Research Biosafety Plan
(EHS Program 3.2)

1.0 Overview

Environmental Health and Safety (EHS) at Weill Cornell Medicine (WCM) has developed this Research Biosafety Program Manual. EHS reviews this manual periodically to include revisions to the biosafety guidelines.

In all situations, the most recent revision of the publications "*Biosafety in Microbiological and Biomedical Laboratories (BMBL)", "Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules and Arthropod Containment Guidelines",* as well as regulations issued by the Occupational Safety and Health Administration (OSHA) regulating biosafety will supplement and supersede this document.

The EHS program manuals covering Waste Disposal Procedures, Biological Spill Planning and Response, and the Bloodborne Pathogen Exposure Control Plan are also considered integral elements of the Research Biosafety Program.

All biomedical research conducted at WCM is reviewed by the Institutional Biosafety Committee (IBC). Principal Investigators are required to register their laboratory research with Environmental Health and Safety (EHS). The Unified Laboratory Safety Registration and Inspection Program allows WCM Principal Investigators (PIs) to complete one document logging all biological, chemical and radiological work occurring in the lab. The IBC focuses on the biological hazards identified in the registration. This registration provides a standardized submission process for PIs to meet the federal, state, and local laboratory safety requirements, including compliance with the National Institute of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

2.0 Emergency Contact Information

Please refer to the following contact list in the event of a fire or other emergency that may involve personnel while working with biological materials.

Environmental Health and Safety (Biological Spills)	**646-962-7233 (1-7233)**
Workforce Health and Safety (8:00AM - 4:00PM)	**212-746-4370 (6-4370)**
Student Health Services (8:00AM - 4:00PM)	**212-746-1450 (6-1450)**
Emergency Medical Services (NYP ER)	**212-472-2222 (2-2222)**
Biosafety Officer	**646-962-7233 (1-7233)**
Engineering & Maintenance (ventilation, power, steam system failures)	
WCM	**212-746-2288 (6-2288)**
NYP	**212-746-1920 (6-1920)**
Security	**212-746-0911 (6-0911)**

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Weill Cornell Medicine
Environmental Health and Safety
TEL 646-962-7233 WEB weill.cornell.edu/ehs EMAIL ehs@med.cornell.edu
Weill Cornell Medicine | 402 East 67th Street, Room LA-0020 | New York, NY 10065

4.0 Applicability

All WCM faculty and research staff using biological agents must read and implement the practices outlined in this manual. Adherence to good microbiological practices and the development of specific research protocols will ensure safety and compliance with standards issued by OSHA, NIH and the CDC.

5.0 Responsibilities

The WCM Research Biosafety Program establishes duties for individuals and groups as described below.

5.1 BIOLOGICAL SAFETY OFFICER

- Ensures that Department Chairs, Principal Investigators, Directors, and Managers comply with the Research Biosafety Program.
- Provides technical advice to Principal Investigators and the Institutional Biosafety Committee on research safety procedures.

5.2 ENVIRONMENTAL HEALTH AND SAFETY (EHS)

- Develops, maintains, and disseminates the Research Biosafety Program.
- Serves as a resource for biosafety procedures and containment.
- Trains laboratory personnel on the principles of this Program.
- Responds to emergency spills or releases.
- Maintains records of training, spills, emergencies, and exposures.
- Inspects laboratories for compliance with this Program.

5.3 PRINCIPAL INVESTIGATORS (PIS) AND OTHER LABORATORY SUPERVISORS

- Ensures that all personnel complies with the WCM Research Biosafety Program during the conduct of biological research.
- Be adequately trained in proper microbiological techniques.
- Provide laboratory research staff with protocols describing potential biohazards and necessary precautions.
- Instruct and train laboratory staff in the practices and techniques required to ensure safety and the procedures for dealing with accidents.
- Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).
- Supervise laboratory staff to ensure the use of all required safety practices and techniques.
- Correct work errors and conditions that may result in the release of recombinant or synthetic nucleic acid materials.
- Ensure the integrity of physical containment (e.g., biological safety cabinets) and biological containment.
- Comply with permit and shipping requirements for hazardous materials, including recombinant or synthetic nucleic acid molecules.
- Adhere to the EHS Biological Spill Planning and Response Program for handling accidental spills and personnel contamination.

5.3.1 PI Responsibilities Prior to Conducting Research

- Submit a Laboratory Registration to the WCM IBC for review and approval.
- Determine whether the research is subject to Section III-A, B, C, D, E, or F of the NIH Guidelines.

- Propose physical and biological containment levels in accordance with the CDC and NIH Guidelines.
- Propose appropriate microbiological practices and laboratory techniques to be used for the research.

5.3.2 PI Responsibilities While Conducting Research

- Consult the WCM IBC before modifying approved recombinant or synthetic nucleic acid research protocols.
- Submit periodic updates and any changes (e.g., changes in the source of DNA or host-vector system) to the IBC for review.
- Report any significant problems regarding the operation and implementation of containment practices and procedures, violations of the NIH Guidelines, or any significant research-related accidents and illnesses to the IBC, OBA, and, as applicable, the Biological Safety Officer, Animal Facility Director, and other appropriate authorities.

5.4 LABORATORY PERSONNEL

- Follow all procedures outlined in this Plan.
- Adhere to recommendations made by the PI, Biological Safety Officer, and EHS.

5.5 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC)

- Conduct initial and periodic review of research conducted at or sponsored by the institution for compliance with the NIH Guidelines.
- Notify the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval.
- Report any significant problems with or violations of the NIH Guidelines and any significant research-related accidents or illnesses to the appropriate institutional official and NIH/OSP.

6.0 Occupational Health

The health screening/monitoring requirements for new research laboratory employees include an initial evaluation , which covers:

- Employee medical screening,
- Blood tests,
- Hepatitis B vaccination,
- TB screening,
- Respirator clearance, and
- Chemo or laser screening as appropriate.

