific information on ventilation requirements, respiratory protection, medical surveillance and training for those personnel who are considered at risk for exposure to tuberculosis. Propagation and/or manipulation of *M. tuberculosis* and *M. bovis* cultures in the laboratory or animal room must be performed at BSL-3 and require IBC approval.

J. CLINICAL LABORATORY GUIDELINES

Clinical laboratories receive clinical specimens with requests for a variety of diagnostic services. The infectious nature of this material is largely unknown. In most circumstances, the initial processing of clinical specimens and identification of microbial isolates can be done safely at BSL-2. A primary barrier, such as a BSC, should be used:

- when it is anticipated that splashing, spraying or splattering of clinical materials may occur,
- for initial processing of clinical specimens where it is suggested that an agent transmissible by infectious aerosols may be present (e.g., *M. tuberculosis),*
- to protect the integrity of the specimen

All laboratory personnel who handle human source materials are included in the *Bloodborne Pathogens Program* as outlined in UNMC/NEBRASKA MEDICINE *Exposure Control Plan.* **"Universal Precautions"** need to be followed when handling human blood, blood products, body fluids or tissues.

The segregation of clinical laboratory functions and restricting access to specific areas is the responsibility of the Laboratory Director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented. Additional recommendations specific for clinical laboratories may be obtained from the Clinical Laboratory Standards Institute (CLSI).

K. USE OF ANIMALS IN RESEARCH AND TEACHING

The use of animals in research and teaching is subject to state and federal laws and guidelines. Policy specifies that:

- All animals under the sponsorship of the Institution will be treated humanely;
- Prior to their inception, all animal projects receive approval by the Institutional Animal Care and Use Committee (IACUC).
- Researchers will comply with state and federal regulations regarding the use and care of animals.

The IACUC should be contacted for questions regarding the use of animals for teaching and research. Principal Investigators planning to use animals for any UNMC/UNO activity must submit an application to the IACUC for review prior to the start of the project, regardless of the source of funding for the project. A copy of the application can be obtained from the IACUC (559-6463). The completed form will include descriptions of experimental protocols, plans for animal care, available facilities, and information on the use of hazardous materials including infectious agents.

All animal protocols involving the use of rDNA and infectious or transmissible agents must be submitted to the IBC for review prior to final approval by the IACUC.

L. TRANSPORTATION OF BIOLOGICAL MATERIALS

All biological materials should be transported in a way that maintains the integrity of the material during normal transport conditions, as well as prevents any accidental release and endangerment of the public and the environment.

Diagnostic and clinical specimens, infectious materials and recombinant DNA molecules need to be packaged in a sealed, leak-proof primary container (e.g., glass tube), which is securely positioned in a secondary leak-proof and closable container (e.g., cooler, ice chest) containing a clearly *visible* biohazard symbol on the outside. The shipment of diagnostic and clinical specimens, biological products, infectious agents and recombinant DNA molecules is regulated by national and international transportation rules. This includes specific procedures for the correct packing and packaging of these materials, necessary documentation and labeling and permits. For more information about specific shipment requirements, contact the UNMC Chemical and Radiation Safety Office at 559-7845.

M. DECONTAMINATION

Methods of Decontamination

Decontamination is defined as the reduction of microorganisms to an acceptable level. Methods applied to reach this goal can vary and most often include disinfecting or sterilization. Disinfecting is used when the acceptable level of microorganisms is defined as being below the level necessary to cause disease. This means that viable microorganisms are present. In contrast, sterilization is defined as the complete killing of all organisms present. Depending on the circumstances and tasks, decontamination of a surface (e.g., lab bench) is accomplished with a disinfectant, while decontamination of biomedical waste is done by sterilization in an autoclave or by incineration.

To select the proper method and tools, it is important to consider, for example, the following aspects:

- Type of biohazardous agents, concentration and potential for exposure
- Physical and chemical hazards to products, materials, environment and personnel.

Physical and chemical means of decontamination fall into five main categories to include heat, liquid, chemical vapors, gases, and radiation.

Disinfection is normally accomplished by applying liquid chemicals or wet heat during boiling or pasteurization. To sterilize, vapors and gases (e.g., ethylene oxide), radiation, and wet heat (steam sterilization in an autoclave) are used. Some liquid chemicals are also applied for sterilization, if used in the right concentration and incubation time. The following paragraphs will focus on some of these methods. See also **Appendix E** for additional information on the properties and applications of disinfectants.

