UWM Biosafety Manual

University Safety and Assurances- Biological Safety Program

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414-588-4261
uwm-biosafety@uwm.edu

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Chapter 1: Introduction and Overview

The University of Wisconsin-Milwaukee (UWM) Department of University Safety & Assurances Biological Safety Program oversees the responsible use of biological hazards in microbiology, tissue culture, recombinant DNA, molecular biology, synthetic biology, and biotechnology at all UWM facilities. The biosafety officer (BSO) and the Institutional Biosafety Committee (IBC) evaluate and approve protocols for research experiments that work with biological hazards. This manual, in part, helps meet the goals of the UWM biological safety program, which include the following:

- **Protection**: Protect personnel, students, staff, and the public from exposure to infectious agents and recombinant nucleic acid materials.
- **Prevention**: Prevent environmental contamination from infectious agents and recombinant nucleic acid materials.
- **Training**: Provide training and outreach to personnel as part of maintaining an excellent research institution while maintaining a safe work environment.
- **Compliance**: Comply with local, state, and federal rules and regulations.

The Principal Investigator (PI) is responsible for the implementation of procedures outlined in this manual. The PI is also responsible for maintaining a laboratory-specific biosafety manual, submitting protocols per NIH Guidelines and University Guidelines, and for the development of lab-specific standard operating procedures (SOPs). It is the responsibility of the laboratory supervisors and laboratory personnel to follow the regulations, policies, and procedures after training, understand their expectations to prevent accidents from occurring, and report any incidents to their PI and to the Biological Safety Program immediately.

Registration with the IBC is required whenever any biological materials are being used that could elicit a potential risk to humans, animals, plants, or the environment. These biological materials may include, but are not limited to: risk group 2 or higher pathogenic microorganisms, toxic chemicals used to elicit a biological response, infectious agents, viruses, viroids, prions, human tissues, human blood and bloodborne pathogens, and in-vitro construction or propagation of recombinant DNA molecules. The Biological Safety Program also asks researchers performing exempt procedures to still submit the Registration Form for University records. All researchers are expected to follow the NIH Guidelines and any other state and federal regulations, regardless of whether they receive any kind of funding for their research. All non-exempt protocol submissions will be required to be approved the IBC. The meeting schedule is posted on the UWM Report Calendar. The BSO will evaluate and approve biosafety protocols that are exempt from IBC registration.

Biosafety Lab Inspections will help the PI and lab researchers determine if there are issues with compliance or SOPs and provide a learning opportunity for both the researchers and the BSO. All laboratories handling any biological materials will be required to have annual or biennial biosafety inspections. In addition to handling biosafety lab inspections, the BSO also oversees the coordination of activities within the IBC and provides records of meeting minutes, approvals, etc. To learn more about the Biological Safety Program and the IBC, visit: http://uwm.edu/safety-health/biosafety/.

Chapter 2: General Biosafety

Biohazardous Materials

A **biohazardous material** is any biological material capable of causing harm to humans, animals or plants, including both biohazardous agents and non-replicating materials such as toxins, and may also be used to refer to material that harbors a biohazardous agent. A biohazardous agent is a pathogen capable of replication and is a disease-causing microorganism (bacteria, chlamydia, fungi, parasites, prions, rickettsia, viruses, etc.) capable of causing diseases in humans, animals, or plants. Toxic, mutagenic, and teratogenic chemicals are not considered biohazards, but rather chemical hazards, and are addressed by the UWM Chemical Hygiene Plan.

Risk Groups

The NIH and WHO recommend four risk groups (RG) based upon the following hazardous characteristics of an agent: its ability to infect and cause disease in a susceptible human or animal host, its virulence as measured by the severity of the disease, and the availability of preventative measures and effective treatments for the disease (US DHHS, 2009). The risk group listing from the NIH Guidelines are the standard, regardless of whether there is use of recombinant DNA (Table 1). This can also be found in the BMBL, page 10.

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)

Table 1 Risk Groups (NIH, 2016, p. 47)

Determination of the appropriate risk group is the first step in determining the appropriate biosafety level (BSL) for working with the agent. The BSL is a reference to the type of containment and PPE necessary for working with the agent. The BSL typically has a parallel numbering of 1-4, thus a RG 1 agent would typically fall into a BSL-1 containment practice. This is not always true though; there are risk group 2 agents that requires some BSL-3 containment practices implemented, particularly if they have the potential to aerosolize or have a low infectious does. This manual will refer to organisms based on their risk groups, and containment requirements by their BSL.

There is also a parallel animal biosafety level (ABSL1 through ABSL4) that specifically pertains to the safe handling of infected or potentially infected animals. See the figure below

from the BMBL for guidance. When working with animals that are recombinant, the biosafety containment levels outlined in the NIH Guidelines are required to be followed.

There is also a plant biosafety level of containment (BSL1-P through BSL4-P). Before working with any biological agent, consult the NIH guidelines, ABSA Risk Group Database, BMBL, Pathogen Safety Data Sheets from the Public Health Agency of Canada, and the BSO to determine containment needs and if protocols need to be filed with the IBC to work with the agent. Plants also have specific containment requirements, as outlined in Appendix P of the NIH Guidelines.

Table 3. Summary of Recommended Animal Biosafety Levels for Activities in which Experimentally or Naturally Infected Vertebrate Animals are Used

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in healthy adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species ■ PPE: laboratory coats and gloves; eye, face protection, as needed	Standard animal facility: ■ No recirculation of exhaust air ■ Directional air flow recommended ■ Hand washing sink is available
2	■ Agents associated with human disease ■ Hazard: percutaneous injury, ingestion, mucous membrane exposure	ABSL-1 practice plus: ■ Limited access ■ Biohazard warning signs ■ "Sharps" precautions ■ Biosafety manual ■ Decontamination of all infectious wastes and animal cages prior to washing	ABSL-1 equipment plus primary barriers: ■ Containment equipment appropriate for animal special ■ PPE: Laboratory coats, gloves, face, eye and respiratory protection, as needed ■	ABSL-1 plus: ■ Autoclave available ■ Hand washing sink available ■ Mechanical cage washer recommended ■ Negative airflow into animal and procedure rooms recommended
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	ABSL-2 practice plus: ■ Controlled access ■ Decontamination of clothing before laundering ■ Cages decontaminated before bedding is removed ■ Disinfectant foot bath as needed	ABSL-2 equipment plus: ■ Containment equipment for housing animals and cage dumping activities ■ Class I, II or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols ■ PPE: Appropriate respiratory protection	ABSL-2 facility plus: ■ Physical separation from access corridors ■ Self-closing, double-door access ■ Sealed penetrations ■ Sealed windows ■ Autoclave available in facility ■ Entry through ante-room or airlock ■ Negative airflow into animal and procedure rooms ■ Hand washing sink near exit of animal or procedure room
4	■ Dangerous/exotic agents which post high risk of aerosol transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments ■ Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level ■ Related agents with unknown risk of transmission	ABSL-3 practices plus: ■ Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting ■ All wastes are decontaminated before removal from the facility	ABSL-3 equipment plus: ■ Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure suit) used for all procedures and activities	ABSL-3 facility plus: ■ Separate building or isolated zone ■ Dedicated supply and exhaust, vacuum, and decontamination systems ■ Other requirements outlined in the text

Viral vectors, even if they are rendered replication-defective, may pose recombination threats with wild-type strains and should be presumed virulent and handled as a virulent agent. When selecting a risk-group, the virulent agent risk group is what is used for the agent, unless indicated otherwise in the BMBL or NIH Guidelines. Most viral vector work falls under Section III-D of the NIH Guidelines, which require IBC approval before beginning work with the vector. All viral vector work is required to be registered with the UWM IBC, regardless of the categorization under NIH Guidelines. PIs should consider requesting viral vector training through the BSO, which can be done for the entire research group in a single session.

Additional considerations need to be made for genetically-modified biological agents. Risk assessment of the wild-type organism should be done. Additionally, addressing the possibility of genetic modification, how it alters pathogenicity of the agent, and its susceptibility to antimicrobial treatments need to be discussed in the risk assessment that would then be attached to the IBC registration form. It is imperative that the PI has researched this information thoroughly and obtained an IBC approval before commencing research with GM agents. It may be possible that this information may not be available for an agent that has recently been developed, making a risk assessment incomplete or hard to complete. Assign these agents a conservative biosafety level containment to exercise the safest practices possible. Re-evaluate the agent when more information is available.

A human and/or animal cell or tissue has enormous potential to harbor potential latent infectious agents. Personnel who handle these are at risk for possible exposure to these agents. Refer to the section "Working with Cell Lines and Tissue Cultures" and refer to the UWM Bloodborne Pathogens Exposure Control Plan. All clinical/patient samples should be considered a minimum of a risk group 2 and only worked with in a BSL-2 containment or higher.

The table below outlines commonly used RG1 agents used at UWM. Note that agents not listed on Risk Groups 2, 3, and 4 are not automatically or implicitly classified in Risk Group 1. A risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

Bacterial Agents	**Viral Agents**	**Fungal Agents**
Bacillus subtilis (asporogenic only) *Bacillus lichenformis* *Escherichia coli* K-12 *Staphylococcus epidermidis*	Adeno-associated virus (AAV) Types 1-4 Recombinant AAV	*Saccharomyces cerevisiae*

Table 2: Risk Group 1 Agents Commonly Used at UWM

RG2 agents should not be assumed to be mostly safe based on their classification alone. All organisms in RG2 have the potential to cause serious harm to the researcher and must be handled accordingly. Some organisms may best be handled in a BSL-3 containment, rather than BSL-2. Complete a risk assessment to determine the best level of containment for the pathogen. The table below identifies commonly used RG2 agents in research.

Type of Agent	Organism
Gram-positive Bacteria	*Arcanobacterium haemolyticum* *Bacillus anthracis* *Trueperella pyogenes* (Formerly: *Actinomyces pyogenes*) *Clostridium botulinum, C.difficile, C. chauvoei, C. haemolyticum, C. histolyticum, C. novyi, C. septicum, C. tetani*- note that Botulinum neurotoxins and Botulinum producing species are Select Agents and subject to regulation from the U.S. Government. *Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale*- Note that the Diphtheria toxin is also to be considered Risk Group 2 and handled as such. *Dermatophilus congolensis (*note: RG 3 in animals) *Erysipelothrix rhusiopathiae* *Listeria,* all species *Mycobacterium* (except those listed in RG3) including *M. avium* complex*, M. asiaticum, M. bovix BCG vaccine strain, M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi* *Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis* *Rhodococcus equi* *Staphylococcus aureus* *Streptococcus* including *S. pneumoniae, S. pyogenes*
Gram-negative Bacteria	*Actinobacillus* *Aeromonas hydrophila* *Arizona hinshawii – all serotypes* *Bartonella henselae, B quintana, B. vinsonii* *Bordetella including B. pertussis* *Borrelia recurrentis, B burgdorferi* *Burkholderia (except those listed in RG3)* *Campylobacter coli, C. fetus, C. jejuni* *Chlamydia psittaci, C. trachomatis, C. pneumoniae* *Edwardsiella tarda* *Escherichia coli* – all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7 *Fusobacterium necrophorum* *Haemophilus ducreyi, H. influenza* *Helicobacter pylori* *Klebsiella*- all species except *K. oxytoca,* which is RG 1 *Legionella,* all species *Leptospira interrogans*- all serotypes *Moraxella,* all species *Neisseria gonorrhoeae, N. meningitides* *Pseudomonas aeruginosa*

	Salmonella including *S. arizonae, S. cholerasuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis,* *S. paratyphi,* A, B, C, *S. typhi, S. typhimurium* *Shigella* including *S. boydii, S. dysenteriae, type 1, S. flexneri, S. sonnei* *Streptobacillus moniliformis* *Treponema pallidum, T. carateum* *Vibrio cholerae, V. parahemolyticus, V. vulnificus* *Yersinia enterocolitica*
Mycoplasma Bacteria	*Mycoplasma*, except *M. mycoides and M. capricolum* (USDA Select Agents)
Fungal	*Blastomyces dermatitidis* *Cladosporium bantianum, aka C. (Xylohypha) trichoides* *Cryptococcus neoformans* *Dactylaria gallopava (Ochroconis gallopavum)* *Epidermophyton* *Exophiala (Wangiella) dermatitidis* *Fonsecaea pedrosoi* *Microsporum* *Paracoccidioides braziliensis* *Penicillum marneffei* *Sporothrix schenckii* *Trichophyton*
Parasites	*Ancylostoma* human hookworms including *A. duodenale, A. ceylanicum* *Ascaris* including *Ascaris lumbricoides suum* *Babesia* including *B. divergens, B. microti* *Brugia* filarial worms including *B. malayi, B. timori* *Coccidia* *Cryptosporidium, including C. parvum* *Echinococcus including E. granulosis, E. multilocularis, E. vogeli* *Entamoeba histolytica* *Enterobius* *Fasciola* including *F. gigantica, F. hepatica* *Giardia* including *G. lamblia* *Heterophyes* *Hymenolepis including H. diminuta, H. nana* *Isospora* *Leishmania including L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica* *Loa loa* filaria worms *Microsporidium* *Naegleria fowleri* *Necator* human hookworms including *N. americanus* *Onchocerca* filaria worms including *O. volvulus*

	Plasmodium including simian species, *P. cynomologi, P. falciparum, P. malariae, P. ovale, P.vivax* *Sarcocystis* including *S. sui hominis* *Schistosoma* including *S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi* *Strongyloides including S. stercoralis* *Taenia solium,* all stages *Toxocara* including *T. canis* *Toxoplasma* including *T. gondii* *Trichinella spiralis* *Trypanosoma* including *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi* *Wuchereria bancrofti* filaria worms
Viruses	Adenoviruses, human – all types Alphaviruses (Togaviridae) – Group A Viruses ➢ Eastern equine encephalomyelititis virus ➢ Venezuelan equine encephalomyelitis vaccine strain TC 83 ➢ Western equine encephalomyelitis virus Arenaviruses ➢ Lymphocytic choriomeningitis virus (non-neurotropic strains) ➢ Tacaribe virus complex Bunyaviruses ➢ Bunyamwera virus ➢ Rift Valley fever virus vaccine strain MP-12 Calciviruses Coronaviruses Flaviviruses (Togaviridae) – Group B Arborviruses ➢ Dengue virus serotypes 1,2,3, and 4 ➢ Yellow fever virus vaccine strain 17D ➢ Other viruses as listed in the reference source(see Section V-C, Footnotes and References of Section I through IV) Hepatitis A, B, C, D, and E Viruses Herpesviruses – except Herpesvirus simiae (Monkey B virus) ➢ Cytomegalovirus ➢ Epstein-Barr virus ➢ Herpes simplex types 1 and 2 ➢ Herpes zoster ➢ Human herpes virus types 6 and 7 Orthomyxoviruses ➢ Influenza viruses types A, B, and C Papovaviruses ➢ All human papilloma viruses Paramyxoviruses ➢ Newcastle disease virus ➢ Measles virus

	➢ Mumps virus ➢ Parainfluenza viruses types 1, 2, 3, and 4 ➢ Respiratory syncytial virus Parvoviruses ➢ Human parvovirus(b19) Picornaviruses ➢ Coxsackie viruses types A and B ➢ Echoviruses – all types ➢ Polioviruses – all types, wild and attenuated ➢ Rhinoviruses – all types Poxviruses- all types except Monkeypox virus, restricted poxviruses including Alastrim, Smallpox, and Whitepox Reoviruses- all types including Coltvirusm, human Rotavirus, and Orbivirus (Colorado tick fever virus) Rhabdoviruses ➢ Rabies virus – all strains ➢ Vesicular stomatitis virus – laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow Togaviruses (see Alphaviruses and Flaviviruses) Rubivirus (rubella)

Table 3 List of Risk Group 2 Agents Commonly Used at UWM

The next table identifies risk group 3 and 4 agents. UWM is neither equipped to conduct research in the BSL-3 and 4 containments required for these pathogens, nor are researchers allowed to work with most of these without additional approvals by the federal government through the Select Agent and Toxin Program, the USDA/APHIS, and Dual Use Research of Concern. Please contact the Biological Safety Officer if you plan to develop a facility to study these organisms.