The medical screening includes occupational and medical history, physical examination, and blood work for measles, mumps, rubella and varicella immunity, as well as vision screening. Research laboratory employees will complete a yearly medical monitoring questionnaire, undergo TB screening, complete chemo screening as required, and complete the animal handler questionnaire.

An additional level of protection for at-risk personnel may be achieved with appropriate prophylactic immunizations. Vaccines for which the benefits (levels of antibody considered to be protective) clearly exceed the risks (local or systemic reactions) should be required for all clearly identified at-risk personnel. Examples of such preparations include vaccines against hepatitis B, yellow fever, rabies, and vaccinia. If special hazards exist for an individual's work, the IBC, EHS and the Director of Workforce Health and Safety should be consulted for other tests.

Individuals with work-related illnesses and injuries or accidental exposures to blood, body fluids, and other potentially infectious materials should report directly to Workforce Health and Safety so that the exposure can be documented and appropriate preventive measures initiated.

The Principal Investigator is responsible for reporting illness among laboratory personnel that affects single individuals repeatedly or multiple individuals, either at the same time or in some close sequence. The PI is also responsible for

reporting even sporadic instances of unusual or life-threatening diseases such as leukemias, lymphoma, or chronic disabilities due to nervous, respiratory, renal, or gastrointestinal illness to the IBC.

7.0 Risk Assessment and Biosafety Level Determination

The selection of an appropriate biosafety level for work with a particular agent or animal study depends upon a comprehensive risk assessment of factors associated with the research.

Risk assessment is the process used to identify hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a Laboratory-Acquired Infection (LAI), and the probable consequences of such an exposure. The NIH Guidelines has established a classification and assigned human etiological agents into four risk groups based on the hazard. The descriptions of the NIH risk group classifications are presented in Section 7.1. They correlate with but do not equate to biosafety levels. The risk assessment will determine the degree of correlation between an agent's risk group classification and biosafety level.

Laboratory directors and principal investigators should use the risk assessment to alert their personnel to the hazards of working with biological agents, and to the need for developing proficiency in the use of selected safe practices and containment equipment. Successful control of hazards in the laboratory also protects persons not directly associated with the laboratory, such as other occupants of the same building and the public. Principal Investigators should make their laboratory personnel aware of the etiological signs and symptoms associated with infection or toxicity of any agents used or stored in their laboratory.

The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: agent hazards and laboratory procedure hazards. The capability of the laboratory staff to control hazards must be considered as well. This capability will depend on the training, technical proficiency, and good habits of all members of the laboratory, and the operational integrity of containment equipment and facility safeguards.

A risk assessment should identify any potential deficiencies in the practices of the laboratory workers. Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are prerequisites for laboratory staff in order to reduce the inherent risks that attend work with hazardous agents. **Laboratory directors or PIs should train and retrain new personnel to the point where aseptic techniques and safety precautions become second nature.**

The principal hazardous characteristics of an agent are:

- Its capability to infect and cause disease in a susceptible human or animal host,
- Its virulence as measured by the severity of disease, and
- The availability of preventive measures and effective treatments for the disease.

Other hazardous characteristics of an agent include probable routes of transmission of laboratory infection, infective dose, stability in the environment, host range, and its endemic nature. Reports of LAIs are also a clear indicator of hazard and often are sources of information helpful for identifying the agent and procedural hazards, and the precautions for their control.

The predominant routes of transmission in the laboratory are:

1. Direct skin, eye or mucosal membrane exposure to an agent;
2. Parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors;
3. Ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure; and
4. Inhalation of infectious aerosols.

A procedure's potential to release microorganisms into the air as aerosols and droplets is the most critical operational risk factor that supports the need for containment equipment and facility safeguards. Procedures and equipment used routinely for handling infectious agents in laboratories, such as pipetting, blenders, non-self-contained centrifuges, sonicators, and vortex mixers are proven sources of aerosols. Most manipulations of liquid suspensions of microorganisms produce aerosols and droplets.

Small-particle aerosols have respirable size particles that may contain one or several microorganisms. These small particles stay airborne for protracted periods and easily disperse throughout the laboratory. When inhaled, the human lung will retain those particles creating an exposure hazard for the person performing the operation, coworkers in the laboratory, and a potential hazard for persons occupying adjacent spaces open to airflow from the laboratory. Larger particle droplets rapidly fall out of the air, contaminating gloves, the immediate work area, and the mucous membranes of unprotected workers.

When work involves the use of laboratory animals, the hazardous characteristics of zoonotic agents also require careful consideration of a risk assessment. Evidence that experimental animals can shed zoonotic agents and other infectious agents under study in saliva, urine, or feces is an essential indicator of hazard. The death of a primate center laboratory worker from Cercopithecine herpesvirus 1 (CHV-1, also known as Monkey B virus) infection following an ocular splash exposure to biologic material from a rhesus macaque emphasizes the seriousness of this hazard.

The identification and assessment of hazardous characteristics of genetically modified agents involve consideration of the same factors used in risk assessment of the wild-type organism. It is particularly important to address the possibility that the genetic modification could increase an agent's pathogenicity or affect its susceptibility to antibiotics or other effective treatments.

In summary, the laboratory director's or PI's biological risk assessment should include the following five points:

1. Identify agent hazards and perform an initial assessment of risk.
2. Identify laboratory procedure hazards.
3. Determine the appropriate biosafety level and select additional precautions indicated by the risk assessment.
4. Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment.
5. Review the risk assessment with a biosafety professional, laboratory animal veterinarian, subject matter expert, and the IBC.

7.1 CLASSIFICATION OF BIOLOGICAL AGENTS

NIH Appendix B reflects the current state of knowledge and should be considered a resource document.