Heat

In order to kill microbial agents, heat can be applied in dry or wet form. The advantage of wet heat

is a better heat transfer to and into the cell resulting in overall shorter exposure *time* and lower temperature. Steam sterilization uses pressurized steam at 121-132°C (250-270°F) for 30 or 40 minutes. This type of heat kills all microbial cells including spores, which are normally heat resistant. In order to accomplish the same effect with dry heat in an oven, the temperature needs to be increased to 160-170°C (320-338°F) for periods of 2 to 4 hours.

Liquid Chemicals

The appropriate liquid disinfectant should be chosen after carefully assessing the biohazardous agent and the type of material to be decontaminated. Liquid disinfectants are preferably used for solid surfaces and equipment. They vary greatly in their efficiency, depending on the chemical constituents and the agents involved. Variables to consider when disinfecting:

- Nature of surface being disinfected - Porous or smooth; the more porous and rough the surface, the longer a disinfectant will need to be in contact with the surface to be effective.
- Number of microorganism present - Higher concentrations require a longer application time and/or higher concentration of disinfectant.
- Resistance of microorganisms - Microbial agents can be classified according to increasing resistance to disinfectants and heat (See **Table 1)**

- Presence of organic material - The proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants.
- Duration of exposure and temperature - Increased exposure time increases the effectiveness of disinfectants. Low temperatures may slow the activity requiring more exposure time.

Table 1. Increasing Resistance to Chemical Disinfectants

Least Resistant		Examples
	Lipid or Medium-Size Viruses	Herpes simplex virus Cytomegalovirus Respiratory syncytial virus Hepatitis B virus Human Immunodeficiency virus
	Vegetative Bacteria	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Salmonella choleraesuis*
	Fungi	*Trichophyton* spp. *Cryptococcus* spp. *Candida* spp.
	Non-lipid or Small Viruses	Poliovirus Coxsackievirus Rhinovirus
	Mycobacteria	*Mycobacterium tuberculosis*; *M. bovis*
Most Resistant	**Bacterial Spores**	*Bacillus subtilis* *Clostridium sporogenes*

There are many different liquid disinfectants available under a variety of trade names; in general, these can be categorized as halogens, acids or alkalines, heavy metal salts, quaternary ammonium compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the most effective disinfectants are often very aggressive (corrosive) and toxic. Some of the more common ones are discussed below:

Alcohols
Ethyl or isopropyl alcohols in concentration of 70°/o to 90% are good general-use disinfectants. However, they evaporate fast and therefore have limited exposure time. They are less active against non-lipid viruses and ineffective against bacterial spores. Concentrations above *90%* are less effective.

Formalin
Formalin is a 37% solution of formaldehyde in water. Dilution of Formalin to 5%, results in an effective disinfectant. Formaldehyde is a human carcinogen and creates respirator problems at low levels of concentration.

Glutaraldehyde
This compound although chemically related to formaldehyde, is more effective against all types of bacteria, fungi, and viruses. Vapors of glutaraldehydes are irritating to the eyes, nasal passages and upper respiratory tract. They should be used always in accordance with the instructions on the label and the appropriate personal protective equipment.

Phenol and Phenol Derivatives
Phenol based disinfectants come in various concentrations ranging mostly from *5%* to 10 *%.* These derivatives including phenol have an odor, which can be somewhat unpleasant. Phenol itself is toxic and appropriate personal protective equipment is necessary during application. The phenol disinfectants are used frequently for disinfecting contaminated surfaces (e.g., walls, floors, bench tops). They effectively kill bacteria including *Mycobacterium tuberculosis,* fungi and lipid-containing viruses. They are not active against spores or non-lipid viruses.

Quaternary Ammonium Compounds (Quats)
Quats are cationic detergents with strong surface activity. They are acceptable for general-use disinfectants and are active against gram-positive bacteria and lipid-containing viruses. They are less active against gram-negative bacteria and are not active against non-lipid-containing viruses. Quats are easily inactivated by organic materials, anionic detergents or salts of metals found in water. If Quats are mixed with phenols, they are very effective disinfectants as well as cleaners. Quats are relatively nontoxic and can be used for decontamination of food equipment and for general cleaning.