Risk Group 3 (RG3) Agents	Risk Group 4 (RG4) Agents
Bacterial Agents *Bartonella* *Brucella including B. abortus, B. canis, B. suis* *Burkholderia (Pseudomonas) mallei, B. pseudomallei* *Coxiella burnetii* *Francisella tularensis* *Mycobacterium bovis (except BCG strain), M. tuberculosis* *Pasteurella multocida type B – "buffalo" and other virulent strains* *Rickettsia akari, R. australis, R. canada, R. conorii, R. prowazekii, R. rickettsii, R. siberica, R. tsutsugamushi, R. typhi (R. mooseri)* *Yersinia pestis* Fungal Agents *Coccidioides immitis* (sporulating cultures; contaminated soil) *Histoplasma capsulatum, H. capsulatum var. duboisii* Parasitic Agents None Viral Agents and Prions Alphaviruses (Togaviruses) – Group A Arboviruses ➢ Semliki Forest virus ➢ St. Louis encephalitis virus ➢ Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83, see Appendix B-II-D (RG2) Arenaviruses ➢ Flexal ➢ Lymphocytic choriomeningitis virus (LCM) (neurotropic strains Flaviviruses (Togaviruses) – Group B Arboviruses ➢ Japanese enchephalitis virus ➢ Yellow fever virus Poxviruses	Bacterial Agents None Fungal Agents None Parasitic Agents None Viral Agents Arenaviruses ➢ Guanarito virus ➢ Lassa Virus ➢ Junin virus ➢ Machupo virus ➢ Sabia virus Bunyaviruses (Nairovirus) Crimean-Congo hemorrhagic fever virus Filoviruses ➢ Ebola virus ➢ Marburg virus Flaviruses (Togaviruses) – Group B Arboviruses ➢ Tick-born encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses Herpesviruses (alpha) ➢ Herpsevirus simiae (Herpes B or Monkey B virus) Paramyxoviruses ➢ Equine morbillivirus

➢ Monkeypox virus Prions ➢ Transmissible spongioform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents) Retroviruses ➢ Human immunodeficiency virus (HIV) types 1 and 2 ➢ Human T cell lymphotropic virus (HTLV) types 1 and 2 ➢ Simian immunodeficiency virus (SIV) Rhabdoviruses ➢ Vesicular stomatitis virus	

Table 4 Risk Group 3 and 4 Agents

Routes of Transmission in the Laboratory and Laboratory-Acquired Infections (LAIs)

There are 4 ways in which an infectious agent may be transmitted in the laboratory:

1. **Direct transmission** through exposure to the agent. Example: splash liquid culture of *S. aureus* in eye while moving it from one bench to another.
2. **Ingestion** of the agent either by accidental ingestion of a liquid suspension or contaminated hand to mouth exposure. Example: Handling of *Cryptosporidium* culture and then failure to wash hands after handling, leading to self-inoculation of *Cryptosporidium*.
3. **Inhalation** of infectious aerosols. Example: Employee working with *M. tuberculosis* has a tear in their mask, and thus, inhales and contracts *M. tuberculosis*.
4. **Parenteral inoculation** from a syringe or contaminated sharp. Example: Researcher uses syringes to inoculate mice with *Streptococcus pneumoniae* and accidentally sticks finger with syringe after inoculating mouse, going through the glove.

There is an increased risk of transmission associated with agents that are transmitted via aerosol or droplet transmission, as well as when high-volume quantities are used in research or teaching laboratories. Both teaching and research laboratories must have appropriate protocols and SOPs in place to minimize the risk of transmission of pathogens. Teaching laboratories are at greatest risk for LAIs, as students have less training and expertise than PIs or research laboratory personnel. In 2011, the American Society for Microbiology (ASM) began developing a framework for laboratory safety in teaching laboratories in microbiology in response to Salmonella outbreaks occurring in teaching laboratories at U.S. academic institutions. The completed ASM project now provides the most current recommendations for teaching laboratories, including PPE, recommended practices, implementation of a laboratory biosafety manual, and more. To learn more, visit the ASM Guidelines for Biosafety in Teaching Laboratories Page.

If an agent may be aerosolized, the lab must have strict protocols in place to prevent transmission. Aerosolized agents are implicated in many of the reported laboratory-acquired infections. Aerosols can spread using air currents, contaminating "clean" areas. For this reason, any agent that can aerosolize must be worked with in a biological safety cabinet (BSC) whenever possible (or fume hood if the agent is a biological toxin) to minimize the spread of the agent. Respiratory PPE, such as a mask, may be recommended when handling the agent outside of the BSC.

Additional measures and considerations may be necessary to prevent laboratory-acquired infections by microorganisms that typically do not cause infection in healthy individuals but are known pathogens in immunocompromised individuals. If there are any PIs or researchers in a lab that may have a compromised immune response and are working with agents that may be of concern to them, they will need to consult their personal physician or health care professional to determine what steps would be most appropriate for their health and safety. It is the responsibility of the PI to communicate the hazards of handling the agent, proper safety practices, proper PPE, and proper disposal of the agent.

All accidental exposures must be reported as an injury that occurred at work using the information provided through the UW System Website. Additionally, the PI must complete a First Report of Biological Exposure or Release Event Form online. It is also the responsibility of the PI (or, in a teaching lab, the laboratory manager and instructor) to ensure all personnel complete the appropriate training so their students in teaching and research laboratories are provided with correct and complete information.

Positive diagnoses of many of RG2 pathogens are required to be reported to public health agencies and will be investigated by the state and with assistance from University Safety and Assurances. A list of notifiable diseases are available online for reference. Animal bites and scratches require additional documentation to the LAI form, located on the UWM Animal Care Program site.

The IBC can effectively carry out its designated functions only if it has adequate prior knowledge of potentially hazardous research projects. Therefore, all instructional, research, and outreach projects involving recombinant/synthetic nucleic acids (including transgenic animals or plants) or any biohazardous materials must be registered with and approved in writing by the Committee.

The following practices are important for disease prevention, avoiding contamination of experimental materials, and safety of the campus and community. Standard microbiological practices are common to all laboratories handling microorganisms. It is the responsibility of the laboratory staff and PI to develop specific procedures unique to their research facility for the safe handling and disposal of the biohazardous material(s) being utilized in the laboratory.

The following information applies to all laboratories housing biological materials. Information for specific biosafety levels is found later in this section. Most LAIs reported in the literature point to accidents during work with some type of infectious agent. These are often due to spills, splashes, or sharps/needlestick accidents. This information should be used as a starting

point for development of a laboratory specific biosafety manual for your research program or teaching laboratory. For more information, guidance, and instruction regarding any type of laboratory biosafety, please visit the UWM Biosafety Page.

Biological Risk Assessment

Biological risk assessment applies biosafety principles to the available options for handling hazardous materials and agents. The following need to be considered by the PI when evaluating a potential biohazardous agent:

1. What is the capability of the biological agent to infect and cause disease in a susceptible host?
2. How virulent is the biological agent?
3. What is the concentration and suspension volume of the agent being used in the experiment?
4. What are the probable routes of transmission?
5. What is the infective dose of the agent?
6. How stable is the agent in the environment?
7. Have there been any reports of laboratory-acquired infections (LAIs) associated with this agent?
8. What is the origin of the agent?
9. What are the procedures in place to prevent the dissemination of this agent?
10. What are the most appropriate methods to inactivate the agent?

Biohazard Signage

Biohazard labels are required for all areas or equipment that house RG-2 or higher agents or in BSL-2 or higher facilities. All labels must be purchased by the laboratory and are required for biohazardous materials. A laminated (or placed in a page protector) door sign indicating the Biosafety Level should be posted at the main entrance door(s) to laboratories and animal rooms and on equipment such as freezers, refrigerators, BSCs, incubators, and transport containers. Signage templates are available online at the UWM Safety and Health Forms Page.

Roles and Responsibilities of Personnel

The following outlines the roles and responsibilities of personnel as they pertain to biological safety at UWM. Contact the Biological Safety Program prior to initiation of a project that involves biological agents to prevent misunderstandings after work begins. This includes research, teaching, and outreach. The Biosafety Program regularly monitors research at UWM involving any biological materials, such as:

- Recombinant (transgenic) or synthetic DNA/ RNA materials, including human gene therapy
- Infectious agent research, including bacteria, viruses, fungi, prions, protozoa, and parasites, including use of proteins and other cell components from infectious agents
- Large scale propagation of cultures consisting of a volume greater than 10L or more in one vessel
- Human cells and cell culture, tissues, organs, or biological samples

- Non-human cells and cell culture, organ, or tissues, or biological samples that are infectious, potentially infectious, or recombinant
- Plants that are recombinant (transgenic), exotic, and/ or grown in association with pathogenic or recombinant microbes and/ or pathogenic or recombinant small animals (insects, etc.)
- Biological toxins

If the Biosafety Program is notified of biological research on-going at UWM that should have a completed registration form, they will reach out the PI and work with them to get this completed as soon as possible. Failure to have a registration form on file and approved can cause delays in research and teaching or denial of funding from the NIH or other governmental agencies.

Biological Safety Officer

It is the responsibility of the BSO to foster safe laboratory practices and ensure compliance with university policies, guidelines, and regulations as established by university administration, the Institutional Biosafety Committee (IBC), and regulatory agencies such as the NIH, CDC, and USDA.

Summary of Responsibilities of the BSO:

- Manage the biological safety program to ensure safety of the campus community, the public, and the environment and to ensure against accidental release of unauthorized biological materials.
- Provide training for biosafety, recombinant DNA work, and bloodborne pathogens.
- Provide initial review of all non-exempt submissions to the IBC.
- Review and approve registration (exempt and non-exempt) with the IBC as an ex-officio member.
- Manage activities and support of the Institutional Biosafety Committee, including the coordination of monthly meetings, public posting of meetings in accordance with Wisconsin Open Meetings Law, maintaining of the meeting minutes, and organizing electronic feedback from IBC personnel regarding protocol submissions.
- Work with IBC chair to appoint members, submit letters for their files, and maintain record of membership.
- Conduct regular research laboratory audits to review biological safety practices to ensure that research is conducted in a manner that protects workers and the community.
- Apply an understanding of Federal regulations and guidelines to provide education and training for UWM faculty, staff, students, and the IBC members.
- Assist with other department compliance activities, including (but not limited to): animal care, human research protections, and radiation safety.

Principal Investigator and Teaching Lead Faculty/ Staff

The principal investigator (PI) is responsible for the training, supervision, and management of their laboratory personnel and equipment. It is the PIs responsibility to understand the contents of this manual and adhere to all guidelines set forth by UWM, the State

of Wisconsin, and the U.S. Federal Government. The PI is responsible for submission of protocols for approval by the IBC, and to update their protocol every three years for re-approval by the IBC. PIs involved in teaching, research, and/or outreach activities involving biohazardous materials have the primary ethical and legal responsibility to ensure the safety of students, faculty, staff, visitors, and the environment. Professors and academic staff that act as course leads are required to train their lab and teaching personnel the same as any research PI. The PI is responsible for staying up-to-date on all current policies and procedures and is required to regularly attend training offered by the Dept. of University Safety and Assurances to be able to effectively train their own personnel.

Summary of Biosafety Responsibilities of PIs/ Teaching Lead Instructors/ Lab Managers:

- Complete a registration form and submit for approval to the IBC and NIH (as applicable) before commencing any work with biological agents that fall under Sections III-A, III-B, III-C, III-D, or III-E of the NIH Guidelines.
- Complete a registration form and submit for approval to the IBC whenever working with biological agents that fall under Sections III-F of the NIH Guidelines
- Train all persons directly involved in potentially hazardous experiments of the potential health risks presented and the safety procedures necessary to minimize exposure.
- Attend biosafety training and stay up to date on biosafety rules and regulations.
- Be responsive and cooperative in scheduling, being present for, and following up on biosafety inspections. Ensure any issues addressed during inspection are corrected in a reasonable time frame to prevent a disruption of research in the facility.
- Maintain a current record of personnel training, a current inventory, and safety information of biological agents being used in the laboratory, and post standard operating procedures (SOPs) for the required biosafety level.
- Establish SOPs for handling of potentially hazardous biological material in the event of a spill or contamination. Post these procedures in a prominent place in the laboratory.
- Immediately report any unusual incident, such as spill, break in containment, or overt contamination, to the BSO and complete an incident report.
- Post working areas and facilities with biohazard warning signs. Standardized signs will be provided by University Safety & Assurances. The PI should consult the BSO if assistance is required in placement of signs.

Laboratory Personnel: Researchers and Students

It is the responsibility of the laboratory personnel to be up to date in biosafety and chemical safety practices. Biological safety training is required every three years and should be completed prior to beginning work in the laboratory.

Summary of Biosafety Responsibilities of Lab Personnel:

- Complete all necessary training and maintain record of the training.
- Adhere to campus, state, and federal policies and regulations.
- Understand the approved protocol(s) for research.

- Perform all tasks using established safety practices and comply with the safety guidelines for the work being performed
- Report any unsafe practices to PI and, if necessary, the IBC.
- Report all accidents and injuries to the PI, emergency personnel, and University Safety and Assurances.

Rules, Regulations, and Guidelines Overview

The University of Wisconsin- Milwaukee follows the rules, regulations, and guidelines set forth by local, state, and federal agencies. Links to resources below, plus additional resources regarding biosafety, can be found at the Biological Safety Resources Page. It is expected that anyone working with biohazardous materials periodically reviews these resources to keep up-to-date on the most current policies and regulations.

National Institute of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules**:** These guidelines provide guidelines for the safe use of recombinant DNA and organisms containing recombinant DNA. The most current edition was revised in April 2019. This document also provides information regarding plant biosafety levels. Use of recombinant or synthetic nucleic acid or organisms containing these are further outlined in the section called Recombinant and Synthetic Nucleic Acids. It is important to note that it does not matter if you receive funding from the NIH; you are required to adhere to these guidelines. The federal policy requires any institution that receives federal funding from the NIH to follow the guidelines in all laboratories.