Appendix B - Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

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)

8.0 Laboratory Biosafety Level Criteria

The description of the four Biosafety levels for activities involving infectious agents, recombinant microorganisms and laboratory animals are summarized here. The levels are designated in ascending order, by the degree of protection provided to personnel, the environment, and the community. The standard and special practices, safety equipment, and facilities that apply to agents assigned to Biosafety Level 1-4 are described in the BMBL.

8.1 BIOSAFETY LEVEL 1 (BSL-1)

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

the environment. Adeno-associated virus, Escherichia coli K-12, Saccharomyces cerevisiae, and Baculovirus are representative of microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals.

BSL-1 work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or related science. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no particular primary or secondary barriers recommended, other than a sink for hand washing.

8.2 BIOSAFETY LEVEL 2 (BSL-2)

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that:

1. Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures;
2. Access to the laboratory is restricted when work is being conducted; and
3. All procedures in which infectious aerosols or splashes may be created are conducted in Biosafety Cabinets (BSCs) or other physical containment equipment.

Biosafety Level 2 practices, equipment, and facility design and construction apply to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. With proper microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, HIV, Salmonella, and Toxoplasma are representative of microorganisms assigned to this containment level.

BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the WCM Bloodborne Pathogen Exposure Control Plan for specifically required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. **Personal protective equipment (PPE) should be used as appropriate, such as splash shields, face protection, gowns, and gloves.** Secondary barriers, such as hand washing sinks and waste decontamination facilities (i.e., autoclave), must be available to reduce potential environmental contamination.

8.3 BIOSAFETY LEVEL 3 (BSL-3)

Biosafety Level 3 practices, safety equipment, and facility design and construction apply to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Mycobacterium tuberculosis, St. Louis encephalitis virus, and Coxiella burnetii are representative of the microorganisms assigned to this level.

BSL-3 laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.

Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

A BSL-3 laboratory has special engineering and design features and a biosafety manual specific to the laboratory and organisms in use. The laboratory director prepares or adopts this manual and incorporates biosafety precautions into standard operating procedures.

The Director of EHS, the Biosafety Officer, and the Senior Associate Dean of Research and Office of Sponsored Research Administration (OSRA) must be consulted before initiating research at this level.

8.4 BIOSAFETY LEVEL 4 (BSL-4)

Biosafety Level 4 practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL-4 agents also should be handled at this level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at BSL-4. The primary hazards to personnel working with BSL-4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals pose a high risk of exposure and infection to laboratory personnel, the community, and the environment.

The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied positive-pressure personnel suit. The BSL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent the release of viable agents to the environment. There are no BSL-4 research facilities at WCM.

Table 8-0: Summary of Recommended Biosafety Levels for Biological Agents

Biosafety Level BSL	Agent Types	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in healthy adults	**Standard microbiological practices**	▪ No primary barriers required. ▪ PPE: laboratory coats and gloves; eye, face protection, as needed.	Laboratory bench and sink required
2	▪ Agents associated with human disease ▪ Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	**BSL-1 practice plus:** ▪ Limited access ▪ Biohazard warning signs ▪ "Sharps" precautions ▪ Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: ▪ BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials ▪ PPE: Laboratory coats, gloves, face, and eye protection, as needed.	**BSL-1 plus:** ▪ Autoclave available
3	Indigenous or exotic agents that may cause serious or potentially lethal disease	**BSL-2 practice plus:** ▪ Controlled access ▪ Decontamination of all	Primary Barriers: ▪ BSCs or other physical containment devices	**BSL-2 plus:** ▪ Physical separation from access corridors

	through the inhalation route of exposure	waste ▪ Decontamination of laboratory clothing before laundering	used for all open manipulations of agents ▪ PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed	▪ Self-closing, double-door access ▪ Exhausted air not recirculated ▪ Negative airflow into laboratory ▪ Entry through airlock or anteroom ▪ Hand washing sink near laboratory exit
4	▪ Dangerous/exotic agents which pose a high individual 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 data are available to designate the level ▪ Related agents with unknown risk of transmission	**BSL-3 practices plus:** ▪ Clothing change before entering ▪ Shower on exit ▪ All material decontaminated on exit from the facility	Primary barriers: ▪ All procedures conducted in Class III BSCs or Class I or II BSCs in combination with a full-body, air-supplied, positive pressure suit	**BSL-3 plus:** ▪ Separate building or isolated zone ▪ Dedicated supply and exhaust, vacuum, and decontamination systems ▪ Other requirements outlined in the text

9.0 Vertebrate Animal Biosafety Level

If experimental animals are used, institutional management must provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, and care.

This guidance is provided for the use of experimentally infected animals housed in indoor research facilities (e.g., vivaria), and is useful in the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. In both instances, the institutional management must provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, security and care for the laboratory animal.

Laboratory animal facilities are a particular type of laboratory. As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents in vivo and in vitro are comparable. However, the animal room can present unique problems, as the activities of the animals themselves can present hazards not found in standard microbiological laboratories. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic agent.

The co-application of Biosafety Levels and the Animal Biosafety Levels are determined by a protocol-driven risk assessment, and the specific practices and containment are documented on the WCM Research Animal Resource Center (RARC) Protection and Control form. For additional information, please refer to the RARC User Guide.

These recommendations presuppose that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g., Guide for the Care and Use of Laboratory Animals and Laboratory Animal Welfare Regulations) and that appropriate species have been selected for animal experiments. The publication by the Institute for Laboratory Animal Research (ILAR), “Occupational Health and Safety in the Care and Use of Research Animals”, is most helpful in this regard. Additional safety guidance on working with non-human primates is available in the ILAR publication, “Occupational Health and Safety in the Care and Use of Nonhuman Primates”.

The recommendations detailed below describe four combinations of practices, safety equipment, and facilities for experiments with animals involved in infectious disease research and other studies that may require containment. These four combinations are, designated Animal Biosafety Levels (ABSL) 1-4, and provide increasing levels of protection to

personnel and the environment, and are recommended as minimal standards for activities involving infected laboratory animals. The four ABSLs describing animal facilities and practices are detailed in the BMBL.