Halogens (Chlorine and Iodine)
Chlorine-containing solutions have broad-spectrum activity. Sodium hypochlorite is the most common base for chlorine disinfectants. Common household bleach (5% available chlorine) can be diluted 1/10 to 1/100 with water to yield a satisfactory disinfectant solution. Diluted solutions may be kept for extended periods (1 week) if kept in a closed container and protected from light. However, it is recommended to use freshly prepared solutions for spill clean-up purposes. Chlorine-containing disinfectants are inactivated by excess organic materials. They are also strong oxidizers and very corrosive. Always use appropriate personal protective equipment when using these

compounds. At high concentrations and extended contact time, hypochlorite solutions are considered cold sterilants since they inactivate bacterial spores. Iodine has similar properties to chlorine; iodophors (organically bound iodine) are recommended disinfectants. They are most often used as antiseptics and in surgical soaps and are relatively nontoxic to humans.

Vapors and Gases

A variety of vapors and gases possess germicidal properties. The most commonly used are formaldehyde and ethylene oxide. Applied in closed systems under controlled conditions (e.g., humidity) these gases achieve sterility. Formaldehyde gas is primarily used in the decontamination of spaces or biological containment equipment like BSCs. Formaldehyde is a toxic substance and a suspected human carcinogen. Considerable caution must be exercised in handling, storing, and using formaldehyde. Ethylene oxide is used in gas sterilizers under controlled conditions. Ethylene oxide is also a human carcinogen and monitoring is necessary during its use.

Radiation

Gamma and X-ray are two principal types of ionizing radiation used in sterilization. Their application is mainly centered on the sterilization of prepackaged medical devices. Ultraviolet (UV) radiation is a practical method for inactivating viruses, *Mycoplasma*, bacteria and fungi. UV radiation is successfully used in the destruction of airborne microorganisms; UV light sterilizing capabilities are limited on surfaces because of its lack of penetrating power.

Room Decontamination

Containment laboratories periodically undergo routine decontamination procedures using a disinfectant gas. Additionally, room decontamination may be required in an area where overt biohazardous agent contamination has occurred. To schedule decontamination, contact Rich Boldt, Coordinator of Special Projects at 559-4100.

N. BIOHAZARDOUS WASTE

The term "biohazardous waste" is used to describe different types of waste that might include infectious agents. Refer to the procedure for the Handling and Disposal of Biohazardous Waste for specific details at "http://www.unmc.edu/ibc/".

Medical waste: Defined as any solid waste, which is generated in the diagnosis, treatment (e.g., provision of medical services), or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biological materials.

Medical waste includes

a) Cultures and stocks of infectious agents and associated biological, including laboratory waste, biological production waste, discarded live and attenuated vaccines, culture dishes, and related devices.
b) Liquid human and animal waste, including blood and blood products and body fluids, but not including urine or feces or materials stained with blood or body fluids.
c) Pathological waste is defined as human organs, tissues, body parts other than teeth, products of conception, and fluids removed by trauma or during surgery or autopsy or other medical procedure, and not fixed in formaldehyde.
d) Sharps are defined as needles, syringes, scalpels, and intravenous tubing with needles attached regardless of whether they are contaminated or not.

e) Contaminated wastes from animals that have been exposed to agents infectious to humans, these being primarily research animals.

O. BLOODBORNE PATHOGENS AND EXPOSURE CONTROL PLANS

All campuses are committed to protecting its employees from risks associated with exposure to bloodborne pathogens through implementation of its *Exposure Control Plan* (ECP). The plan follows the requirements established by the U.S. Occupational Safety and Health Administration in December 1991 (29 CFR 1910.1030). All employees that have a reasonable anticipated risk for exposure to bloodborne pathogens need to be included in the Bloodborne Pathogens Program. As outlined in the ECP, these employees need to be identified and provided with the appropriate means to safely conduct their individual jobs. The following principles must be followed when employees are potentially exposed to bloodborne pathogens:

- Minimize all exposure to bloodborne pathogens.
- Institute as many engineering and work practice controls as possible to eliminate or minimize employee exposure to bloodborne pathogens.
- Routinely employ "Universal Precautions" when exposure to blood or potentially infectious materials is anticipated.