Centers for Disease Control and Prevention (CDC) and the National Institute of Health (NIH) Biosafety in Microbiological and Biomedical Laboratories (BMBL): The CDC and NIH have published this comprehensive guide that provides information pertaining to biological safety. This includes standard and special microbiological practices, safety equipment, facilities maintenance and design, and requirements for animal biosafety levels. The most current edition is the sixth edition, published in 2020. Much of the UWM Biosafety Manual has been developed from the comprehensive information provided in this guide.

State of Wisconsin Infectious Waste Regulations: These are state regulations that are utilized to ensure that we comply with State Statutes 289, 299, 500.03, and NR 526.04, under the guidance of the University of Wisconsin System, the UWM Waste Management Specialists, and contracted waste management vendors. For more information regarding waste disposal, visit the UWM Environmental Protection Page.

Occupational Safety and Health Administration Bloodborne Pathogen Standard 1910.1030:
In 1992, the Occupational Safety and Health Administration (OSHA) set a standard to address the occupational health risk associated with the exposure to human blood and other potentially infectious human materials. State and local government employees in Wisconsin are covered under the Department of Safety and Professional Services (DSPS) which serves as the enforcement agency for all OSHA standards. For more information about the UWM Bloodborne

Pathogens Training, please visit the UWM Biological Safety Resources Page, or contact the Biological Safety Program, Engelmann Hall Room 270.

Federal Select Agent Program: The Federal Select Agent Program is a collaborative effort comprised of the CDC HHS Division of Select Agents and Toxins (DSAT), and the USDA APHIS Division of Agricultural Select Agents and Toxins. The Federal Select Agent Program oversees the possession, use and transfer of biological select agents and toxins, which have the potential to pose a severe threat to public, animal or plant health or to animal or plant products. Refer below for more information regarding select agents and toxins.

A current list of HHS and USDA Select Agents and Toxins can be found on the FSAP website here: https://www.selectagents.gov/sat/list.htm

Dual Use Research of Concern (DURC): Dual Use Research of Concern is research that can reasonably be anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants/animals/environments, or national security. Research under this definition is subject to additional federal oversight.

Packaging, shipment and transportation requirements for infectious substances, diagnostic specimens, biological products and genetically modified organisms (GMOs):

- United Nations 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
- U.S. Department of Transportation 49 CFR Parts 171-177 Hazardous Materials Regulations (DOT)
- U.S. Public Health Service 42 CFR Part 72 Interstate Shipment of Etiologic Agents 1
- U.S. Postal Service 39 CFR Part 111 General Information on the U.S. Postal Service
- U.S. Department of Labor, OSHA 29 CFR 1910.1030 Bloodborne Pathogens
- U.S. Public Health Service, 42 CFR Part 71 Quarantine, Inspection, Licensing.
- In addition, the 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.

Personnel Training

Trained PIs and laboratory personnel will be the primary means to preventing an accident from occurring in the laboratory.

It is the responsibility of the PI to coordinate training for working with plants, arthropods, specific lab equipment, autoclaves, biological safety cabinets, etc. It is the responsibility of the PI to coordinate training with the Animal Care Program for their research team if working with animals. Contact the ARC manager to arrange this training. Laboratory safety training can be coordinated through the Laboratory Safety Coordinator or the Research Safety Coordinator. Radioactive materials training can be coordinated through the Radiation Safety Officer.

A training matrix with details on current biosafety training requirements is available on the Biosafety Program website here: https://uwm.edu/safety-health/biosafety-training/

A variety of biological safety trainings are available for researchers through CITI program online. Visit the Biosafety Training Page for more information about the Biosafety Training opportunities.

Medical Surveillance of Lab Personnel

It is important that personnel are regularly being monitored to identify any health concerns that could increase their risk for contracting a laboratory-acquired infection. Some agents may require vaccination prior to handling (such as personnel working in a laboratory handling blood- personnel must be offered a Hepatitis B vaccination). Accidental exposure requires an illness and injury report to be complete through the UW System HR Page by the employee and employer, as well as a follow-up with a primary-care physician for treatment for exposure.

It is the responsibility of the PI to inform their personnel and any visitors to their laboratory of risks associated with the biological materials being used in their lab- including routes of transmission, signs and symptoms of the disease, and risks for those who are immunocompromised or immunosuppressed. It is also the responsibility of the PI to put in place restricted access policies for those at elevated risk of infection. Please contact the BSO to work with your lab to determine the best safe practices.

Whenever a vaccine is available for a biological agent being studied in the laboratory, personnel should be recommended to receive the vaccine prior to working with the infectious material to minimize the risk of a laboratory-acquired infection. The PI should determine these needs and set the guidelines for their research facility. Vaccine requirements or recommendations must be included on the entry door to the lab to communicate the risks associated with the pathogen being studied.

Safe Handling of Specimens and Cultures

The following outlines the safe handling of research specimens, cultures, animals (for purposes of biosafety), and plants. Safe Practices, SOPs and more can be found online at the Biosafety Program SOPs Page.

Personal Protective Equipment (PPE)

Personal protective equipment is used to protect laboratory personnel from contact with hazardous materials and biological agents. Appropriate lab attire also helps prevent materials from being contaminated. Safety equipment, personal protective devices, and training use of these devices must be provided by the PI or laboratory supervisor prior to use. It is the responsibility of the PI to ensure personnel are selecting and using PPE appropriately. The following is a short guide to selecting the appropriate PPE. Consultation of government resources, the BSO, and other literature regarding research with the biological agents being used will help in best determining needs for the lab personnel. Additional information can be found on the UWM Laboratory Safety PPE Page.

- Eye and Face Protection: It is required that lab personnel wear safety glasses whenever procedures involving a possibility of a splash, work with low hazard chemicals, or impact hazard research is being conducted. These should optimally be performed in a BSC or fume hood (dependent on material), but when this is not available, the following is required for eye and face PPE:
    - Splash goggles: These are required whenever there is any probability (no matter how low) of splash may occur- including when cleaning with bleach solutions. The UWM bookstore carries a variety of splash goggles.
    - Full face protection (such as a face shield): Required whenever there is an anticipated splash or spray of hazardous materials or a high potential for aerosol generation. These are not a replacement for eye protection, so splash goggles should also be worn. These are available from the UWM bookstore.
    - Safety glasses: If the work involves an impact hazard, with low probability of splashes and chemicals that are of a low hazard, safety goggles are an appropriate choice. These are available from the UWM bookstore.

The eyes and mucous membranes are two potential routes of transmission of pathogens. Eye protection should always be worn in the laboratory. Dependent upon the other materials being handled, contact lenses may or may not be worn. Refer to the UWM Chemical Hygiene Plan for determining the best choice in protective eyewear for the laboratory. Additionally, the OSHA Lab Standard is a good reference.

Laboratory Attire: Coats, Aprons, Scrubs, Smocks, Gowns, Foot Covers

Laboratory attire includes coats, scrubs, smocks, gowns, and foot covers. The proper lab attire is important in prevention of accidental exposure or contamination. Lab coat selection should be made carefully. Aprons are not appropriate for the lab as long sleeves are necessary for arm protection. If splashes may occur, the lab coat should be resistant to liquids.

It is the recommendation of the BSO that all labs use disposable lab coats, which are disposed of in an autoclavable bag monthly (bi-monthly max.) and autoclaved prior to disposal. This minimizes the risk of accidental release of pathogens into the environment or contamination of lab personnel. They are readily available from the UWM bookstore and cost less than $10. If fabric lab coats are used, they should be autoclaved monthly and laundered after they have been

autoclaved. Both are available through the UWM bookstore. In student teaching laboratories, students handling biological agents are required to use disposable lab coats that are stored in the lab for the duration of the semester and then disposed of by the student in an autoclavable bag and autoclaved prior to disposal by the lab manager. Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas.

Do not go into non-research areas or other labs wearing lab coats worn in BSL lab facilities. **Do not take lab coats home to wash**; this is an accidental release risk that could expose the community and environment to pathogens. Disposable lab coats are to be made available for visitors, facilities personnel, and service workers. This is to protect them and to protect your work from contamination. Please keep extras available in the laboratory for this purpose.

Gloves

Gloves are selected by the PI and lab personnel based on the hazards involved and the type of work being done. Gloves are required whenever working with biohazards, toxic substances, and hazardous chemicals. If hot materials or dry ice are being handled, temperature resistant gloves must be worn. Work that requires an elevated level of precision necessitates the use of thin-walled gloves. It is recommended that nitrile, not latex gloves, are used for this purpose, due to the high incidence of allergies associated with the use of latex gloves. Powdered gloves are banned by the U.S. Food and Drug Administration (FDA) due to negative reactions to the starch powder.

If gloves are contaminated, they need to be changed immediately and hands should always be washed after removing gloves using soap and warm water for a minimum of 30 seconds. If you find that a glove has been torn or punctured while working with BSL-2 or higher pathogens, this needs to be documented and reported to the BSO as an accidental exposure. Visit the First Report of Biological Exposure or Release Event to report the incident and complete any applicable work the UWM HR.

When transporting potentially infectious materials between lab rooms, such as cultures or waste to be autoclaved, one gloved hand should be used to handle the infectious material and the other hand should remain ungloved to touch common surfaces, such as elevator buttons or door knobs.

Respirators

Aerosol exposure is a continued concern in laboratories. If there is a risk of aerosol exposure that cannot be mitigated using alternative procedures or containment equipment, then respiratory protection, such as a respirator, should be considered. Respirators are selected based on the hazards the researcher will encounter and the protection required. Please contact the UWM Environmental Health, Safety, and Risk Management program for assistance in determining options and appropriate types to purchase or use in your laboratory. It is strongly recommended that you seek out training in respirator use prior to using the selected one; an error could create a very dangerous situation for the researcher wearing it. If respiratory protection is

determined to be required by risk assessment, any individuals using respiratory protection must enroll in the Respiratory Protection Program and be fit tested for use of a respirator. There are a variety of options, but none have been tested against any pathogens except *Mycobacterium tuberculosis.* Review Respiratory Safety under Occupational Health on the University Safety and Assurances Page for determining needs regarding respiratory protection.

Integrated Pest Management Plan

Having an integrated pest management plan (IPM) is a major component of protecting both the researchers in the lab and the external community. Any kind of presence of any kind of insects, whether they are pests or innocuous, is of a concern for spread of pathogens as a mechanical vector on the insect. If you have any kind of pest issue, including flies, cockroaches, mice, and the like, contact the building manager, custodial staff, and professional pest controllers (if necessary) to remove the issue immediately. The best way to prevent a pest issue is to keep your laboratory facility clean, organized, and well-secured always. See Appendix G of the BMBL for more information.

Inventory Log and Physical Inventory

It is the responsibility of the PI and their research personnel to keep a complete inventory of chemicals and biological agents being used. A physical inventory should be available in the lab. A running log of biological agents and chemicals should be maintained electronically or written to minimize the risk of anything being taken without being noticed from the lab. The biological agents must be closely monitored always to be able to recognize if materials are missing, what those missing materials are, the quantity of the missing materials, and the potential hazard associated with those materials. Use the Risk Group Database, ATCC, and the Canadian Pathogen Safety Data Sheets to keep an up to date log of information regarding biological agents.

Use of Radioisotopes in Research

Some investigators may work with radioisotopes in conjunction with their work with some biohazardous agents. All work with radioisotopes conducted at UWM must be authorized through the campus Radiation Safety Program. Visit the Radiation Safety Page for more guidance, information, and training.

Use of Plants in Research or Teaching Laboratories

The use of plants in biological research only necessitates IBC approval when plants are being inoculated with plant pathogens, when transgenic plants are being researched, or when plants may be considered harmful to humans or the environment (e.g., produce toxins, are invasive). Plants have a system for containment unique to only plants (BSL1-P through BSL4-P) developed by the NIH, and can be found on pp. 129-138, Appendix P, of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, April 2016.

Transgenic Plants

Transgenic plants should be given consideration as well; identification on the door of the facility housing these plants should be posted to indicate need for preventing accidental release from the facility. A customizable sign is available on the UWM Safety and Health Forms Page. The development of transgenic plants requires a full approval of the IBC before commencing. Please complete the IBC registration form for approval.

Plant Containment and Accidental Release

Containment practices should be developed with the greenhouse director and should be approved by the IBC. An inadvertent release of plants or spill of microorganisms must be reported to the BSO and treated immediately. Complete a First Report of Biological Exposure or Release Event Form for accidental release records. Failure to submit a report of accidental release from a greenhouse research facility may result in a review by the IBC of the research and suspension of the research until the appropriate biocontainment practices are obtained. Contact the BSO for guidance, training, discussion of facilities and greenhouse, and rules and regulations involving plants and plant biocontainment. All plant policies and procedures should be made available to all working on experiments in the greenhouse in their laboratory specific safety manual.

Use of Animals in Research and Teaching Laboratories

The use of animals for pathogen research poses numerous risks and require additional safety practices. Refer to the UWM Animal Care Program for details on handling animals, becoming certified in animal care at UWM, and to submit protocols specific to handling animals. In addition to following procedures and policies set forth by the UWM Institutional Animal Care and Use Committee (IACUC), protocols must also be submitted to the IBC for approval in the event infectious pathogens are being used in animal research or if animals themselves may harbor zoonoses. Visit the UWM IBC Page for the appropriate forms to file with the IBC.

In the event an investigator is bitten or scratched by an animal infected with a pathogen, an accidental biological release form must be filed with the IBC, in addition to the accident forms that are filed with animal care and the University. Individuals who handle bedding and animal waste must also take additional precautions and must follow the policies set forth by the animal care program, as well as policies in place for the appropriate biosafety level based on the animal hazards. All bedding from BSL-2 animal research labs must be autoclaved prior to disposal. Contact the BSO and animal care to determine how to develop a protocol for handling animals and pathogen(s) in the laboratory prior to their use.

Laboratory Animal Facilities

Animal facilities are assigned to a containment level based on their risk assessment and risk group, just like a standard biological laboratory. There are additional factors that need to be considered when working in animal facilities, including:

- Route of excretion of agents (if any)

- Zoonotic diseases to which the animals are susceptible and humans are susceptible
- Natural parasites that could be a problem for the animals used
- Nature of the animals (do they bite, scratch, spit, etc.)
- Possible allergen considerations
- Design features required for safety and containment

Working with Genetically Modified Animals

The National Institutes of Health (NIH) requires review of all recombinant DNA research proposals that fall under their scope of approval. The University of Wisconsin-Milwaukee requires all biological research that involves genetic modifications to be filed using an IBC protocol submission, regardless of whether it is exempt from NIH oversight. As a condition for NIH funding of recombinant or synthetic nucleic acid molecule research, institutions shall ensure that such research conducted at or sponsored by the institution, irrespective of the source of funding shall comply with the NIH Guidelines (NIH, 2016, p. 10). Only a limited number of experiments are NIH Exempt. Visit the IBC Page to learn more about work with Genetically-Modified Animals.