In addition to the animal biosafety levels described in this section, the USDA has developed facility parameters and work practices for handling agents of agriculture significance. Appendix D in the BMBL includes a discussion on Animal Biosafety Level 3 Agriculture (BSL-3-Ag). USDA requirements are unique to agriculture because of the necessity to protect the environment from pathogens of economic or environmental impact. Appendix D also describes some of the enhancements beyond BSL/ABSL-3 that may be required by USDA-APHIS when working in the laboratory or vivarium with specific veterinary agents of concern.

Facility standards and practices for invertebrate vectors and hosts are not addressed explicitly in this section; however, the Arthropod Containment Guidelines are available online via the American Society of Tropical Medicine and Hygiene (ASTMH).

9.1 ANIMAL BIOSAFETY LEVEL 1 (ABSL1)

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

Standard practices, safety equipment, and facility requirements apply to ABSL-1. ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required. Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

9.2 ANIMAL BIOSAFETY LEVEL 2 (ABSL2)

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. **ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. I**t also addresses hazards from ingestion, as well as from percutaneous and mucous membrane exposure.

ABSL-2 requires that:

1. Access to the animal facility is restricted;
2. Personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents;
3. Personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and
4. BSCs or other physical containment equipment is used when processes involve the manipulation of infectious materials, or where aerosols or splashes may be created.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. An employee occupational health program is in place for researchers conducting work with animals.

9.3 ANIMAL BIOSAFETY LEVEL 3 (ABSL3)

Animal Biosafety Level 3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing severe or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2. The ABSL-3 laboratory has special engineering and design features.

ABSL-3 requires that:

1. Access to the animal facility is restricted;
2. Personnel must have specific training in animal facility procedures, the handling of infected animals, and the manipulation of potentially lethal agents;
3. Personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations, and husbandry procedures; and

4. Procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in BSCs or by use of other physical containment equipment.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment and an employee occupational health programs are implemented.

Animal Biosafety Level 3 requires a specially designed facility, with unique engineering and design features, a biosafety manual specific to the laboratory and organisms in use, which includes written standard operating procedures, and specialized training in handling these agents. Prior consultation with the Director of the Research Animal Resource Center (RARC), the Director of EHS, the Biosafety Officer and the Senior Associate Dean of Research and Office of Sponsored –Research Administration (OSRA) must be made before initiating research at this level.

9.4 ANIMAL BIOSAFETY LEVEL 4 (ABSL4)

Animal Biosafety Level 4 is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission.

There are no ABSL-4 research facilities at Weill Cornell Medicine.

Table 9.0 – Summary of Recommended Animal Biosafety Levels for Activities in which Experimentally or Naturally Infected Vertebrate Animals Are Used

Animal Biosafety Level (ABSL)	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 standard care of each species ▪ PPE: laboratory coats and gloves; eye, face protection, as needed	Standard animal facility: ▪ No recirculation of exhaust air ▪ Directional airflow 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 the facility ▪ Entry through ante-room or airlock

				▪ Negative airflow into animal and procedure rooms ▪ Hand washing sink near the exit of animal or procedure room
4	▪ Dangerous/exotic agents which pose a high individual 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 data are available to re-designate 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 a 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

10.0 Tissue Culture Procedures

Workers who handle or manipulate human or animal cells and tissues are at risk for possible exposure to potentially infectious latent and adventitious agents that may be present in those cells and tissues. This risk is well understood and illustrated by the reactivation of herpes viruses from latency, the inadvertent transmission of disease to organ recipients, and the persistence of human immunodeficiency virus (HIV), HBV, and hepatitis C virus (HCV) within infected individuals in the U.S. population. There also is evidence of accidental transplantation of human tumor cells to healthy recipients, which indicates that these cells are potentially hazardous to laboratory workers who handle them.

In addition, human and animal cell lines that are not well characterized or are obtained from secondary sources may introduce an infectious hazard to the laboratory. For example, the handling of nude mice inoculated with a tumor cell line unknowingly infected with lymphocytic choriomeningitis virus resulted in multiple LAIs. The potential for human cell lines to harbor a bloodborne pathogen led the Occupational Health and Safety Administration (OSHA) to interpret that the occupational exposure to bloodborne pathogens final rule would include primary human cell lines and explants.

Although the risk of laboratory infection from working with cell cultures, in general, is low, risk increases when working with human and other primate cells, and primary cells from other mammalian species. Potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens HBV, HIV, HCV, HTLV, EBV, HPV and CMV as well as agents such as Mycobacterium tuberculosis that may be present in human lung tissue. Cells immortalized with viral agents such as SV-40, EBV adenovirus or HPV, as well as cells carrying viral genomic material also present potential hazards to laboratory workers. Tumorigenic human cells also are potential hazards due to self-inoculation. There has been one reported case of development of a tumor from an accidental needle-stick. **Laboratory workers should never handle autologous cells or tissues.**

Other primate cells and tissues also present risks to laboratory workers. Non-human primate (NHP) cells, blood, lymphoid and neural tissues should always be considered potentially hazardous. B virus occurs as a natural infection of Asiatic macaque monkeys. Personnel working in the laboratory with potentially infected cells or tissues from macaques must always follow additional precautions (access to NHP exposure kit, NHP training). Exposure of mucous membranes or through skin breaks provides B virus access to a new host, whether the virus is being shed from a macaque or present in contaminated cells or tissues.

BSL-2 practices and containment are recommended for handling human and other primate cells. All work should be performed in a BSC, and all material decontaminated by autoclaving or disinfection as detailed in the waste

management manual. BSL-2 recommendations for personal protective equipment such as laboratory coats, gloves and eye protection should be rigorously followed.