All employees covered under the ECP need to attend an initial training class on bloodborne pathogens as well as an annual refresher course. In addition, employees have to be provided with Hepatitis B vaccination free of charge. The specific requirements and responsibilities of Principal Investigators, laboratory supervisors, health care managers, employees and others are outlined in the ECP. Please consult this plan for further information.

P. HAND HYGIENE IN RESEARCH LABORATORIES

Hand-washing with soap and water has been considered an important measure of personal hygiene whether working within the confines of a research laboratory or within the everyday private environment. Washing of hands when handling biohazarouous agents, is the major method for the prevention of disease transmission. In the research and health care setting, a number of developments have lead to new guidelines designed to improve hand hygiene practices in the research laboratory. Most of the reports describe hand-washing practices in the healthcare setting; however, these guidelines also have application to the research laboratory.

For an in-depth review of hand hygiene practices, refer to the published report by the CDC. (Guidelines for Hand Hygiene in Health Care Settings: Recommendation of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA hand hygiene task force. *MMWR* 2002; 51 [No. RR-16]).

Appendix A

Select Agents (CDC)

In order to prohibit the unlawful use and distribution of certain infectious biological agents and toxins (BSATs), the CDC and USDA have established certain restrictions. All BSATs included in the following list must be registered with the CDC/USDA. To receive any of these BSATs , all acquisition requests need to be handled by the CDC. This includes transfers in-between workgroups, universities or laboratories, purchasing from chemical manufactures as well as any other shipment or acquisition. Laboratories and PIs need to be approved prior to receiving and working with these agents. The CDC is required to track these BSATs from the time of acquisition to final disposal. Contact the Biosafety Safety Officer or the Responsible Official for BSATs for more information. (Some of the agents listed below are classified as RG-4 and require containment procedures and facilities not available on the UNMC/UNO/NEBRASKA MEDICINE campuses.) BSAT exclusions can be found on the Federal Select Agent Program website at: http://www.selectagents.gov/SelectAgentsandToxinsExclusions.html

A. HHS Select Agents and Toxins

1. Viruses
   - Crimean-Congo haemorrhagic fever virus
   - Eastern Equine Encephalitis virus
   - Ebola virus*
   - Lassa fever virus
   - Lugo virus
   - Marburg virus*
   - Monkeypox virus
   - Reconstructed replication competent forms of the 1981 pandemic influenza virus
   - SARS-associated coronavirus
   - South American Haemorrhagic fever viruses
     (Junin, Machupo, Sabia, Flexal, Guanarito)
   - Tick-born encephalitis complex (flaviviruses)
     (Central European tick-born encephalitis, Far-Eastern tick-borne encephalitis [Russian spring and summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever])
   - Variola major virus (Smallpox virus)*
   - Variola minor virus (Alastrim)*

2. Bacteria
   - Botulinum neurotoxin producing species of *Clostridium**
   - *Coxiella burnetii*
   - *Francisella tularensis**
   - *Rickettsia prowazekii*
   - *Yersinia pestis**

3. Toxins
   - Abrin
   - Botulinum neurotoxins*
   - Conotoxins
   - Diacetoxyscirpenol
   - Ricin
   - Staphylococcal enterotoxins A, B, C, D, E subtypes
   - Saxitoxin
   - T-2 toxin
   - Tetrodotoxin

4. Genetic elements, recombinant nucleic acids, and recombinant organisms

-Select agent viral nucleic acids (synthetic or naturally derived, contiguous or fragments, in host chromosomes or in expression vectors) that can encode infectious and/or replication competent forms of any of the select agent viruses.

-Nucleic acids (synthetic or naturally derived) that encode for the functional form(s) of any of the toxins listed in part 4 of this section if the nucleic acids (1) are in a vector or host chromosome, (2) can be expressed in vivo or in vitro or, (3) are in a vector or host chromosome and can be expressed in vivo or in vitro.

-Viruses, bacteria, fungi, and toxins of this section that have been genetically modified.

5. Exclusions

-Excluded from select agent classification are any select agent or toxin that is in its naturally occurring environment provided it has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.