Invertebrate Research Special Considerations

Invertebrates are assigned to an Animal Biosafety Level similar to vertebrate animals, but have additional considerations. Even if arthropods are not infected with a human pathogen, they can become a risk to the external environment if they get outside of the lab, especially if they can complete a transmission cycle for a disease in which they act as a biological vector. Invertebrates can also act as mechanical vectors and transmit pathogens, such as house fly transmission of *E.coli* or *Salmonella*, and should therefore be tightly managed. Please contact University Safety & Assurances for assistance with determining the needs for working with invertebrates. Work with recombinant DNA or synthetic nucleic acid-modified arthropods requires IBC approval prior to commencing work. Visit the IBC Page to learn more about work with Genetically-Modified Animals.

Aquatic Animal Special Considerations

Aquatic pathogens have different considerations than those of the terrestrial animals and their pathogens. Biocontainment necessitates a separate set of considerations, because they are a "wet" facility, which can increase the risk of spread of potential pathogens. There are no U.S. national standards set forth for aquatic biocontainment systems. Some concerns related to work in aquatic systems include generation of aerosols from water spray, improper sterilization of equipment which could contaminate multiple tanks, centralized water that could introduce pathogens to water and re-circulate throughout the facility, and outside personnel tracking in pathogens that could spread to tanks. The following are recommendations based on Canadian standards and current recommendations in literature (Bailey, 2008) (CCAC, 2005).

- Containment facility:

    - Physical separation from other holding rooms and facilities.
    - Quarantine: separate area and protocol for incoming fish from external environment to prevent spread of disease.
    - All entry and exit points have foot baths or disinfection mats and hand wash stations.
    - Controlled access into the secured entry areas.
    - Separate clothing transfer and locker area adjacent to facility for preparing for entry to lab.
    - Location and design should prevent accidental release in event of a natural disaster.
    - Pest control management plan developed for prevention of introduction of pests into facility.
    - Minimize use of materials that can withstand rigorous decontamination.
    - Immersion disinfection buckets should be available for regular sanitation of room-specific equipment.
- Water source
    - Water system should be independent and the distribution lines should be separated for zones within the fish room to minimize spread of anything through the water source.
- Tanks
    - Closures and seals should be installed and maintained to prevent spills or splashing.
- Air supply
    - Sterile air supply when possible, including use of UV air sterilizers
- General Design
    - Mechanical and accessory systems are accessible without having to enter the containment area from outside.
    - Room surfaces - including floor, walls, and ceiling - must be easy to sanitize, smooth, and impervious to moisture.
    - Ventilation and temperature control permits drying conditions and air mixing but prevents airborne pathogens from escaping through air movement or condensation.
    - Automated system to monitor ventilation and temperature.
    - Fail-safe backup pathogen control in event of failure of automated system.
    - Water collected into treatment tanks and disinfected; release meets local, state, and federal regulations.
    - Flood drains routed to holding reservoir to process water with disinfectant system that has an alarm system to monitor it.
    - Doors and walls are sealed with raised dams along doors and floors, which can hold water in containment room in the event of a leak or spill.
    - Plumbing prevents backflow from animal holding tanks and effluent handling systems.
    - Pipes are hard-plumbed with removable access points to clean and do QC checks following research studies. Exposed piping should be easily accessible for cleaning.

  - Electrical fixtures should be ground fault interrupted, have gaskets, be sanitized, and be provided with an emergency back-up power source.
  - Wall switches are sealed and waterproof so they can be disinfected.
  - Ceiling fixtures have gaskets, are waterproof, and can be sanitized.
  - Outlets are positioned well above floor level and water supply lines.
  - Anywhere that could leave potential for water to penetrate building should be caulked, sealed, and have a gasket.
  - Spill kits should be stored up and away from the floor and from water sources.

- Waste Disposal
  - A means of sterile disposal of carcasses and other contaminated biological wastes, including incineration, autoclaving or rendering, should be considered following animal care rules, biological safety procedures, and facility safety considerations.

Recombinant DNA and Synthetic Nucleic Acid Use in Teaching and Research

All recombinant DNA (rDNA) research proposals, regardless of funding sources, require the PI to determine the physical and biological containment level, complete an IBC protocol submission, and receive approval from the IBC. There are six categories of experiments covered by the NIH guidelines. The following is a summary based on these guidelines. The comprehensive NIH Guidelines for Research Involving Recombinant DNA or Synthetic Nucleic Acid Molecules was most recently updated in April 2019.

Research that Requires NIH Approval (and IBC)

Section III-A: Intentional Drug Resistance in Microorganisms

Per Section III-A of the NIH Guidelines, experiments falling under this category require the approval of the NIH Director prior to commencing the research. Experiments that fall in this category include those that involve the transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally from their environment, specifically if this could compromise the ability to control the disease agent.

The UWM IBC will not approve a protocol that falls in this category until it has received approval from the NIH Director, which should be submitted with the IBC Registration Form. After reading and reviewing the NIH Guidelines, contact University Safety & Assurances if your research falls in this category for assistance.

Section III-B: Cloning of Toxin Molecules

Per Section III-B of the NIH Guidelines, research that falls in this category cannot be initiated without submission and approval by the NIH Office of Science Policy (OSP). Review the NIH Guidelines for exceptions. Experiments in this category include experiments involving the cloning of toxin molecules, including botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin.

The UWM IBC will not approve a protocol that falls in this category until it has received approval from the NIH, which should be submitted with the IBC Registration Form. After reading and reviewing the NIH Guidelines, contact University Safety & Assurances if your research falls in this category for assistance.

Research that Requires IBC Approval Before Initiation

Section III-C: Human Gene Transfer

Section III-C experiments cover experiments involving human gene transfer. In addition to having IBC approval, these experiments require Institutional Review Board (IRB) approval. These include all experiments that involve the deliberate transfer of rDNA or synthetic nucleic acid molecules, or DNA/ RNA derived from rDNA or synthetic nucleic acid molecules to one or more human research subjects.

See the IRB page for more details regarding IRB approvals. An IBC registration form needs to be approved. After reading and reviewing the NIH Guidelines, contact University Safety & Assurances if your research falls in this category for assistance.

Section III- D: RG2/3/4 Agents and DNA, Viruses in tissue culture, Animals, Plants, Large-Scale Cultures, and Influenza Viruses

Section III-D covers whole animal or plant experiments, experiments involving the use of infectious DNA or RNA viruses, or use of defective DNA or RNA viruses in the presence of a helper virus in tissue culture, experiments involving DNA from Risk Group 2, 3 or 4 agents, experiments involving greater than 10 liters of culture, and experiments involving Influenza viruses. Prior to the commencing an experiment in this section, the PI must submit a Protocol to the Institutional Biosafety Committee. The IBC reviews and approves all experiments in this category prior to initiation. Additionally, IACUC will require filing of appropriate documentation for approval for animal experiments.

Section III-E: Require Approval Concurrent with Research

Section III-E experiments include experiments that do not fall under the section III-A, III-B, III-C, III-D, or III-F, and fall in one of the following: Experiments that involve forming rDNA or synthetic nucleic acids containing no more than two-thirds of the genome of any eukaryotic virus, genetically modified plants, transgenic rodents (ABSL-1 only), breeding of transgenic rodents (ABSL-1 only). The Institutional Biosafety Committee reviews and approves all such proposals, but Institutional Biosafety Committee approval prior to initiation of the experiment is not required. PIs must submit a Biosafety Protocol Form before commencing any work in this category.

Section III-F: NIH Exempt Experiments

Section III-F experiments are exempt from the NIH Guidelines; however; they must still be registered with the IBC who will verify the exempt status of the registration. It is the responsibility of the PI to file the paperwork in a timely manner in accordance with NIH Guidelines. See the IBC Page for the appropriate registration forms.

Transport and Shipping of Biological Materials

The proper packaging, labeling, and transportation methods are essential in minimizing an accidental exposure or release of biological material on campus during transport. The following should be considered when transporting and shipping biological materials around campus.

Transportation of Biological Materials

- Primary containment: Select an appropriate primary container that is designed for transporting the material. Do not use food containers or other containers that have not been designed for the explicit use as a laboratory storage container.
- Primary sample containers should be placed in a secondary container for transport. For example, if a bag full of inoculated culture plates need to be transported to the autoclave for disposal, the bag should be placed in a labeled biohazard container. Do not use red biohazard bags for disposal unless necessary- they cannot go in the regular garbage and must go through medical waste.
- Bubble wrap, newspaper, etc. may be used inside the secondary containment to act as shock-absorbers and to stabilize the primary containers from rupturing due to shifting around in the secondary containment.
- Secondary containers should be clearly labeled with a description of contents and an emergency contact name and phone number. If it is a biohazard, a biohazard label should also be affixed to the container.
- If the material must be transferred to another part of campus that is further than walking distance and must be transported in a vehicle, a UWM vehicle should be used for transport. When transporting in a vehicle, secure the container using bungee cords, belts, or other means.

Shipment of Biological Materials

Shipping hazardous materials requires training for shipping the materials and falls under U.S. Department of Transportation (DOT), International Air Cargo Organization (IACO), and International Airport Transport Association (IATA) federal regulations. Contact the Department of University Safety and Assurances to determine training needs and safe handling practices.

If the material being moved off-campus is biohazardous waste, it must be handled by approved vendors or the Waste Management Specialist. It should not be moved by researchers or PIs from the laboratory. Please contact Waste Management for additional assistance.

Chapter 3: BSL-1 Laboratory Procedures

The following are some key techniques and safety considerations for work at BSL-1. Remember that risk group organisms generally fall into their parallel containment level; so RG1 organisms most likely need to be handled at BSL-1 containment. It is the responsibility of the PI to determine the appropriate BSL and submit a complete IBC registration form.

BSL-1 Standard Microbiological Technique and Hygiene
(UW Biosafety, 2017)

The following are recommendations for BSL-1 labs based on the BMBL 5th edition. Please note that there may be additions to this list, and it is only intended to be a starting point for determining safety needs in the laboratory. A registration form should be filed with the IBC for BSL-1 to ensure there is a record of research with UWM.

- ✓ Do not eat, drink, chew gum, use tobacco, apply cosmetics, or handle contact lenses in the laboratory.
- ✓ Do not store food for human consumption in the laboratory.
- ✓ Do not store items such as coats, handbags, dishes, or other personal items in the laboratory.
- ✓ Wash hands frequently after handling infectious materials, after removing personal protective equipment (PPE), and always before leaving the laboratory.
- ✓ Keep hands away from mouth, nose, eyes, face, and hair.
- ✓ Do not pipet by mouth.
- ✓ Wear pants and close-toed shoes in the laboratory.
- ✓ Wear the appropriate PPE for BSL-1 containment: at a minimum, a lab coat; with gloves, eye protection, respiratory protection, face protection, etc. used when appropriate.
- ✓ Keep laboratory doors closed and locked.
- ✓ Aerosol generating procedures should not be performed in equipment corridors not located in the laboratory suite.
- ✓ Plants or animals not associated with the research being conducted are not permitted in the laboratory.

ABSL-1 Facility

Most stock animals will fall into this level after quarantine. In addition, any animals inoculated with Risk Group 1 (RG1) agents fall in this level of containment. The following are items that must be followed in an ABSL-1 lab:

- Approval from the UWM Institutional Animal Care and Use Committee (IACUC) and the Animal Care Program.
- Training with safe handling of animals, coordinated through animal care (visit their UWM page for more information).

- Research lab-specific biosafety manual (separate from this manual), containing: specific PPE, location of supplies, training requirements for personnel, waste handling practices, autoclave procedures, operation and decontamination of equipment used in facility, disinfectants to use in lab (appropriate concentration, contact time and shelf life), and any of the SOPs for research. It is the responsibility of the PI to coordinate training with the on-site veterinarian and animal care manager and to ensure that personnel have been adequately trained in biosafety practices. PIs and their personnel are required to follow the policies set forth by the UWM Animal Care Program. You will not be allowed to do animal research in the animal facility without IACUC and Animal Care approvals.
- All lab personnel handling animals must go through the Animal Care Program training. Contact the Animal Care Manager for more information and to coordinate the training. The PI must ensure that all lab personnel have additional training in laboratory safety, biological safety, and bloodborne pathogens. Contact the Department of University Safety and Assurances to set up training.
- All personnel involved in animal research are required to complete an Occupational Health Animal Care Program Questionnaire. This is available on the Animal Care Occupational Health Page.
- Door signage: Entrances to all animal areas must have signage that indicates restricted access, applicable occupational health requirements, PPE, contact information for the PI or their lab manager, and any specific procedures to follow for entry and exit.
- The animal facilities are tightly controlled. Animals used in research at UWM are housed in approved Animal Research Facilities that are closely monitored by the campus veterinarian. The access to these facilities is restricted and are to remain locked always.
- PPE: Please contact the Animal Care Program to learn about PPE options that they have set forth for use in research facilities.
- Minimize splashes and aerosols through using safety features on equipment, mechanical pipettors, use of a biological safety cabinet, etc. No mouth pipetting is allowed.
- Handwashing must be done before leaving the laboratory or touching any common use surfaces.
- Sharps must be disposed of in approved containers and removed for disposal through coordination with the Waste Management Specialist. Contact the Dept. of University Safety and Assurances for coordination of sharps removal.
- Work surfaces must be decontaminated after work is complete to minimize the risk of accidental release. Work with the Animal Care Program to select an appropriate disinfectant.
- No plants or animals that are not part of the research should be in the facility.
- Contact the Dept. of University Safety and Assurances if there are ever issues with pest management.
- All cultures, stocks, etc. are to be decontaminated before disposal. The Animal Care Program will coordinate use of the autoclave. If additional assistance is needed, contact University Safety & Assurances. Any time materials are being moved out of a room, they need to be contained in a leak proof secondary container and preferably only moved using a cart.
- A biological safety cabinet (BSC) is not generally required in an ABSL-1 lab. But, risk is minimized if one is available for use. The Animal Care Program has specific requirements for entry and exit of animal research facilities. Long hair must be tied back.

Goggles must be worn when there's a splash risk. If lab personnel wear contact lenses, safety glasses or other eye protection should be worn to prevent airborne particles from encountering the eyes. Gloves are required and should only be disposed of in the animal room.

- Secondary barriers:
    - Located in area of a building not open to unrestricted personnel.
    - Self-closing, self-locking external doors.
    - Doors should remain closed (do not prop open).
    - Sink must be available for handwashing with soap and paper towel available.
    - Floors- slip-resistant, impervious to liquids, chemical resistant.
    - Bench tops- impervious to water, easy to clean, non-porous, chemical resistant.
    - Chairs- non-porous material, easily cleaned and disinfected.
    - Windows- if the facility has windows, they must be break resistant. If they can open, screens must be put on them to prevent accidental release.
    - Airflow- inward flow of air without recirculation of exhaust air. Contact Animal Care for details.
    - Proper lighting is necessary to keep animals comfortable and to keep the research area safe when working in it.
    - Floor drain traps should be filled with water or disinfectant.
    - Cages- see Animal Care for details. There are automatic cage washers available.
    - Eyewash stations and chemical shower must be readily available.

BSL-1 P Facility Overview

BSL1-P is designed to provide a moderate level of containment for plant experiments for which there is convincing biological evidence that precludes the possibility of survival, transfer, or dissemination of recombinant DNA into the environment, or in which there is no recognizable and predictable risk to the environment in the event of accidental release.