All laboratory staff working with human cells and tissues should be enrolled in an occupational medicine program specific for bloodborne pathogens and should operate under the policies and guidelines established in the WCM Bloodborne Pathogen Exposure Control Plan. Laboratory staff working with human cells and tissues, NHP blood/tissues, other body fluids, and other tissues should be offered hepatitis B immunization and must be evaluated by a health care professional following an exposure incident.

11.0 Recombinant DNA Research

11.1 RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES

The purpose of the NIH Guidelines is to specify the practices for constructing and safe handling of research involving recombinant and synthetic nucleic acid molecules, by determining appropriate biosafety practices and procedures for research involving the construction and handling of either recombinant DNA molecules or organisms and viruses that contain recombinant DNA.

In the context of the NIH Guidelines, recombinant and synthetic nucleic acids are defined as:

(i) Molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids;

(ii) Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, (i.e., synthetic nucleic acids), or

(iii) Molecules that result from the replication of those described in (i) or (ii).

As an NIH-funded institution, Weill Cornell Medicine adopts all practices recommended under the Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, as published by the National Institutes of Health. **Compliance with the NIH Guidelines is mandatory for all investigators conducting recombinant or synthetic nucleic acid molecule research funded by the NIH, or performed at or sponsored by any public or private entity that receives any NIH funding.** This broad reach of the NIH Guidelines aims to instill biosafety practices throughout the institution.

The Weill Cornell Medicine Institutional Biosafety Committee (IBC) is a faculty-led committee of experts in biosafety-related fields. The IBC, established under the NIH Guidelines, is responsible for providing review and oversight to ensure that all forms of research are conducted at Weill Cornell Medicine (WCM) in compliance with the applicable Federal, State, and local health and safety standards and Institutional policies.

The IBC supervision of research includes projects involving:

- Recombinant or synthetic nucleic acid molecules,
- Biological agents classified as Risk Group 2, 3, and 4 in the NIH Guidelines,
- Research involving select agents as listed by the USDA/CDC,
- Human gene transfer research, and
- Clinical trials involving the use of biohazardous agents in human subjects.

The guidelines have specific sections addressing risk assessment concerning the hazards associated with recombinant and synthetic nucleic acid molecules and manipulations of microorganisms. The appendices of the guidelines should be reviewed prior to designing experiments. Four biosafety levels are described in the Guidelines for Research Appendix G, Physical Containment. These biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed, and are based on the potential hazards imposed by the agents used and for the laboratory function and activity.

Experiments involving recombinant and synthetic nucleic acid molecules lend themselves to a third containment mechanism, namely, the application of highly specific biological barriers. Natural barriers exist that limit either:

(i) The infectivity of a vector or vehicle (plasmid or virus) for particular hosts, or
(ii) Its dissemination and survival in the environment.

Vectors, which provide the means for recombinant and synthetic nucleic acid molecules and/or host cell replication, can be genetically designed to decrease, by many orders of magnitude, the probability of dissemination of recombinant and synthetic nucleic acid molecules outside the laboratory.

Since these three means of containment are complementary, different levels of containment can be established that apply various combinations of the physical and biological barriers along with constant use of standard practices. Categories of containment are considered separately in order that such combinations can be conveniently expressed in the NIH Guidelines.

For research involving plants, there are four biosafety levels (BL1-P through BL4-P) described in the Guidelines for Research Appendix P, Physical and Biological Containment for Recombinant or Synthetic Nucleic Acid Molecule Research Involving Plants. **If your research involves plants, plant pathogens, or related recombinant DNA, EHS must be contacted prior to conducting experiments.**

For research involving animals which are of a size or have growth requirements that preclude the use of conventional primary containment systems used for small laboratory animals, four biosafety levels (BL1-N through BL4-N) are described in the Guidelines for Research Appendix Q.

Copies of the NIH Guidelines are available online in the NIH Website. For additional information, please contact the Office of Sponsored Research Administration (OSRA) or Environmental Health and Safety.

12.0 Disinfectants and Sterilization

12.1 DISINFECTANTS

The information presented in this Section will provide a general guideline for selecting a particular disinfectant for use in the laboratory.

The best way of ascertaining if a disinfectant is suitable for a particular agent is to challenge that agent with the disinfectant in a dose-versus-viability study. In general, this is not necessary due to a large volume of literature already existent for many organisms and disinfectant combinations. A brief description of the mode of action of each class of disinfectant is given below.

Although physical methods are superior to chemical disinfection, it is not practical to autoclave everything especially if denaturation of equipment or explosion can occur from such treatment.

Treatment of inert surfaces and heat-labile materials can be accomplished by use of chemical disinfectants, provided the following factors are considered:

- Concentration of active ingredient,
- Duration of contact,
- pH,
- Temperature, Humidity, and
- Presence of extrinsic organic matter.

The interplay of these factors will dictate whether minimal inactivation or final disinfection is achieved. In all situations, review the manufacturer's recommendations for formulation and use.

Do not attempt to use a chemical disinfectant for a purpose it was not designed for.

12.2 DISINFECTANT GROUPS

12.2.1 Aldehydes (Formaldehyde, Paraformaldehyde, Glutaraldehyde)

Formaldehyde and its polymerized solid, paraformaldehyde, is effective for surface and space decontamination. Formaldehyde gas is used to decontaminate large spaces and biological safety cabinets. As a liquid (5% concentration) it

is an effective liquid decontaminant. It is not stable at refrigeration temperatures and has a pungent, irritating odor. In addition, **formaldehyde is presently considered a cancer-suspect agent by regulatory agencies, and its use is not recommended for decontamination spaces or equipment.**

Formaldehyde gas is explosive between 7.0 - 73% concentration in air, and has to be used with extreme caution due to its high vapor pressure. Action is accomplished through alkylation of carboxyl, hydroxyl and sulfhydryl groups on proteins. Cidex, a commercial glutaraldehyde disinfectant, is used for cold surface disinfection of clinical instruments.