-Excluded from select agent classification are nonviable select agent organisms or nonfunctional toxins,
-Vaccine strain of Junin virus.
-Purified form of the toxins listed in Part 4 if the aggregate amount under the investigator does not, at any time exceed the amount specified: 100 mg of Abrin; 100 mg of Conotoxins; 1,000 mg of Diacetoxyscirpenol; 100 mg of Ricin; 100 mg of Saxitoxin;; or 100 mg of Tetrodotoxin.
-The HHS Secretary may exclude attenuated strains if it is determined they do not pose a severe threat to the public health and safety.

*Denotes a Tier 1 Agent.

B. HHS / USDA Overlap select agents and toxins

The viruses, bacteria fungi, toxins, genetic elements, recombinant nucleic acids, and recombinant organisms specified below are overlap (regulated by both the HHS and the USDA) select agents and toxins.

1. Viruses

Nipah virus
Hendra virus
Rift Valley fever virus
Venezuelan equine encephalitis virus

2. Bacteria

*Bacillus anthracis**
Bacillus anthracis Pasteur strain
Brucella abortus
Brucella melitensis
Brucella suis
*Burkholderia mallei**
*Burkholderia pseudomallei**

*Denotes a Tier 1 Agent.

Appendix B
USA PATRIOT Act of 2001

(Uniting and Strengthening America by Providing Appropriate Tools Required to Intercept and Obstruct Terrorism Act of 2001, Public Law 107-56, HR 3162, October 26, 2001, composed of 10 Titles containing 159 Sections)

Title VII - Strengthening the Criminal Laws Against Terrorism
Section 817- Expansion of the Biological Weapons Statues

Overview: The first provision of this law makes it unlawful for an individual to possess certain "biological agents, toxins, or delivery systems" in a quantity or of a type that "is not reasonably justified by a prophylactic, protective, bona fide research, or peaceful purpose". The second provision states that persons who meet the definition of "restricted persons" are prohibited from having access to or possessing any amount of the biological agent, or any of the toxins listed on the CDC's list of Select Agents.

What "biological agents" are covered by the Act?
The term "biological agent" is defined by the Act as any microorganism, virus, infectious substance, or biological product that may be engineered as a result of biotechnology, or any naturally occurring or bioengineered component of any such microorganism, virus, infectious substance, or biological product, capable of causing death, disease, or other biological malfunction in a human, an animal, a plant, or another living organism; deterioration of food, water, equipment, supplies, or material of any kind; or deleterious alteration of the environment.

What "toxins" are covered by the Act?
A "toxin" means the toxic material of plants, animals, microorganism, viruses, fungi, or infectious substances, or a recombinant molecule, whatever its origin or method of production, including any poisonous substance or biological product that may be engineered as a result of biotechnology produced by a living organism; or any poisonous isomer or biological product, homolog, or derivative of such a substance.

What are the "Select Agents"?
See Appendix A of the *Biosafety Manual*.

Who is a "restricted person"?
According to the Act, the term "restricted person" means an individual who meets any one or more of the following criteria:

-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 United States;
-has been adjudicated as a mental defective or has been committed to any mental institution;

-is an alien (other than an alien lawfully admitted for permanent residence) 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 (As of April 30, 2001, these countries were Iran, Iraq, Syria, Libya, Cuba, North Korea, and the Sudan.); or
-has been discharged from the Armed Services of the United States under dishonorable conditions.

What is the responsibility of the Principal Investigator?
The PI is responsible to comply to the requirements of the Institute for the reporting and securing of agents that fall within the bounds of the Act. The law does not create an affirmative duty on any individual's part to seek out information from current employees or students as to whether the "restricted persons" criteria apply to them.

Appendix C
Definitions from the *NIH Guidelines* for the Use of Exempt rDNA Molecules

Section III-F. Exempt Experiments

The following recombinant DNA molecules are exempt from the *NIH Guidelines* and registration with the IBC using only the form described in Appendix B is required (a completed IBC protocol is not required to register this type of experimentation):

Section III-F-1. Those that are not in organisms or viruses.
Interpretation/Examples:

Ligation of recombinant molecules and study of these molecules without transferring to a bacterium, virus, or creating a virus.
Southern blot of plasmid DNA.
Synthetic DNA encapsulated in a synthetic delivery vehicle intended for injection into animals.
Generation of a DNA segment using PCR.
Radiolabeling a probe for *in situ* hybridization.