Chapter 4: BSL-2 Laboratory Procedures

BSL-2 Standard Microbiological Practices
(UW Biosafety, 2017)

Many laboratories operate at BSL-2. Any research requiring this level of containment requires a filed and approved registration form with the IBC.

- ✓ A site-specific laboratory manual containing SOPs, activities performed, and a copy of this manual should be available on site.
- ✓ Employees and students should be trained and informed of biohazards.
- ✓ Plan and organize materials and equipment before starting work.
- ✓ Keep laboratory doors closed; limit access to necessary personnel.
- ✓ Post a biohazard sign at the laboratory entrance when RG2 pathogens are being used. Identify the agent in use, and the appropriate emergency contact personnel.
- ✓ A lab coat and eye protection are required at a minimum for laboratory entry. A fully fastened lab coat, gloves, and eye protection are required when working with all RG2 organisms, human blood, fluid, or tissues, or human cells.
- ✓ Remove all protective clothing, including gloves, before exiting the laboratory and wash hands thoroughly.
- ✓ When practical, perform aerosolizing procedures in a certified biological safety cabinet (BSC). Some equipment cannot be handled in a BSC, because it will disturb the air curtain, so this may not always be an option.
- ✓ Centrifuge materials in unbreakable, closable tubes. Use a rotor with a sealed head or safety cups, and load it in a BSC. After centrifugation, open the rotor and tubes in a BSC.
- ✓ Avoid using hypodermic needles whenever possible. If they must be used, discard in approved sharps containers without removing or re-capping needles. Refer to the bloodborne pathogens plan for more information.
- ✓ Cover countertops where biohazardous materials will be used with plastic-backed disposable paper to absorb spills; discard after work session.
- ✓ Routinely wipe work surfaces with an appropriate disinfectant after experiments and immediately after spills. Routinely decontaminate all infected materials by appropriate methods before disposal.
- ✓ Report all accidents and spills to the PI or laboratory safety manager. All laboratory personnel should be familiar with the emergency spill protocol, where/ how to clean up equipment, and how to report the incident.
- ✓ Good housekeeping practices are essential in laboratories engaged in work with infectious microorganisms. Establish a habit of weekly cleaning.
- ✓ Be sure to advise custodial staff of hazardous areas and places they are not to enter. Use appropriate warning signs.

ABSL-2 Facility

All procedures and protocols mentioned in the ABSL-1 facility above are required in ABSL-2 facilities. Animals infected with RG2 pathogens typically require ABSL-2 containment. In

addition to the requirements for ABSL-1 facilities listed above, additional components include the following:

- Door signage: Entrances to all animal areas must have signage that indicates restricted access, applicable occupational health requirements, PPE, contact information for the PI or their lab manager, and any specific procedures to follow for entry and exit. Additionally, the lab entrance must have an Animal Biosafety Level 2 door sign. Signs must include any occupational health requirements, PPE requirements, contact information, and entry/ exit procedures.
- Medical surveillance of animals, lab personnel, and support personnel is required.
- A currently certified biological safety cabinet (BSC) is **required** when there is any potential for creating infectious aerosols, including (but not limited to): pipetting, centrifuging, sonicating, blending, mixing, shaking, opening of containers, intranasal inoculation of animals, and harvesting tissues. Centrifugation can be done outside of a BSC if it has safety cups or sealed rotors.
- All wastes must be disinfected- including all cultures, stocks, wastes, carcasses, tissues, bedding, feed, sharps, etc. - before disposal and transported in a secondary container with a biohazard label.
- Lab equipment must be decontaminated after every procedure.
- A Biological Spill Kit must be housed in the lab facility. This includes: disinfectant, waste container(s), PPE, tools for picking up broken glass (tongs, dustpan, broom), spill-cleanup procedures, and barrier tape.
- In addition to a BSC, it is required that cages are washed in a cage washer, windows are be sealed (cannot open to outside), and an autoclave be available in the facility (not necessarily in the room).

BSL2-P Overview

BSL2-P is designed to provide a greater level of containment for experiments involving plants and certain associated organisms for which there is a recognized possibility of survival, transmission, or dissemination of recombinant DNA-containing organisms, but the consequence of an inadvertent release has a predictably minimal biological impact.

Chapter 5: BSL-3 and 4 Recommendations

BSL-3 Recommendations

There are currently no RG3 organisms necessitating a BSL-3 facility at UWM; however, some RG2 organisms may necessitate BSL-3 containment depending on specific strains or procedures used. The UWM biosafety manual will be updated to reflect BSL-3 policies and procedures if needed for new research on campus. It is the responsibility of a PI's home Department or School/College to provide facilities for accommodating BSL-3 research if needed.

Some key elements to keep in mind regarding BSL-3 are as follows:

- ✓ Special consideration for all sharps required.
- ✓ Elimination or reduction of use of glassware in laboratory.
- ✓ Hazard communication and training for microbes handled in laboratory.
- ✓ A special BSL-3 laboratory-specific manual is required.

- ✓ All procedures for infectious materials must be done within a BSL-3 approved BSC.
- ✓ Researchers are required to wear solid-front gowns, scrub suits, or coveralls that are not worn outside of the laboratory.
- ✓ Eye and face protection is worn for anticipated splashes.
- ✓ Gloves are always worn in the laboratory and disposed of in the laboratory.
- ✓ The laboratory doors must be self-closing and have restricted access.
- ✓ The laboratory has a ducted ventilation system and personnel must be able to identify direction of airflow.
- ✓ Facility design will include decontamination, engineering controls, operational parameters, SOPs, and manuals specific to the BSL-3 laboratory space.

ABSL-3 Facility Guidelines

ABSL-3 facilities are suited for animals infected with RG3 agents. Currently there are no active ABSL-3 facilities at UWM. Should an ABSL-3 facility be needed, it should be developed by the PI in coordination with University Safety & Assurances, and more specific guidelines related to ABSL-3 requirements will be developed. In addition to ABSL-1 and ABSL-2 requirements, ABSL-3 facilities include the following:

- Door signage for ABSL-3: Entrances to all animal areas must have signage that indicates restricted access, applicable occupational health requirements, PPE, contact information for the PI or their lab manager, and any specific procedures to follow for entry and exit.
- Very controlled access (minimal entry/ exit by personnel).
- Lab coats/ gowns/ uniforms required, face protection and splash goggles required when there is any potential for splash, respirators must be worn as appropriate, hair should be up in a hair net. Disposable PPE should be disposed of in an appropriate biohazard container. Two pairs of gloves should be worn as appropriate. Reusable PPE should be decontaminated after each use.
- Containment caging systems should be used to reduce the risk of infectious aerosols from encountering animals and bedding. They must be ventilated to prevent escape of microbes from the cage.
- Exhaust systems should be sealed and HEPA filtered with an alarm system for malfunctions.
- Wastes are to only be decontaminated in the facility and transported to waste disposal using an approved secondary container labeled “BIOHAZARD” with a biohazard symbol.
- Secondary Barriers:
    - Entry is through a double-door entry.
    - Showers should be considered; determine need through risk assessment prior to set-up of facility.
    - Sinks are to be hands-free or automatically operated and stocked with soap and water, located near the exit. If there are segregated areas for manipulation of infected animals or materials, there needs to be a sink available at each exit. Sink traps must be filled with water or disinfectant.
    - External windows are discouraged. If there are windows, they must be break-resistant and sealed.

- Ventilation requires careful monitoring- must be inward flow without recirculation of exhaust air, exhaust must be dispersed away from air intake or occupied areas, or it must be HEPA filtered.
- Design and operational procedures must have written documentation, and facility must be tested prior to commencing research, and annually thereafter to verify that all ABSL-3 parameters are being met.

BSL3-P and BSL4-P Overview

BSL-3 and BSL4-P describe additional containment conditions for research with plants and certain pathogens and other organisms that require special containment because of their recognized potential for significant detrimental impact on managed or natural ecosystems (UW Biosafety, 2017). UWM currently does not have any facilities for working in BSL3-P or BSL4-P containment.

BSL-4 Recommendations

UWM does not allow RG4 organisms or biohazardous materials requiring BSL-4 containment or facilities on the campus or at any of its outlying units or off-campus locations. This type of work may only be conducted at approved and certified BSL-4 facilities, such as those at the Centers for Disease Control and Prevention in Atlanta, GA, and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRID) in Fort Detrick, MD.

Chapter 6: Equipment and Facility Management

Laboratory Design

As a pathogen increases in its virulence, its physical containment level also increases. In addition to PPE, it is imperative to have proper safety equipment, as this provides the primary means of containment of a pathogen. The laboratory design is secondary to the equipment. Please contact the BSO and University Safety and Assurances when developing renovations, additions, or new facilities. Additional information can be found in the BMBL.

Laboratory Ventilation

Be sure you know the differences between chemical fume hoods, clean benches, biological safety cabinets, and isolators (UW Biosafety, 2017). Several types of ventilation provide distinct types of protection. These protections include:

- *Product protection:* protection of product/ experiment.
- *Personal protection:* protection of personnel working in laboratory.
- *Environmental protection:* protection of the environment outside of the laboratory.

Please contact University Safety & Assurances to determine your laboratory facility needs. You may require both a chemical fume hood and a biological safety cabinet. This can be determined through risk assessment.

Laboratory air pressure must be lower than that in adjacent spaces for laboratory containment to be effective. Negative air pressure is what ensures that air stays in the lab and doesn't carry pathogens into hallways or adjacent spaces. The primary way to effectively maintain this pressure is keep the doors to the laboratory closed. Ensure that exhaust air from biohazardous laboratories does not recirculate in the building, but rather is ducted to the outside only and leaves through a stack remote from the building air intake. The use of HEPA filters may be employed in particularly hazardous facilities.

Chemical Fume Hoods

Chemical fume hoods are not typically used for biological agents. They are intended for work with chemical hazards. They exhaust air to the outside, do not filter air, and directly draw air from the laboratory environment. Do not use a chemical fume hood in place of a biological safety cabinet. For more details on chemical fume hoods, refer to the UWM Chemical Hygiene Plan or contact the laboratory safety coordinator.

Clean Benches, Clean Air Devices

Clean benches and clean air devices provide product protection. The airflow from benches and devices goes through a HEPA filter, and discharged air will then flow back across the work surface and directly into the worker. They are appropriate choices for working with products that are not hazardous but need to remain contaminant free, when preparing nonhazardous mixtures and biological media, and for particulate-free assembly of sterile

equipment and electronic devices. They are not appropriate for working with pathogens and should never be used for work with potentially hazardous biological or chemical materials.

Biological Safety Cabinet

Biological Safety Cabinets (BSC) are designed to work with biological hazards and allow for the handling of products in a clean environment. BSCs are designed with an inward flow to protect the researcher, HEPA-filtration exhaust to protect the surrounding environment, and HEPA-filtered supply air for protection of the product (except for Class I) (UW Biosafety, 2017).

There are three classes of BSCs: Class I, Class II (Type A1/ A2/ B1/ B2), and Class III (glove box, isolator). BSCs are the primary means of containment in working with infectious microorganisms. Details on the proper selection, installation, and use of Biological Safety Cabinets are provided in the BMBL. If a BSC is being used in a laboratory, it should be certified. It is required to be certified annually if it is used for work at BSL2 or BSL3 containment. Development of a SOP for the BSC used in your research facility is required and will be requested when registering research with the IBC. A template is available for use on the UWM Safety and Health Forms Page.

Handling of Environmental, Clinical, and Pathological Specimens

Every environmental, clinical, and pathological specimen taken from a human, animal, or plant should be considered a biological hazard and handled following guidelines for their safe handling. In laboratories that handle human blood or body fluids, Universal Precautions must be followed. All personnel handling human blood or body fluids are required to complete bloodborne pathogen training and are advised to possess the HBV vaccine.

Laboratories working with human blood or other potentially infectious materials (OPIM) must have a written exposure control plan in place (UW Biosafety, 2017). OPIM include semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva, and any other body fluids that may be mixed in origin. Additionally, any unfixed human tissues, organs, primary cell cultures, cultures containing HIV or HBV, human stem cells, and experimental animals infected with HIV or HBV are included in these regulations. Contact the Biological Safety Officer for more information regarding regulations and regulatory requirements for the safe handling of PIM.

Cultures

Aerosol formation from culture samples continues to be an area of concern when performing routine procedures in the laboratory. The following are means by which cultures could be released via aerosol formation (UW Biosafety, 2017):

- Removing stoppers from culture vessels
- Opening vessels after vortexing or shaking
- Flame-sterilizing utensils
- Electroporation

- Centrifugation
- Sonication, homogenization, blending or grinding tissues
- Expelling final drop from pipette

Cultures should be handled carefully to avoid aerosols. When using centrifugation, ensure that the tubes and rotors are gasket-sealable. Microplate lids need to be sealed with tape or use an adhesive backed Mylar film in place of the lid. Use a fume hood or BSC to load, remove, and open tubes, plates, and rotors. Take care to minimize risk of accidental spilling on benches, floors, and during transport.

Research equipment used to manipulate infectious materials needs to be evaluated to best determine secondary containment, as well as issues one may encounter with decontamination. Development of a SOP for disinfection of each item of equipment may be necessary and should be included in the laboratory safety manual for the research facility. It is the responsibility of the PI to train their personnel in safe use of all equipment.

Use of human or animal cell cultures requires an additional set of considerations, and protocols on safe handling of these cell cultures should be included in the laboratory safety manual. Cell or tissue cultures themselves are typically not biohazardous; however, when inoculated with a pathogen experimentally or naturally, they are classified and handled at the same biosafety level as the agent. At UWM, BSL-2 containment is required for all cell lines of human origin and for all human clinical material; there are no exceptions to this rule. Any cell lines exposed to oncogenic viruses, primate cell cultures originating from lymphoid or tumor tissues, and all nonhuman primate tissues are to be handled using BSL-2 practices. Do not use a laminar flow cabinet to perform manipulations that could create aerosols; only a certified BSC is appropriate for these materials. Post a labeled biohazard symbol at the door to the laboratory. The PI is required to ensure that all proper containment is available and training occurs before handling of these types of cultures.

Long-Term Storage of Cultures

Some laboratories maintain cultures and/or archival samples of biohazardous agents. In these instances, a “generic” registration for maintenance and storage of reference or other samples/cultures should be submitted to the biosafety program office. An inventory of agents should be attached and updated whenever significant changes are made, either additions or deletions. Significant changes include additional species or additional strains of inventoried agents that demonstrate a need for more stringent containment.

Equipment

Centrifuge Equipment

Centrifuges are commonly used in laboratories that handle biohazardous materials. Centrifuges can create aerosols if there is a mechanical failure. Aerosols can be generated in the process of filling centrifuge tubes, removing the supernatant from centrifuge tubes, and resuspending pellets. The greatest concern with centrifugation is if a tube breaks during the

centrifugation process, which can cause a release of aerosols upon opening the centrifuge. These risks can be minimized by using sealed tubes and safety buckets that are sealed with O-rings; filling tubes, rotors, and accessories inside of a BSC; properly balancing the buckets, tubes, and rotors; and working in a BSC to decant supernatant or re-suspend pellets. The following are procedures that should be implemented and documented in the laboratory-specific biosafety manual. These procedures will help minimize the risk of injury or accidental exposure from use of a centrifuge.