12.2.2 Halogens (Iodine, Chlorine)

Both halogen compounds behave similarly, by binding to protein and acting as oxidizing agents. Specifically, chlorine as sodium hypochlorite (household bleach), at 5.25% provides 52,500 ppm available chlorine. It must be used the same day of preparation and must be made fresh.

Care must be taken in using chlorine-based disinfectants, which can corrode metal, rubber and other surfaces. Free organic matter (protein etc.) will decrease the available chlorine, making this disinfectant concentration-dependent with respect to organic load. It should not be used in autoclaves without reducing first with sodium thiosulfate or sodium bisulfite to a neutral solution.

Hypochlorite is an oxidizer and should not be used on paper and other flammables. Chloramine T (from sodium hypochlorite and p-toluenesulfonamide) is a more stable, odorless and less corrosive chlorine compound, but has decreased germicidal activity.

Wescodyne, Betadyne, Povidone-Iodine and other iodophors are commercially available and give good control when used according to the manufacturer's recommended dilution instructions.

Both bleach and iodophors should be made up in cold water in order to prevent the breakdown of the disinfectant.

12.2.3 Quaternary ammonium compounds: (Zephirin, CDQ, A-3)

These compounds are basically "-static" in nature, and are generally ineffective as disinfectants against viruses, spores, and Mycobacterium tuberculosis. Reduced activity occurs when used with soaps, detergents, acids, and when in the presence of heavy organic matter loads; which makes it insufficient for any type of terminal disinfection. Their mode of action is by membrane damage and leakage, followed by protein denaturation. Many of these compounds are used in water baths, incubators and other areas where halide or phenol residues are not desired.

12.2.4 Phenolics: (O-phenophenoate - base compounds)

These compounds act through membrane damage and are effective against viruses, rickettsia, fungi, and vegetative bacteria. They are not as adversely affected by organic matter as other disinfectants. Cresols, hexachlorophene, alkyl-and-chloro derivatives, and biphenyls are more active than phenol itself. Available commercial products are Amphyl, O-syl, Tergisyl, Lysol, Vesphene, and Expose.

12.2.5 Acids/Alkalies

Strong mineral acids and alkalies have disinfectant properties proportional to the extent of their dissociation in solution. Some hydroxides are more effective than would be predicted from their values. In general, acids are better disinfectants than alkalis. Their mode of action is attributed to increase of H+ and OH- species in solutions which interfere with certain microbial functions, however the total effect is not dependent on pH alone.

Weak organic acids are more potent than inorganic acids, despite low dissociation rates in solution. Benzoic acid, lactic acid, and propionic acid are commonly used as preservatives in food.

12.2.6 Alcohols

Alcohols solubilize lipids and denature proteins slowly by acting directly on S-H functional groups. The compounds are effective against lipid-containing viruses and a broad spectrum of bacterial species, but ineffective against spore-forming bacteria. They can be combined with phenolics and iodine to enhance activity.

Alcohols evaporate quickly and leave no residue. They are essentially non-corrosive. Higher molecular weight alcohols are more effective but are less miscible with water. Ethanol and isopropanol are used as 70-80% aqueous solutions. Absolute alcohols are not as effective, indicating that some water is needed in the disinfection process.

12.3 STERILIZATION

12.3.1 Autoclave

Autoclaving at temperature 121°C (steam under pressure), at 20 psi, is one of the most convenient and effective means of sterilization available. Care must be taken to ensure that the steam can circulate articles in order to provide even exposure to heat. The success of sterilization is very time-dependent in liquid media, with large volumes requiring longer times to reach the effective temperature within the media itself.

Additionally, there should be no empty spaces in the load that could insulate against the steam. This condition could prevent the steam from reaching the container and transferring the heat to the vessel. In dry loads, small amounts of water should be included inside the autoclave bag to ensure moisture content within the open bag.

A Diack or commercial Bacillus stearothermophilus or Bacillus subtilis var. niger test strips to check for autoclave efficiency should be used on each terminal sterilization of pathogenic cultures. Autoclave tape (non-lead containing) can be used for routine runs involving sterile media and glassware.

12.3.2 Dry Heat

Ovens operating at 160-170°C for periods of 2-4 hours are efficient for sterilizing glassware, or other non-porous, heat-conductive materials. It is not suitable for organic and inorganic materials that can act as insulation, or for heat labile materials.

Incineration is a very efficient means of final sterilization and disposal and is used for "spot" sterilization of inoculating loops and needles, as well as flaming glassware during microbiological procedures. Loaded materials must be flamed carefully since this practice can release aerosols of viable (infectious) material.

12.3.3 Radiation

Ionizing radiation is not used for general laboratory sterilization. However ultraviolet radiation (U.V.) is used to control airborne microorganisms and environmental surface contamination. Ultraviolet light sources are used in Biological safety cabinets for partial contamination control. It is extremely limited due to its poor penetrating power and is not entirely documented as a control method.

12.3.4 Vapors and Gases (Ethylene Oxide, Paraformaldehyde, H_2O_2, ClO_2)

From a practical point of view, formaldehyde, beta-propiolactone, and ethylene oxide are not routinely used in laboratory sterilization applications. These sterilants are used in hospital and commercial facilities where closed systems controlling temperature, humidity, and concentration are required to achieve sterilization using these agents.

Biological safety cabinets have been decontaminated using Paraformaldehyde heated to decomposition to release formaldehyde gas (explosive). This procedure is performed only by National Sanitation Foundation-certified individuals trained in this technique.

12.3.5 Hydrogen Peroxide Vapor

Hydrogen peroxide can be vaporized and used for the decontamination of biological safety cabinets, glove boxes, and small room areas. Vapor-phase hydrogen peroxide has been shown to be an effective sporicide at concentrations ranging from 0.5 mg/L to <10 mg/L.