Section III-F-2. Those that consist entirely of DNA segments from a single non-chromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
Interpretation/Example:

The use of SV40 DNA in tissue culture experiments or lambda bacteriophage DNA in *E. coli* (do not carry a foreign insert but can lead to alterations [mutations] in the sequence.
The cloning of the 5' ends of cDNA (from mRNA) to determine transcriptional start site.

Section III-F-3. Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.
Interpretation/Example:

Cloning *Escherichia coli* DNA using vector (plasmid) derived from *E. coli* or other *Enterobacteriaceae* (i.e. pBR322, pUC19, etc.) and using *E. coli* as a transforming host. The statement "or when transferred to another host by well established physiological means" is not interpreted to mean that "host" is another species and "host" may refer to another *E. coli* isolate/strain.

Section III-F-4. Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).
Interpretation/Example:

Same interpretation as section III-F-3 above, except using a eukaryotic host (i.e. a yeast such as *Saccharomyces cerevisiae*)

Section III-F-5. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV-C-1-b-(1)-(c), *Major Actions*). See Appendices A-I through A-VI, *Exemptions Under Section III-F-5--Sublists of Natural Exchangers*, for a list of natural exchangers that are exempt from the *NIH Guidelines*. **Interpretation/Example:**

Certain microorganisms are known to exchange genetic information through a variety of mechanisms including conjugation, transduction, etc. Recombinant DNA experiments are exempt if cloning DNA from, for instance, *Pseudomonas aeruginosa* and transferring that DNA to *E. coli.* These two species are known to exchange DNA naturally. A list of those organisms that are known to exchange DNA are shown below (Identified as Appendix A by the *NIH Guidelines*, Exemptions under section III F-5 sub-lists of natural exchanges).

Sublist A

Genus *Escherichia*
Genus *Shigella*
Genus *Salmonella* - including *Arizona*
Genus *Enterobacter*
Genus *Citrobacter* - including *Levinea*
Genus *Klebsiella* - including *oxytoca*
Genus *Erwinia*
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescens, and Pseudomonas mendocina
Serratia marcescens
Yersinia enterocolitica

Sublist B

Bacillus subtilis
Bacillus licheniformis
Bacillus pumilus
Bacillus globigii
Bacillus niger
Bacillus nato
Bacillus amyloliquefaciens
Bacillus aterrimus

Sublist C

Streptomyces aureofaciens
Streptomyces rimosus
Streptomyces coelicolor

Sublist D

Streptomyces griseus
Streptomyces cyaneus
Streptomyces venezuelae

Sublist E

One way transfer of *Streptococcus mutans* or *Streptococcus lactis* DNA into *Streptococcus sanguis*

Sublist F

Streptococcus sanguis
Streptococcus pneumoniae
Streptococcus faecalis
Streptococcus pyogenes
Streptococcus mutans

Section III-F-6. Those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), *Major Actions*), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See below, *Exemptions under Section III-F-6* for other classes of experiments which are exempt from the *NIH Guidelines*.

Interpretation/Example:

Recombinant DNA experiments associated with specific host systems are not considered a public health threat and are therefore exempt. These exemptions are listed below (Identified as Appendix C by the *NIH Guidelines*, Exemptions under section III-F-6).

Exemption C1: Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome that are propagated and maintained in cells in tissue culture are exempt from these *NIH Guidelines* with the exceptions listed below

Exceptions to Exemption C1:

1) **Cloning a drug resistance marker not naturally known to be found in the organism.**
2) **Cloning of toxins with an LD_{50} of less than 100 ng/kg (botulinum toxin, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin)**
3) **Cloning of DNA from any Risk group 3 or 4 pathogen or cloning from cells known to be infections with a risk group 3 or 4 pathogen.**
4) **Experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates.**
5) **Whole plants regenerated from plant cells and tissue cultures are covered by the exemption provided they remain axenic cultures even though they differentiate into embryonic tissue and regenerate into plantlets.**

Exemption C2: Recombinant DNA experiments which use *E. coli* K12 host-vector systems (almost all *E. coli* strains purchased from molecular biology sources are from the K12 lineage) provided that: 1) the *E. coli* strain does not contain conjugative plasmids or prophages that are able to undergo transduction. 2) Lamboid or non-conjugative plasmids are used as cloning vectors (this includes pUC19, pGEM, and most all cloning vectors used in *E. coli* genetic experiments). However, a conjugative plasmid may be used if the DNA that is inserted is from organisms that naturally exchange DNA with *E. coli* (see Appendix A-1 sublist A above).