- Review the operating manual before using the centrifuge. Keep the manual with the biosafety manual in lab.
- Do not deviate from the manufacturer instructions; maintain and operate the centrifuge following the manufacturer instructions only.
- Examine the centrifuge on a regular basis for damage or poor maintenance, and keep a log of use and maintenance.
- All users should be trained by the PI prior to first use, and an SOP should be made available for the lab.
- Post operating instructions that include safety precautions on the unit.
- Keep the vendor information handy in case an issue arises so they can be contacted quickly.

The PI or laboratory manage must document safety training for use of a centrifuge. In this documentation, include the following:

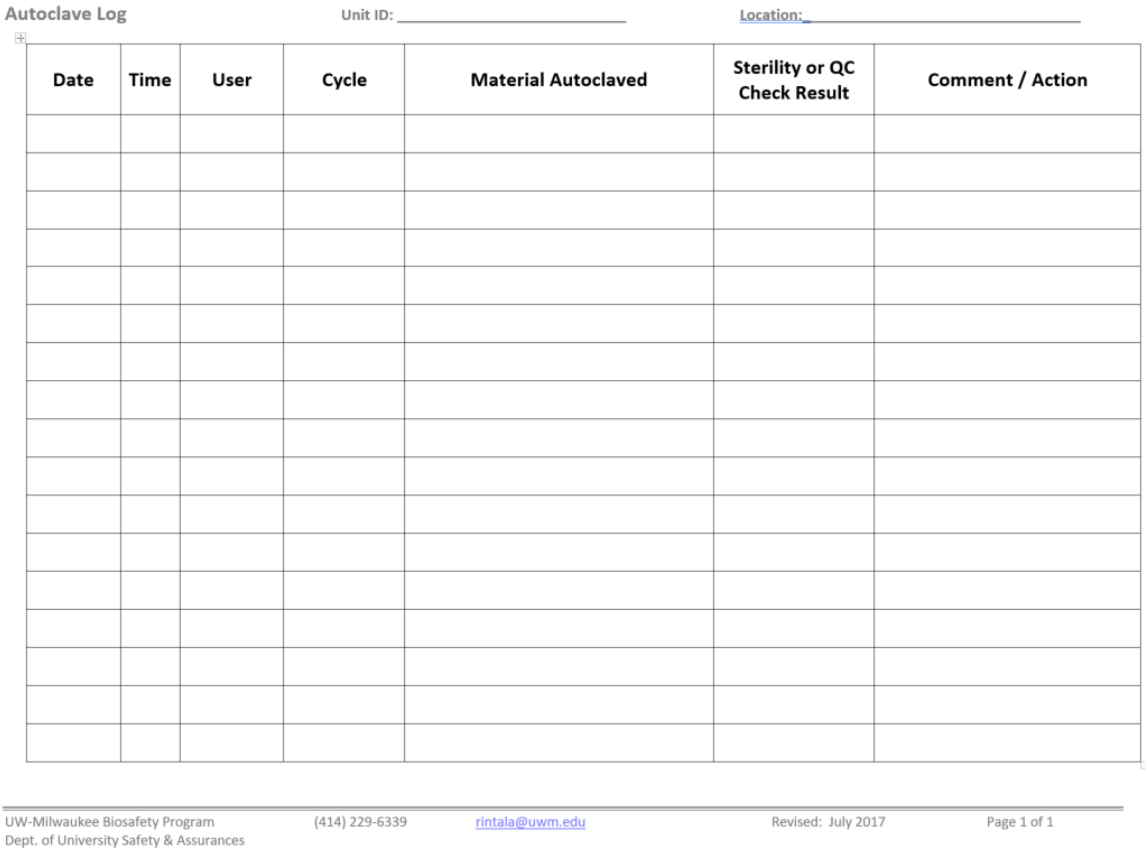
Possible routes of exposure of material used in centrifuge (e.g., skin, eyes, inhalation)
Proper PPE and engineering controls
Safe use
SOP
Date researcher(s) received training
Name of researcher(s)
Signature of researcher(s)

Autoclaves

Autoclaving, or steam sterilization, is the use of pressurized steam to kill infectious agents. This form of "wet heat" is an effective means for sterilizing standard laboratory equipment and decontaminating biohazardous waste generated in teaching and research laboratories. Autoclave use should only be performed by those trained in the use of the autoclave. It is the responsibility of the PI to ensure that all lab personnel know how to properly use the autoclave for their facility. Lab managers need to ensure that teaching assistants know how to use the autoclave properly as well.

Standard autoclave cycles for sterilization/decontamination include steam pressure of about 15 psi to and a temperature of 121°C for 30-60 minutes, depending on the material being autoclaved. In addition to proper function of the autoclave, preventing entrapment of air is important to ensure all the material is properly being sterilized. An SOP needs to be in place for labs that use an autoclave. Each autoclave on campus is required to maintain an autoclave use

log. The autoclave log should include the columns indicated in Figure 1 below. A Word version and fillable PDF of this form is available under Biological Forms on the University Safety and Assurances website.



Autoclave Log Unit ID: _____ Location: _____

Date	Time	User	Cycle	Material Autoclaved	Sterility or QC Check Result	Comment / Action

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Dept. of University Safety & Assurances

Figure 1 Sample Autoclave Log.

All autoclavable waste should be in approved bags and a rigid, autoclavable secondary container. Orange or clear autoclavable biohazard bags should be used. Follow the guidelines provided by the manufacturer for setting cycle time. Keep a log for each autoclave. Check the autoclave monthly using a sterilizing indicator (biological or chemical). If the waste being autoclaved is relatively dry waste (e.g., agar plates), add a cup of water to the bag before autoclaving and keep the bag slightly open while autoclaving; otherwise the steam will not penetrate the waste completely, leaving potential pathogens alive. After the cycle has been completed, let the waste cool before removing from the autoclave. If autoclaved waste is in a bag, seal it after removing from the autoclave. Treated autoclave bags should go into an opaque black garbage bag and then be moved to the general trash.

Wear heat-resistant gloves when loading and unloading the autoclave. Under the heat-resistant gloves, wear fluid-resistant gloves before autoclaving to prevent hands from being contaminated from untreated waste. Wear a lab coat to protect clothing, and splash goggles if a splash hazard is present. A general standard operating procedure (SOP) for autoclave use that may be used in your facility can be found on the UWM Biosafety Program website.

Flow Cytometers

Teaching and research laboratories using flow cytometers should operate under the same containment conditions in which the cells would normally be handled. For example, if human cells are being sorted in a flow cytometer, they need to be handled at a BSL-2 containment. If the cells being sorted are potentially infectious unfixed cells, potentially infectious aerosols will be generated when using a flow cytometer, particularly if the cell sorter fails to operate in a normal manner. Higher speeds generate higher numbers of aerosols. Wear the proper PPE when working with a flow cytometer.

Pipettes and Pipetting Aids

Pipetting must be done by mechanical means, never by mouth. Ideally, pipet work should be done in a BSC. If one is not available, minimize hazard by using cotton-plugged pipettes and pipette tips; do not use suction and propulsion pipettes with biohazardous materials; and store used pipettes for disposal in a rigid container that fits the pipette in its entirety. Use plastic over glass whenever possible. The use of a plastic garbage bag is not acceptable for collection of pipettes and pipet tips. When the waste container of pipettes becomes full, it may be autoclaved.

Sharps

The use of sharps should be restricted as much as possible. The only times sharps should be used is when injections, phlebotomy techniques, and fluid aspiration from diaphragm bottles are performed. Some sharps may be used when doing dissections as well- in these cases disposable sharps should be considered for scalpels, biopsy punches, etc., to minimize accidental exposure hazards. If researchers are using sharps in their research, they should complete bloodborne pathogens training (to review information on needlestick injury prevention) in addition to biosafety training.

PIs and lead instructors are responsible for training their respective personnel in safe sharps handling and disposal practices. Sharps containers should be situated closely to where the sharps are being used. Sharps may be disposed of in a hard-sided container that can be completely sealed. If a container designed for sharps disposal is not used, deface all labels and clearly label as a sharps container, including a biohazard symbol, prior to using for sharps disposal. Never overfill a sharps container; when it is 2/3 full, seal the container and request a pick-up. Never try to push waste down to make space for more waste, as this increases the risk of a needle stick injury. Request pick-up of sharps containers using the online form. Refer to the disposal section for more information.

Any research facility that uses needles runs a risk of needlestick injury and will need to complete bloodborne pathogens training, as well as maintain a Bloodborne Pathogens Exposure Control Plan following the UWM Bloodborne Pathogens Exposure Control Plan template.

Loop Sterilizers and Bunsen Burners

The sterilization of an inoculation loop or needle in an open flame generates aerosols that can contain viable microbiological agents. It is strongly encouraged that laboratories use a shielded electric incinerator or a hot bead sterilizer to minimize the risk of aerosol production while sterilizing a loop or needle. Another recommended option is to use disposable (one-time use) plastic inoculation loops and needles for culture work and collecting the waste loops and plastic needles in a hard-sided container that fits them in their entirety. They can be autoclaved and disposed of after autoclaving in general waste. The use of continuous flame gas burners, such as Bunsen burners, in a BSC is prohibited, as they can produce turbulence that interferes with the airflow of the cabinet and can damage the HEPA filter.

Biohazardous Waste Disposal

The following biohazardous waste disposal guidelines are intended to protect the public, the environment, laboratory personnel, custodial personnel, waste haulers, and landfill/incinerator operators. Workers that generate biohazardous waste in the laboratory need to follow the appropriate labeling, packaging, and intermediate disposal of waste that conforms to guidelines set forth by the Biological Safety Program to ensure the safety of all individuals who may encounter the waste. Signage templates are available on the UWM Safety and Health Page for any facilities that have biohazardous materials.

Decontamination means reducing the number of disease-producing microorganisms and rendering an object safe for handling. Please note, if the waste is mixed, containing both chemical hazards and biological hazards, the hazardous chemical or radioactive materials take precedence over the biological hazards and need to be handled by a Waste Management Specialist for disposal in accordance with the relevant chemical/radioactive hazards.

Biohazardous waste must be stored in a secondary container until it is moved for decontamination. The secondary container must be hard-sided (cannot leak through), possess a secure fitting lid, and possess the following symbol (or similar):

BIOHAZARDOUS WASTE

Figure 2 Biohazardous Waste Symbol

The following are examples of biohazardous materials that must go through the proper decontamination prior to disposal:

- Microbiological Laboratory Wastes, including
  - Cultures derived from clinical specimens and pathogenic microorganisms
  - Laboratory equipment that has encounter microbiological waste
- Human materials: tissues, liquid blood, cells, body fluids
- Animal materials: tissues, liquid blood, cells, body fluids from animal carrying an infectious agent that can be transmitted to humans
- Any materials containing animal or human pathogens
- Plants:
  - Exotic/invasive plants
  - Virulent plant pathogens
- Contaminated sharps
- Animal bedding/waste as pre-determined by the Animal Care Program.

Infectious and Medical Waste Disposal

Contaminated materials from teaching labs, research laboratories, and animal research facilities must be decontaminated prior to disposal or washing for reuse. These include all cultures, tissues, media, plastics, glassware, instruments, and laboratory coats. Materials should be collected in leak-proof containers containing the universal biohazard symbol. Use only an autoclavable biohazard bag for waste, contained in an autoclavable secondary container for autoclaving purposes.

After waste has been decontaminated, place decontaminated waste in a regular black trash bag. For reusable materials (i.e. plastics, glassware, and instruments that are reusable), they may be washed normally and reused only after they have been autoclaved. Laboratory coats should be autoclaved regularly to minimize the risk of accidental exposure, or disposable laboratory coats should be used and disposed of monthly.

Sharps must be collected in an approved medical sharps container. These include syringes with needles, lancets, and razor blades. It does not matter what they were used for; they must be disposed of as medical waste. The Waste Management Specialist handles the processing for the medical waste through the University of Wisconsin System contracted vendor Madison Environmental Resourcing, Inc. (MERI) and is not handled by general custodial services. Please contact the Waste Management Specialist to coordinate removal of sharps containers. Fragile glass, glass slides, and cover slips that have encountered infectious materials should also be disposed of in a sharps container.

Plastic pipettes and pipette tips that have encountered infectious materials should be disposed of in a hard-sided container to reduce the possibility of puncture through a biohazard bag during handling or disposal. This container can then be placed inside an autoclavable biohazard bag to be autoclaved, double bagged, and disposed of in the regular trash.

Liquid Waste

Any liquid waste, such as cultures or media, that have been contaminated/inoculated with biological agents or toxins must be rendered safe through chemical or autoclave treatment. An SOP for inactivating the agent is required in the protocol form that is to be submitted to the IBC for all biohazardous materials.

Animal Waste

Animal waste (i.e., bedding, feces, urine, etc.) may require disinfection or inactivation and will be outlined in the approved IBC protocol. Disinfected waste can be disposed of in the trash or by other approved means after disinfection. Animal waste that does not require disinfection/inactivation may be disposed of in the regular trash or other approved means. It is the responsibility of the PI to coordinate appropriate waste disposal with the Animal Care Program.

Animal carcasses that contain recombinant or synthetic nucleic acid molecules or were inoculated with organisms containing recombinant/synthetic nucleic acids are required to be decontaminated prior to disposal; they may not be discarded in regular trash. Carcasses are sent for disposal via incineration through our contracted medical waste service. Contact the BSO or Campus Research Veterinarian to determine disposal means prior to IBC approval based on the animals being used in research.

Animal carcasses from preserved dissection specimens should be disposed of according to chemical hazard first. If they contain less than the 2% threshold of formalin, they may be carefully double bagged and handled according to the preservation company instructions.

Noninfectious Waste

There are items in the laboratory that may fall under noninfectious waste, but do require containment. These items can be placed in plastic garbage bags and disposed of in the regular trash unless they have been contaminated with any infectious waste. If they have been contaminated with any kind of infectious waste, then they must be treated as such. The following are a list of items that may fall under noninfectious waste (UW Biosafety, 2017):

- Items that are soiled or spotted with human blood or body fluids not known to be infected with any infectious agents. Examples include gowns, gloves, dressing, and surgical drapes.
- Laboratory equipment, non-fragile waste glass, containers, packaging materials, and any other materials that did not have any contact with blood, body fluids, clinical cultures, or infectious agents.
- Noninfectious animal waste, including feces, bedding, tissues, blood, body fluids, or cultures not suspected to be carrying an infectious agent transmissible to humans.
- Fragile glass, glass slides, cover slips, pipettes, and pipette tips that have not encountered blood, body fluids, clinical cultures, or infectious agents. These items should be disposed of in a hard-sided container that, when full, is closed and disposed of in the regular trash.

Choosing a Method of Decontamination

Determination of the appropriate method for decontaminating your materials may be challenging. There may be multiple SOPs in place for your research laboratory as there may be multiple means of decontamination based on the type of material being decontaminated and what equipment is available. Work with the BSO to determine what methods of decontamination may work best for your needs.