The optimal concentration of this agent is about 2.4 mg/L with a contact time of at least one hour. This system can be used to decontaminate glove boxes, walk-in incubators, and small rooms. An advantage of this system is that the end products (i.e., water) are not toxic. Low relative humidity can be used.

12.3.6 Chlorine Dioxide Gas

Chlorine dioxide gas sterilization can be used for decontamination of laboratory rooms, equipment, glove boxes, and incubators. The concentration of gas at the site of decontamination should be approximately 10 mg/L, with a contact time of one to two hours.

Chlorine dioxide possesses the bactericidal, virucidal, and sporicidal properties of chlorine, but unlike chlorine, does not lead to the formation of trihalomethanes or combine with ammonia to form chlorinated organic products (chloramines). The gas cannot be compressed and stored in high-pressure cylinders but is generated upon demand using a column-based solid phase generation system. Gas is diluted to the use concentration, usually between 10 and 30 mg/L. Within reasonable limits, a chlorine dioxide gas generation system is unaffected by the size or location of the ultimate destination for the gas. Relative humidity does need to be controlled, and high humidities are optimal. Although most often used in closed sterilizers, the destination enclosure for the chlorine dioxide gas does not need to be such a chamber. Because chlorine dioxide gas exits the generator at a modest positive pressure and flow rate, the enclosure also need not be evacuated and could be a sterility-testing isolator, a glove box, or sealed BSC, or even a small room that could be sealed to prevent gas egress.

Chlorine dioxide gas is rapidly broken down by light; care must be taken to eliminate light sources in spaces to be decontaminated.

Instruments and optics that may be damaged by other sterilization methods, rooms, building, and air-handling systems are also sterilized using these agents. All of these agents are extremely toxic and regulated under Federal OSHA and EPA guidelines.

Note: *the desired result for any treatment is to arrive at a significant reduction in the numbers of infectious agents (several orders of magnitude), with the effect that there is no longer a risk of acquiring an infection while handling the materials after treatment. Complete sterility except media preparation is not required for the disposal of infectious waste.*

12.4 USEFUL DILUTIONS OF WESCODYNE AND COMMON HOUSEHOLD BLEACH

12.4.1 Standard Wescodyne Solution

3 OZ=90cc (1.2 cc/5 gallons = 1ppm)

90cc	=	36cc / 5gal	=	18cc / 2gal	=	2.37cc / 1gal	= 75 ppm available iodine 500ml
180cc	=	72cc / 5gal	=	36cc / 2gal	=	4.8cc / 1gal	= 150 ppm available iodine 500ml
1800cc	=	720cc / 5gal	=	360cc / 2gal	=	48.0cc / 1gal	= 500 ppm available iodine 500ml

12.4.2 Bleach Solutions

1/100 dilution of 5.25% Bleach ~ 525ppm

1/10 dilution of 5.25% Bleach ~ 5,250ppm

1.0 (straight) of 5.25% Bleach = 52,500ppm available chlorine

1/8 = 6562.5ppm ~ Dakin solution

(500 ppm recommended for most uses)

12.4.3 Phenolics

Follow the directions of the manufacturer for proper dilution of the concentrate. Any deviation from the quantities recommended will result in less than satisfactory results. The preparation as formulated at a given concentration was tested using A.O.A.C. protocols, and proven effective against the organisms listed on the table.

13.0 Biological Safety Cabinet (BSC)

Biological safety cabinets (BSC) are among the most effective, as well as the most commonly used primary containment devices in laboratories working with infectious agents. The three general types available (Class I, II, III) have performance characteristics and applications which are described below. The type of cabinet used will depend on application, type of agents used in the lab, and whether product sterility, personal protection, or both are critical considerations in the research environment.

Properly maintained Class I and II BSC s, when used in conjunction with **proper microbiological techniques,** provide an effective containment system for safe manipulation of moderate and high-risk microorganisms (Biosafety Level 2 and 3 agents).

As with any other piece of laboratory equipment, **personnel must be trained in the proper use of the biological safety cabinet.** Of particular note are those activities which may disrupt the inward directional airflow through the work opening of Class I and II BSCs. Repeated insertion of the worker's arms in and from the work chamber, or briskly walking past the BSC while it is in use are demonstrated causes of the escape of aerosolized particles from within the cabinet. Class I and II BSCs should be located away from traffic patterns and doors. Fans, heating, and air conditioning registers, and other air handling devices can also disrupt airflow patterns if located adjacent to the BSC. Strict adherence to recommended practices for the use of BSCs and proper placement in the laboratory is essential in attaining the maximum containment capability of the equipment, as is the mechanical performance of the equipment itself.

It is imperative that BSCs are tested and certified in situ at the time of installation within the laboratory, any time the BSC is moved, and at least annually thereafter. Certification at locations other than the final site may attest to the performance capability of the individual cabinet or model but does not supersede the critical certification prior to use in the laboratory. A list of BSC certifiers is available on the EHS website.

13.1 CLASS I BSC

The Class I BSC provides **personnel and environmental protection, but no product protection**. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. In the Class I BSC, unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow as long as a minimum velocity of 75 linear feet per minute (lfpm) is maintained through the front opening. Because product protection is provided by the Class II BSCs, general usage of the Class I BSC has declined. However, in many cases, Class I BSCs are explicitly used to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures with potential to generate aerosols (e.g., cage dumping, culture aeration or tissue homogenization).

The classical Class I BSC is hard-ducted (i.e., direct connection) to the building exhaust system, and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter

as it enters the cabinet exhaust plenum. A second HEPA filter may be installed in the terminal end of the building exhaust prior to the exhaust fan.

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

Some Class I models used for animal cage changing are designed to allow recirculation of air into the room after HEPA filtration and may require more frequent filter replacement due to filter loading and odor from organic materials captured on the filter. The re-circulating Class I BSC should be annually certified for sufficient airflow and filter integrity.