Exceptions to Exemption C2:

1) **Experiments involving DNA from Risk Groups 3 or 4 organisms.**
2) **Large-scale experiments (e.g., more than 10 liters of culture).**
3) **Experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates.**

Exemption C3: Recombinant DNA experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems.

Exceptions to exemption C3:

1) **Experiments involving DNA from Risk Groups 3 or 4 organisms.**
2) **Large-scale experiments (e.g., more than 10 liters of culture).**
3) **Experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates.**

Exemption C4: Recombinant DNA experiments involving asporogenic *Bacillus subtilis* or *Bacillus lichemiformis* host-vector systems. *Bacillus* strains used must not form spores at a frequency greater than 10-7.

Exceptions to exemption C4:

1) **Experiments involving DNA from Risk Groups 3 or 4 organisms.**
2) **Large-scale experiments (e.g., more than 10 liters of culture).**
3) **Experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates.**

Exemption C5: Recombinant DNA molecules derived entirely from extrachromosomal elements (i.e. plasmids) of the gram-positive organisms listed below (including shuttle vectors comprised of vectors listed in Exemption C2 above), propagated and maintained in organisms listed below are exempt from the *NIH Guidelines*.

Bacillus amyloliquefaciens
Bacillus amylosacchariticus
Bacillus anthracis
Bacillus aterrimus
Bacillus brevis
Bacillus cereus
Bacillus globigii
Bacillus licheniformis
Bacillus megaterium
Bacillus natto
Bacillus niger
Bacillus pumilus
Bacillus sphaericus
Bacillus stearothermophilis
Bacillus subtilis
Bacillus thuringiensis
Clostridium acetobutylicum
Lactobacillus casei
Listeria grayi
Listeria monocytogenes
Listeria murrayi
Pediococcus acidilactici
Pediococcus damnosus
Pediococcus pentosaceus
Staphylococcus aureus
Staphylcoccus carnosus
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus avium
Streptococcus cremoris
Streptococcus dorans
Streptococcus equisimilis
Streptococcus faecalis
Streptococcus ferus
Streptococcus lactis
Streptococcus ferns
Streptococcus mitior
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarious
Streptococcus sanguis
Streptococcus sobrinus
Streptococcus thermophylus

Interpretation/Example:

Recombinant DNA and cloning experiments using shuttle plasmids constructed from *Staphylococcus aureus* (i.e. pE194) and *Escherichia coli* (pUC19) are exempt if the plasmids are maintained in one of the species listed above. These recombinant DNA molecules can also be transferred to *E. coli* K12 lineage strains as discussed in exemption C2 above (through the use of the shuttle plasmids).

Appendix D

Biosafety Risk Assessment Summary

(Refer to Risk Factor Assessment Outline to complete the RG classification)

Risk Factor	**Risk Group**
Pathogenicity/virulence	_____
Infectious Dose	_____
Route of Spread	_____
Communicability	_____
Environmental stability	_____
Host range	_____
Economic impact	_____
Availability of prophylactic/treatment	_____
Vectors	_____
Concentration/volume	_____

Risk Factor Assessment Outline

Pathogenicity/virulence

RG1 Unlikely to cause disease, low individual and community risk.

RG2 Mild or moderate disease with moderate individual risk and low community risk; any pathogen that can cause disease but under normal circumstances, is unlikely to be a serious hazard to a healthy worker, the community, livestock, or the environment.

RG3 Serious livestock, poultry or wildlife disease with high individual risk and low community risk; any pathogen that usually causes serious disease or can result in serious economic consequences or does not ordinarily spread by causal contact from one individual to another.

RG4 Severe livestock, poultry or wildlife disease with high individual risk and high community risk; any pathogen that usually produces very serious and often fatal disease, often untreatable and may be readily transmitted from one individual to another or from animal to human or vice-versa, directly or indirectly, or by casual contact.