If you are working with biological waste that contains any volatile, toxic, or carcinogenic chemicals, radioisotopes, or explosive substances, these take precedence over the biological material. These should not be autoclaved and need to be handled as hazardous or radioactive waste, as appropriate based on the material. Contact the Laboratory Safety Coordinator to determine how to handle the material, and contact the Radiation Safety Officer for radioactive materials safe-handling.

Biohazardous Waste Disposal Decision Tree

The following is a decision tree that can help guide you in determining the best way to handle the biohazardous waste you generate in your lab (Figure 3). Please remember the following when disposing of hazardous waste:

Autoclavable bags that you want to throw in the trash after decontamination should not be red. Red bags should only be used for items that cannot be autoclaved and require other methods of decontamination (e.g., incineration).

All sharps go into sharps containers. The best way to determine if something should go in a sharps container is to think about whether it may be able to puncture human skin. If it could puncture human skin, it should go in a sharps container.

Autoclave Use

Steam sterilization by means of a properly functioning autoclave is the ideal method for decontamination of many biohazardous materials. To ensure that the autoclave is effectively decontaminating materials, they are to be tested monthly using a biological (*Geobacillus stearothermophilus* spore test) or chemical indicator that can verify adequate times and temperatures are being used to decontaminate a full load containing biohazards. The use of indicator tape is advised whenever using an autoclave to ensure that the load has reached sterilization temperature. Please note that the bigger the load, the longer the exposure time necessary to properly decontaminate the biohazards. Larger loads of solid waste should be autoclaved at a minimum of 121 ℃ and 15 PSI for one hour.

Chemical Disinfection

If an autoclave is unavailable or not appropriate for the material, the alternative is to use a chemical disinfectant that has been freshly prepared at a concentration known to be effective against the biohazards that need to be inactivated (UW Biosafety, 2017). Determining the appropriate disinfectant can be a complex process; it is strongly recommended to discuss your choice and process for chemical disinfection with the Laboratory Safety Coordinator and the BSO. Consideration of level of resistance should be considered . Table 5 includes a brief overview of options available, but ultimately, additional references should be sought out to determine what will fit your facility needs. It is recommended that teaching laboratories use 10% bleach (1:10 bleach: water) solution for routine lab bench disinfection after handling biological agents.

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DEPARTMENT OF UNIVERSITY SAFETY AND ASSURANCES

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BIOLOGICAL & MEDICAL WASTE DECISION TREE

July 2017

Figure 3 Biohazard Decision Tree

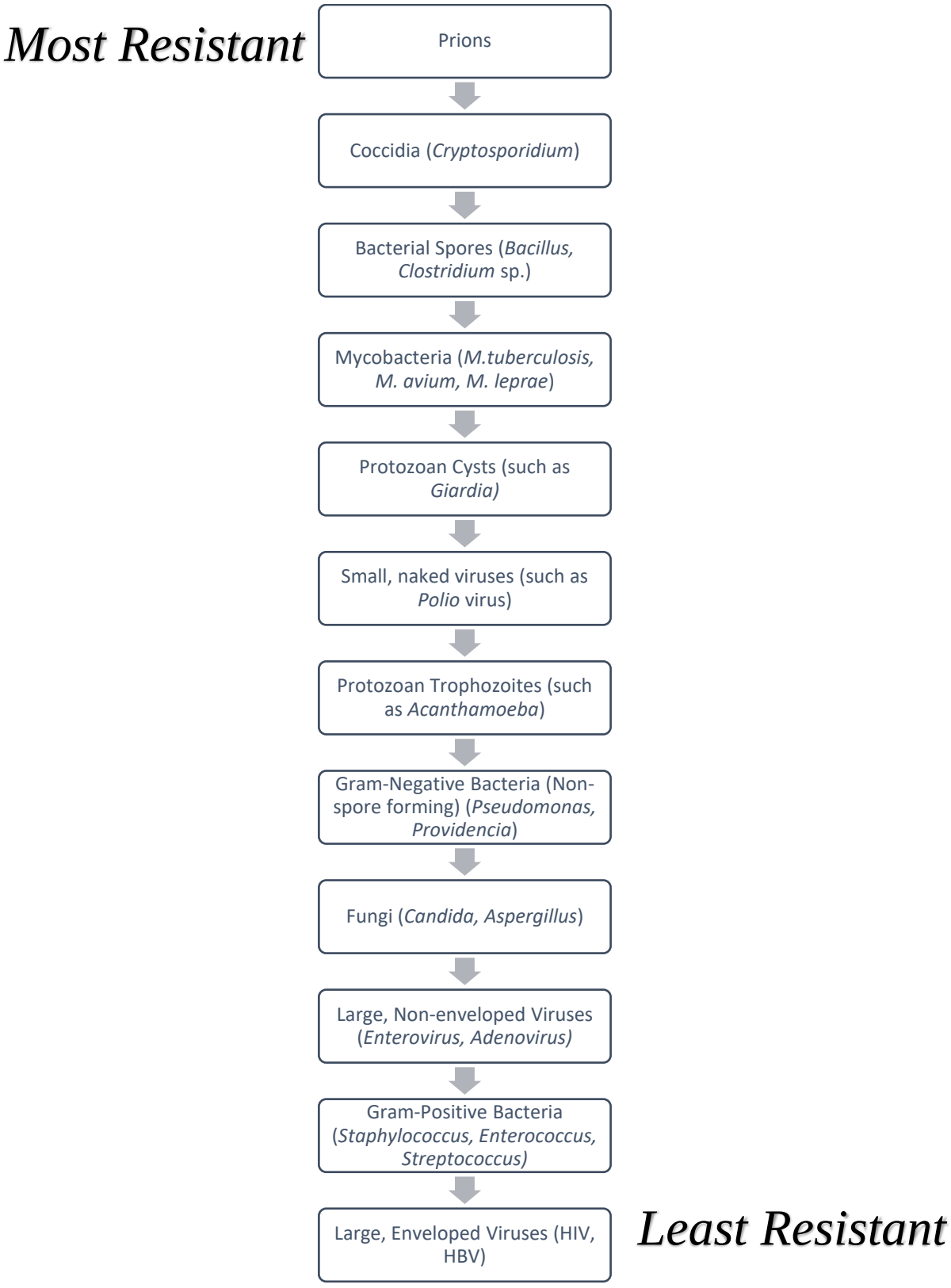

Figure 4 Descending level of germicidal resistance of pathogens

Chemical	Best Used for Inactivation Of…	Applications	Level of Activity	Considerations
Alcohol Ethanol Isopropanol	Vegetative bacteria, Mycobacteria, vegetative fungi, enveloped viruses	Instruments, surfaces that have low organic burden, lightly soiled hands if hand-washing isn't readily available	Intermediate	Flammable, does not penetrate protein-rich materials, rapid evaporation, ineffective against naked viruses and spores
Aldehydes Cidex Wavicide-01	All microorganisms	Non-porous surfaces	High	Very toxic to animals and humans
Peroxygen Compounds Ethylene oxide Virkon	Wide range of bacteria, viruses, and fungi; variable against bacterial spores and Mycobacteria	Heat-sensitive equipment	High	Ethylene oxide is a human carcinogen, and restricted use
Halogens Clidox Clorox Other household bleach	Vegetative bacteria, enveloped viruses	Benchtop surfaces, blood spills	Medium-High	Inexpensive, highly effective in decontaminating large spills Short shelf life, easy binding to nontarget organic substances, corrosive, cannot cross paths with autoclaving process
Iodophors Povidine Bentadine	Mycobacteria, viruses, fungi, most fungi, varying for fungal and bacterial spores	Antiseptic	Medium-High	Low toxicitiy, Low irritant Needs additional time for certain fungi and bacterial spores
Phenolic Compounds	Vegetative Bacteria (Gram-Positive), Enveloped viruses	In combination with detergents, excellent choice for cleaning benchtops, general purpose surfaces	Medium-High	Can be used with detergents Generally safe
Quaternary ammonia disinfectants	Most fungi, vegetative Gram-positive bacteria	Added to handwashing compounds	Low-Medium	Low toxicity, but ineffective against mycobacteria, spores, and most viruses Can cause contact dermatitis

Table 5 Chemical Methods of Microbial Control

Incineration

The ultimate means of sterilization of medical and microbiological waste is incineration. Animal carcasses treated with preservatives such as formalin and medical sharps are examples of materials that are shipped for incineration. Contact the waste management specialist to determine the needs for your laboratory.

UV Treatment

UV light is not recommended as a primary means of disinfection because there are several factors that could influence the efficacy of its ability to disinfect materials. UV light does not penetrate organic material well and works best when used on surfaces that it encounters. Because UV light can cause erythema (sunburn) and eye injury, personnel that are using UV light should avoid exposure. This includes the use of UV light in a biosafety cabinet as a means of disinfection. UV light alone is neither recommended nor an acceptable means of disinfection as a standalone treatment in biosafety cabinets. It is recommended that 70% ethanol be used as a primary means of biosafety cabinet disinfection, or other stainless-steel safe decontaminant agents.

Equipment Malfunction

In the event of a mechanical malfunction, systems breakdown, equipment/mechanical shutdown, or preventive maintenance of primary containment equipment or components, the BSO must be notified immediately. In the case of an unplanned event and if Physical Plant mechanical staff is not already on the scene, the BSO will notify appropriate Physical Plant staff. Proper precautions must be taken immediately. All experiments must be halted and the biological agents secured (e.g., containers sealed or containers placed in freezer or refrigerator). The area must be cordoned off during the entire time of the shutdown. No further activities will be allowed until University Safety and Assurances staff certify that the facility is safe to use.

Food and Drink Guidelines

Food and drink used for human consumption are not allowed in any research or teaching laboratories at any time. This includes at student work desks. The only acceptable barrier is a physical wall and door separating the non-lab work space from the lab work space. Students are required to find a safe area to consume their food and drink outside of the laboratory. It is the responsibility of the PI to ensure lab personnel and students are not eating or drinking anything in the laboratory, and enforce this policy.

In addition to food and drink, gum chewing, applying cosmetics, smoking, and taking medication are strictly prohibited in teaching and research laboratories handling biological agents. Water bottles are included in this guideline; all water bottles need to be stored out of the research/teaching facility in a backpack or separate room. All backpacks should be housed in cubbies or on shelves, never on the floor. PIs should set the example; they too should not be eating/drinking in the lab facilities, and they need to enforce this in their labs. The chemical hygiene plan is required to reflect your food and drink policy. The lab safety coordinator and the biological safety officer will check that this policy is included in the chemical hygiene plan and clearly posted in the laboratory during inspections.

Housekeeping

Laboratory personnel and the PI are expected to maintain good housekeeping in their facilities. BSL-2 labs should NEVER have a custodial staff member entering to clean the lab. Laboratory personnel should move all non-hazardous waste to be disposed of outside of the lab. Regular decontamination of benches, washing of glassware, and keeping the lab free of clutter

are important in minimizing additional risks of contamination or injury in the lab. Contact the laboratory safety coordinator for help evaluating your lab to ensure it is safe and orderly.

Chapter 7: Emergency Management and Biosecurity

Biosecurity

When an experiment is in progress, lab doors should be closed. If there is no one present in the lab, the doors are to remain locked. Unauthorized/unapproved people are not permitted in the laboratory. If anyone requests access to a laboratory and you do not know who the person is, request identification (Panther Card ID or Driver's License) and their purpose for entering the facility. This is for your safety and their safety. Unauthorized personnel should never be in the research or teaching laboratories, as it exposes an accidental release hazard and threatens the biosecurity of UWM. If you feel that unauthorized personnel are trying to gain unapproved access to your lab facility, contact UWM Public Safety immediately at 9911 on a campus phone or (414) 229-9911 from a cell phone or other non-campus phone.

The University of Wisconsin-Milwaukee is committed to protecting students, employees, and the public from any possible bioterrorism agents or accidental release of biological agents. The following identifies the list of steps taken by UWM to prevent biosecurity incidents. There is also discussion of Select Agents and Dual Use Research of Concern (DURC) in this section. Biosecurity measures taken by University Safety and Assurances are listed below.

- Inspection: The BSO conducts biosafety inspections annually or biennially, and the laboratory safety personnel conduct lab safety inspections. These identify any areas of concern and address them so corrective actions can be taken.
- Security of biologically sensitive materials: access controls, including locked doors, restricted animal facilities, and key card access (some labs) help restrict non-authorized personnel from entering facilities. Materials are locked up and stored securely by PIs to prevent theft.
- Inventory: each PI is responsible for maintaining a biological and chemical inventory for their lab, and holds their personnel accountable for tracking usage, transfer, and decontamination of biological materials. Visit the UWM Safety and Health Forms page for a sample of an inventory log that can be used in research labs.
- Transport of biological agents: PIs and laboratory personnel follow state and federal regulations regarding the transport and shipment of biological agents. See the section below for more information regarding transport.
- Approval of Use: All research and teaching labs involving the use of any kind of biohazardous material must be registered and approved by the IBC. Visit the IBC Page for more information.
- Reporting: If an accidental release occurs, University Safety & Assurances and emergency personnel are contacted immediately and an accidental release form is completed.
- Training: providing up to date biosafety training helps the University ensure staff and students are trained properly to handle biohazardous materials.

Select Agents

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002, Subtitle A of Public Law 107–188 requires the Department of Health and Human Services (HHS) to establish and regulate a list of biological agents and toxins that have the potential to pose a severe threat to public health and safety (DHHS, 2017). In addition, it is required under the Agricultural Bioterrorism Protection Act of 2002 that the USDA establishes and regulates a list of biological agents that pose a severe threat to animal health and safety, plant health and safety, and/or to the safety of animal or plant products (DHHS, 2017). Work with any of these select agents requires special registration and inventory. Visit www.selectagents.gov for more information and a complete list of regulated agents & toxins, or contact the BSO to discuss your research if you believe it may fall into this category of research.

Dual Use Research of Concern (DURC)

The University of Wisconsin-Milwaukee is subject to the United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern (DURC). Thus, the UWM Biological Safety Program must review all potential dual use research to determine whether it meets the criteria outlined in this policy for DURC. Dual Use Research of Concern (DURC) is life sciences research that could be utilized to provide knowledge, information, products, or technologies that could be intentionally misused to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, material, or national security (NIH OCP, 2017).

Even if your laboratory does not receive federal grant funds from the U.S. Government, you still need to have a protocol approved by the IBC. If you think that you may have research that could potentially be DURC, contact the biological safety program. Review the DURC policy for a list of current DURC agents.

Emergency Plans

There are emergency plans implemented by the Safety and Industrial Hygiene Program focused on the safe handling of chemicals, good laboratory practices, and other general procedures that you may need education/training in to work safely on campus. Each laboratory should have their own emergency plan that has been developed through working closely with the University Safety & Assurances Department and through biosafety protocol development.

General Emergency Plan

The key information that should be included regarding biological hazards includes (but is not limited to) the following (UW Biosafety, 2017):

- If a spill occurs, leave the affected area immediately. Even if the spill is small, aerosols may be generated that could lead to pathogen exposure. If clothing is contaminated, remove clothing if possible. Exposed skin should be washed for 15+

minutes with soap and water. A splash to the eyes should be treated using an eyewash station for at least 15 minutes.

- If the spill may be dangerous to people in and out of the lab and staff cannot contain it, the spill needs to be reported to UWM police.
- Close the laboratory door, and mark it with a "NO ENTRY" sign. Notify the PI (if not present) and the biological safety officer.
- Seek medical treatment for anyone who has been exposed.
- If necessary, call 9-1-1.
- Complete an accidental release/exposure form within 24 hours of the incident.
- Do not reenter the room until any aerosols have settled (minimum 30 minutes) and the extent of the hazard and its dissemination has been determined.
- Each person who enters the laboratory for cleanup should wear (at a minimum) a lab coat, gloves, and eye protection.
- Use an appropriate concentrated disinfectant to decontaminate. Ensure that a supply of stock disinfectants is always readily available in the laboratory.
- Decontaminate anything used in cleanup.

BSL-3 facilities have a different plan to follow. If a BSL-3 facility is developed at UWM, new plans will be implemented to reflect additional safety procedures necessary.

Exposure Response

PIs are asked to consider the potential consequences of an exposure to the biological hazards they are working with and to have a developed response procedure for this potential exposure on file with their protocol. Complete the First Report of Biological Exposure or Release Event Form online. Information that should be kept on file in case of accidental exposure should include the following (UW Biosafety, 2017):

- A description of the pathogen(s), including signs and symptoms of an infection
- Distinct characteristics of the strain(s) used in the laboratory, including antibiotic resistance, transmissibility, atypical tissue tropism, foreign genes that alter pathogenicity, etc.
- Recommendations for treatment, including effective medications, quarantine, etc.
- A detailed record of a history of exposure to the agent(s) in question for some pathogens from start of employment (work with BSO to determine need for this)
- Completion of an accidental exposure/ release form within 24 hours of the incident, submitted to the BSO. This form must be used when any of the following occur:
    - Potential exposures or releases of organisms or biological toxins on the UWM campus and UWM off-site facilities.
    - Potential exposures include needle sticks, animal bites, aerosol exposures, and other incidents potentially resulting in disease.
    - Potential releases including spills outside of primary containment as well as potential releases to the environment.
    - Unauthorized releases of transgenic animals or plants should also be reported on this form.

It is the PI's responsibility to ensure that an exposure/release form is submitted after any potential exposure or release event. After completing the form, select "Submit" at the bottom of the form. The information on the form will be sent to designated individuals at the UWM Biological Safety Program. The information provided is used to determine how our offices may help you and your laboratory and for mandatory federal reporting purposes. The submitter will be contacted for incident follow-up. If you need assistance completing the form or reporting an incident, please call the BSO at 414-588-4261.

Spills Inside a BSC

A properly functioning and certified BSC should contain potentially hazardous biological aerosols from spills within its unit on its own. Therefore, it is extremely important to have your biosafety cabinet certified annually. It is the responsibility of the PI to have a well-developed SOP in place for operation and cleanup of a BSC, as well as spill procedures, which are required in any approved research protocol.

Recommended Clean-Up Materials for Lab Facilities

The following should be kept in the laboratory. All personnel should be trained in where spill kit materials are housed and how to use them. An SOP for accidental spill should also be available

- **Disinfectants**: Selection should be made based on the biological agent(s) it would be used against (See Table 5). If dilutions are made, such as with bleach, fresh solutions should be made on a schedule depending on the materials used and the manufacturer's recommendations.
- **Absorbent materials**: There should be, at a minimum, a sufficient quantity of paper towels on hand to soak up the maximum volumes handled in the laboratory. Other absorbent pads may also be used, but paper towels will typically suffice.
- **Extra PPE**: This is dependent upon the biological agent, but when handling a spill, a gown, gloves, and eye protection should always be worn to prevent additional accidental exposure from occurring.
- **Signage**: Signage available for posting until aerosols have settled after a spill.

Volunteers and Minors in the Laboratory

In general, children or adult volunteers should refrain from entering the laboratory facilities unless the appropriate paperwork has been filed and approved. This includes a volunteer application, agreement for assumption of risk, indemnification, release, and consent for emergency treatment; volunteer action plan completed by PI, signed laboratory safety sheets, and SOPs for the lab facility.

Additionally, minors must have a letter sent to their legal guardian(s), and there needs to be a completed background check on anyone working near the student in question. For more information regarding volunteers in the laboratory, please contact University Safety & Assurances. Note that minors and volunteers must complete biosafety training if working in

BSL-2 facilities as well, and they are not permitted in any lab that is deemed a high hazard by the University Safety and Assurances staff. Please visit the Laboratory Forms on the UWM site to view and complete forms for minors to work in laboratories.

Chapter 8: Institutional Biosafety Committee

The Institutional Biosafety Committee (IBC) is charged by the University Chancellor to formulate guidelines and procedures related to the use of biohazardous agents, including: human, animal, and plant pathogens, other infectious agents, toxins, and recombinant DNA (rDNA). As mandated by the NIH, experiments involving human gene therapy, formation of transgenic animals or plants, and the generation and/or use of rDNA must be registered and approved by the IBC. UWM also requires IBC registration and approval for use of Risk Group 2 or higher biohazardous agents. Roles and duties specific to the NIH Guidelines can be found in the Section IV-B-2 of the NIH Guidelines (NIH, 2016).

The Chancellor, upon the recommendation of the Vice Chancellor for Research and Dean of the Graduate School, will appoint members to the IBC and designate one member to serve as chairperson. To provide the quality of input needed for in depth consideration of research activities presenting real or potential hazards, the membership shall be composed of the following:

- Faculty: Faculty shall be selected based on experience and expertise in infectious disease research, experience and expertise in rDNA technology, and the capability to assess the safety of biological research and to identify any potential risk to public health or the environment. Research academic staff with PI status are considered faculty for this guideline.
- Community Members Not Otherwise Affiliated with the University: A minimum of two outside members who represent the interest of the surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community) shall be appointed.
- Laboratory Staff: A minimum of one member representing laboratory research staff, such as a research associate/research assistant, medical technologist, or laboratory technician, shall be appointed.
- Continuing Members: The following will be continuing (ex-officio) committee members.
    - Asst. Director, University Safety and Assurances
    - Biological Safety Officer
    - Campus Veterinarian

The IBC has the responsibility of assessing risks and potential environmental impacts associated with investigations involving biological agents and making recommendations for safe conduct of such studies. It also functions on behalf of the institution to ensure that the experimental work is performed in compliance with current policies and guidelines promulgated by government granting and regulatory agencies. The Committee does not monitor activities which are appropriately the concern of other established programs, e.g., Radiation Safety Program or Animal Care Program; however, it will closely interact with these groups in a concerted effort to minimize health risks to university personnel, students, and the public.

The current protocol submission forms, information on the UWM IBC, and more can be found at the UWM IBC website. Registrations approved by the IBC will be active for 3 years from the date of approval. Written notification that the registration will expire will be sent out at least 30 days prior to the expiration date. The PI will then be required to submit an updated registration application for review and approval by the IBC. The IBC will meet monthly to conduct business during the year. Registration forms/protocols will be discussed and determination of approval/revision/rejection will be decided at these times.

IBC Meeting Procedures and Protocol Reviews

Biosafety protocols are evaluated based on risk assessment in accordance with NIH Guidelines. Regardless of the status of the project (NIH exempt or non-exempt) it is expected that all protocols adhere to state and federal regulations and recommendations. The following are the actions the IBC will take regarding a protocol, following Robert's Rules of Order.

- APPROVE: >50% of the IBC approves the protocol as submitted.
    - Biological Safety Officer (BSO) sends final electronic copy with approval to PI. Work can begin once approval is received..
- APPROVE WITH CONTINGENCY(IES). The PI is required to complete additional steps as outlined by the IBC before the protocol is to be approved. A revised protocol must then be submitted for approval.
- TABLE. If the IBC is unable to come to a majority approval, it will be tabled; deficiencies will be addressed by the PI before re-submission.
- REJECT. This action is only taken when there are significant issues with the protocol. A new protocol must be developed and include recommendations provided from the BSO and IBC.

The following projects must have a protocol approved and on file before commencing.

- Recombinant (transgenic) or synthetic DNA/RNA materials, including human gene therapy, proteins, and enzymes of infectious biological agents
- Microbes and disease-causing agents including bacteria, viruses, fungi, prions, protozoa, and parasites
- Large-scale propagation consisting of a volume greater than 10L in a single vessel
- Human cells and cell culture, organs or tissues, or biological samples
- Non-human cells and cell culture, organ or tissues, or biological samples that are infectious, potentially infectious, or recombinant
- Animals (vertebrate and/or invertebrate) that are recombinant (transgenic), exotic, and/or grown in association with pathogens and/or recombinant materials
- Plants that are recombinant (transgenic), exotic, and/or grown in association with pathogenic or recombinant microbes and/or pathogenic or recombinant small animals (insects, etc.)
- Biological Toxins (this does not include toxic chemicals or antibiotics)
- Select Agents and Toxins
- Dual Use Research of Concern

All research that is subject to III-E of the NIH Guidelines is also required to be submitted to and reviewed by the IBC when initiating the research. Once a protocol form has been submitted, research in this category can begin before receiving approval from the IBC. These include experiments that involve the formation of recombinant or synthetic nucleic acid molecules containing no more than two-thirds of the genome of any eukaryotic virus, experiments involving whole plants that do not already fall under III-A, III-B, III-D, or III-F, or experiments involving transgenic rodents. These projects must fall under BSL-1 containment.

Protocols that are Exempt from NIH Guidelines and consist only of work suitable in a BSL-1 laboratory are reviewed administratively by the BSO; the IBC receives notification of the review and status of these protocol submissions, but does not conduct formal review. Other minor modifications to already approved protocols may also be administratively approve, such as:

- Personnel amendments
- Grant additions

All other protocols must be approved by the IBC. All projects must be registered for teaching and research, even if they do not require a full review by the IBC.

All approved protocols are required to be re-submitted for review every three years to the IBC. If a change is made to the experiment in which NIH Guidelines apply or differ from what was previously approved, this will also be reviewed and approved by the IBC. Protocol changes that require significant changes to safety precautions, such as PPE, administrative or engineering controls, will also be reviewed and approved by the IBC (UW Biosafety, 2017). If there are smaller changes, such as personnel additions/deletions, the approval can be done by the BSO, who will then notify the IBC.

The BSO and the Dept. of University Safety and Assurances withhold protocols from IBC agenda that are deemed not ready for review. PIs may be asked to attend the meeting to clarify their protocol information and answer questions during protocol review. If a PI is unable to attend, and the IBC is unable to understand the protocol, it may be tabled until the PI can attend a meeting. If a protocol is tabled, the research described in the protocol cannot be conducted during that time. A PI can send a lab manager or researcher in their place to answer questions, but the PI is responsible for the contents of the protocol and must sign off before it can be submitted or re-submitted.

Meetings may be digitally recorded so there is an accurate record of the meeting on file and so the BSO can accurately prepare minutes for review. All meetings are conducted following Robert's Rules of Order. Thus, the IBC cannot act on a protocol without a quorum present, which is one more than half of the voting members. Therefore, it is important for IBC members to attend meetings regularly to ensure that the IBC will meet quorum; otherwise, the meeting will be cancelled and all protocols scheduled to be approved at that session will be held until the next scheduled meeting. If a protocol is left unapproved, the research described in that protocol cannot be conducted during that time.

The IBC is subject to the Wisconsin Open Meetings Law. Actions may only be taken at meetings that have been announced and are open to the public. Notices will be posted in advance at https://uwm.edu/news/ under open meetings. Some sessions may go to closed session.

Protocols that contain information that must be protected due to confidentiality agreements, disclosure, safety and security, DURC, select agents/toxins, protocol violations, or repeated biosafety violations in the research laboratory will be discussed in closed session pursuant to Wisconsin Statues sections 19.85(1)(d) and 19.85(1)(e). More details can be found on the IBC website at the UWM Biosafety Page. Meetings are held monthly, typically during the last week of the month. These meetings are held online and will last 1-3 hours, depending on the number of protocols submitted and other items that need to be discussed. Agendas are made available to the public upon request and can be obtained through the BSO, who acts as the UWM Contact and Recorder for the IBC.

Protocol Review Questions

The PI and the IBC must concur on all matters relating to containment requirements, safe practices, and handling and disposal procedures for biohazardous agents. In event of non-concurrence, the recommendations of the Committee shall prevail until they are modified or rescinded by appellate decision of an administrative review which may include outside reviewers.

The IBC will use an evaluation form to review the criteria found on the IBC registration form. All comments will be compiled and used to discuss the protocol at a formal meeting. Personnel involved in the submitted protocol are invited to discuss their submission at the meeting.

Visit the UWM IBC Page for more information.

Teaching Laboratories and IBC Registration

The University of Wisconsin-Milwaukee offers a variety of teaching laboratories that work with recombinant DNA, animals, animal or human cells/tissues, and biological agents. The UWM Biosafety Program requires that all teaching laboratories that handle agents subject to NIH Guidelines have an approved protocol on file for the safety of the students, staff, and public. The UWM IBC Page has instructions for submission of teaching protocol forms. The IBC is not responsible for how the content is taught, simply for evaluating the safety and efficacy of using biological agent(s) in the course as outlined in the protocol form.

Termination of Unsafe Research

The Biological Safety Officer, with concurrence from the Chair of the IBC, or with concurrence of three (3) members of the IBC if the Chair is unavailable, may stop any work with microbial agents or any hazardous research project that creates an unreasonable hazard to personnel or involves experiments prohibited by the institution. The entire IBC then will review the problem and will complete the review within a working week. A written recommendation will then be forwarded to the Vice Chancellor for Research and Dean of the Graduate School and the Provost for final action. It is required that any unlawful research is reported to the federal government.

Standard Operating Procedures (SOPs)

The UWM Biosafety Program has developed generally accepted standard operating procedures (SOPs) for general research practices on the UWM Biosafety website. Anyone working with a biohazardous agent or biohazardous material at any facility of UWM is expected to follow these SOPs. PIs should work with the BSO to develop specific SOPs for their research facility. A general blank SOP is available for use on the UWM Biosafety website.

Bibliography

Bailey, A. (2008). *Redefining "Containment" for Aquatic Facilities.* ALN.

CCAC, C. C. (2005). *Guidelines on: the Care and Use of Fish in Research, Teaching, and Testing.* CCAC.

DHHS, D. o. (2017, June 7). *Federal Select Agent Program Select Agents* . Retrieved from Federal Select Agent Program: https://www.selectagents.gov

NIH. (2016). *NIH Guidelines for Research involving Recombinant or Synthetic nucleic Acid Molecules.* Washington D.C.: NIH.

NIH OCP, N. I. (2017, June 7). *Biosecurity Dual Use Research Concern* . Retrieved from National Institutes of Health Office of Science Policy : http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/dual-use-research-concern

US DHHS, U. D. (2009). *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition.* Washington, D.C.: HHS.

UW Biosafety, U. o. (2017). *University of Wisconsin Researchers Biosafety Manual.* Madison, WI: University of Wisconsin.

WDHHS. (2017, June 16). *Disease Reporting*. Retrieved from Wisconsin Department of Health and Human Services: https://www.dhs.wisconsin.gov/disease/diseasereporting.htm