Infectious dose

RG1	Not applicable	(rare cause of human disease)
RG2	High	(>1,000 organisms)
RG3	Medium	(10-1,000 organisms)
RG4	Low	(1-10 organisms)

Route of spread

RG1 Not applicable (rare cause of human disease)

RG2 Primary exposure hazards are through ingestion, inoculation, and mucous membrane route

RG3 May be transmitted through airborne route; direct contact or via vectors

RG4 Readily by aerosol transmission

Communicability

RG1 Not applicable (rare cause of human disease)
RG2 Geographical risk of spread if released from the laboratory is limited.
RG3 Geographical risk of spread if released from the laboratory is moderate
RG4 Geographical risk of spread if released from the laboratory is high.

Environmental stability

RG1 Not applicable
RG2 Short term survival (days), can survive under ideal conditions
RG3 Moderately resistant (days to months)
RG4 Highly resistant (months to years), e.g. spores.

Host range

RG1 Not applicable
RG2 Infects a limited number of species
RG3 Infects multiple species
RG4 Infects many species

Economic aspects

RG1 Not applicable
RG2 Limited economic impact
RG3 Severe economic impact
RG4 Extreme economic impact

Availability of prophylactic and therapeutic treatments

RG1 Not applicable
RG2 Effective treatment and preventative measures are available
RG3 Prophylactic and/or treatments may or may not be readily available
RG4 Prophylactic and/or treatments are not available

Vectors

RG1 Not applicable
RG2 Do not depend on vectors or intermediate hosts for transmission
RG-3 May depend on vectors or intermediate host for transmission
RG4 May depend on vectors or intermediate host for transmission.

Concentration/volume

RG1 Not applicable
RG2 Low quantity of high titer
RG3 High quantity (10 liters or more) of high titer as described by the BMBL
RG4 Not applicable

Recombinant properties

RG1 Recombinant is a RG1 organism and modifications have not changed the risk; low probability of
RG2 replication-incompetent virus becoming competent
RG2 Recombinant is a RG2 organism and modifications have not changed the risk, DNA from RG2 or
RG3 organism is transferred into RG1 organism but not the whole genome, DNA from RG4 organism is transferred into RG1 organism, or the recombinant is a RG3 or RG4 organism and the modification has resulted in proven attenuation; moderate probability of RG2 replication-incompetent virus becoming competent
RG3 Recombinant is a RG3 organism and modifications have not change the risk, the recombinant is based on a RG2 organism; however, the modifications have increased to RG3 organism.
RG4 Recombinant is a RG4 organism and modifications have not changed the risk, DNA from RG4 organism is transferred into RG1organism in absence of demonstration of lack of virulence or pathogenicity.

Appendix E

Properties and Applications of Disinfectants

Disinfectant Category*	Use Dilution	Requirements	Inactivates
Liquid			
Quat. Ammonium Cmps.	(0.1%-2.0%)	10 m contact	VB, LV
Phenolic Cmps.	(1.0%-5.0%)	10 m contact	VB, LV
Bleach .	(0.5%-10%)	30 m contact	VB, LV, NLV, MYC, BS
Iodophor	(0.5%-5%)	30 m contact	VB, LV, NLV
Alcohol, ethyl	(75%-85%)	30 m contact	VB, LV
Alcohol, isopropyl	(70%-85%)	30 m contact	VB, LV
Formaldehyde+	(0.2%-8.0%)	10 m contact	VB, LV, NLV, MYC, BS
Glutaraldehyde	(2%)	30 m contact	VB, LV, NLV, MYC, BS
Gas			
Ethylene oxide++	(8-23 g/ft^3)	60 m, 37 C, 30% hum	VB, LV, NLV, MYC, BS
Paraformaldehyde+	(0.3 to 0.6g/ft^3.)	4 hrs, <23 C, >60% hum	VB, LV, NLV, MYC, BS

Abbreviations: VB = vegetative bacteria, LV = lipoviruses, NLV = nonlipid viruses, MYC = *Mycobacterium*, BS = bacterial spores, m = minutes, hum = humidity, comps = compounds

.*Small volumes of pourable disinfectant can be disposed in the sanitary sewer system. Contact Chemical Safety for advice on the disposal of larger volumes.

+These chemicals are known carcinogens and require special procedures for disinfection. Contact Chemical Safety for recommendations on use.

++This chemical is extremely flammable and requires special precautions for use.