13.2 CLASS II BSC

As biomedical researchers began to use sterile animal tissue and cell culture systems, particularly for the propagation of viruses, cabinets were needed that also provided product protection.

The Class II (Types A1, A2, B1, and B2) BSCs provide **personnel, environmental and product protection**. Airflow is drawn into the front grille of the cabinet, providing personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination across the work surface of the cabinet. Because cabinet exhaust air is passed through a certified HEPA filter, it is particulate-free (environmental protection), and may be recirculated to the laboratory (Type A1 and A2 BSCs) or discharged from the building via a canopy connection. Exhaust air from Types B1 and B2 BSCs must be discharged to the outdoors via a hard connection.

HEPA filters are effective at trapping particulates and thus infectious agents, but do not capture volatile chemicals or gases. Only Type A2-exhausted or Types B1and B2 BSCs exhausting to the outside should be used when working with volatile, toxic chemicals, but amounts must be limited.

Class II BSCs provide the microbe-free work environment necessary for cell culture propagation and may be used for the formulation of nonvolatile antineoplastic or chemotherapeutic drugs.

13.2.1 The Class II, Type A1 BSC

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

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

The air is discharged through the front and rear grilles under negative air pressure into a blower and pushed into the space between the supply and exhaust filters. Due to the relative size of these two filters, approximately 30% of the air passes through the exhaust HEPA filter, and 70% recirculates through the supply HEPA filter back into the work zone.

13.2.2 The Class II, Type A2 BSC

Note: Only when this BSC is ducted to the outdoors does it meet the requirements of the former Class II Type B3. The Type A2 cabinet has a minimum calculated or measured inflow velocity of 100 lfpm. All positive pressure biologically contaminated plenums within the cabinet are surrounded by a negative air pressure plenum, thus ensuring that any leakage from a contaminated plenum will be drawn into the cabinet and not released to the environment. Minute quantities of volatile toxic chemicals or radionuclides can be used in a Type A2 cabinet only if it exhausts to the outside via a properly functioning canopy connection.

It is possible to exhaust the air from a Type A1 or A2 cabinet outside of the building. However, it must be done in a manner that does not alter the balance of the cabinet exhaust system, and thereby disturbing the internal cabinet airflow.

The proper method of connecting a Type A1 or A2 cabinet to the building exhaust system is through use of a canopy hood, which provides a small opening or air gap (usually 1 inch) around the cabinet exhaust filter housing. The airflow of the building exhaust must be sufficient to maintain the flow of room air into the gap between the canopy unit and the filter housing. The canopy must be removable or be designed to allow for operational testing of the cabinet. Class II Type A1 or A2 cabinets should never be hard-ducted to the building exhaust system.

13.2.3 The Class II, Type B1 BSC

Some biomedical research requires the use of small quantities of hazardous chemicals, such as carcinogens. The powdered form of these carcinogens should be weighed or manipulated in a chemical fume hood or a static-air glove box equipped with a double-door airlock. Carcinogens used in cell culture or microbial systems require both biological and chemical containment.

The Class II, Type B cabinet originated with the National Cancer Institute (NCI)-designed Type 2 (later called Type B) BSC, and was designed for manipulations of minute quantities of these hazardous chemicals with in vitro biological systems. The NSF International NSF/ANSI Standard 49 - 2002 definition of Type B1 cabinets includes this classic NCI design Type B, as well as cabinets without supply HEPA filters located immediately below the work surface, and/or those with exhaust/recirculation down flow splits other than exactly 70/30%.

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

The room air is drawn through the face opening of the cabinet at a minimum measured inflow velocity of 100 lfpm. As with Type A1 and A2 cabinets, there is a split in the down-flowing air stream just above the work surface. In the Type B1 cabinet, approximately 70 percent of the downflow air exits through the rear grille, passes through the exhaust HEPA filter and is discharged from the building. The remaining 30 percent of the downflow air is drawn through the front grille. Since the air flowing to the rear grille is discharged into the exhaust system, activities that may generate hazardous chemical vapors or particulates should be conducted towards the rear of the cabinet work area.

Type B1 cabinets must be hard-ducted, preferably to a dedicated, independent exhaust system, or a properly designed laboratory building exhaust. Fans for laboratory exhaust systems should be located at the terminal end of the ductwork. A failure in the building exhaust system may not be apparent to the user, as the supply blowers in the cabinet will continue to operate.

A pressure-independent monitor and alarm should be installed to provide warning and shut off the BSC supply fan, should a failure in exhaust airflow occur. Since not all cabinet manufacturers supply this feature, it is prudent to install a sensor such as a flow monitor and alarm in the exhaust system as necessary. To maintain critical operations, laboratories using Type B1 BSCs should connect the exhaust blower to the emergency power supply.

13.2.4 The Class II, Type B2 BSC

This BSC is a total-exhaust cabinet; no air is recirculated within it. It provides simultaneous primary biological and chemical containment.

Consideration must be given to the chemicals used in BSCs, as some chemicals can destroy the filter medium, housings and/or gaskets causing loss of containment. The supply blower draws either room or outside air in at the top of the cabinet, passes it through a HEPA filter and down into the work area of the cabinet. The building exhaust system draws air through both the rear and front grills, capturing the supply air, plus the additional amount of room air needed to produce a minimum calculated or measured inflow face velocity of 100 lfpm. All air entering this cabinet is exhausted and passes through a HEPA filter (and perhaps some other air-cleaning device such as a carbon filter if required for the work being performed) prior to discharge to the outside.

The Class II, Type B2 cabinet exhausts as much as 1200 cubic feet per minute of conditioned room air, making this cabinet expensive to operate. The higher static air pressure required to operate this cabinet also results in additional costs

associated with heavier gauge ductwork and higher capacity exhaust fan. The need for using this type of BSC should, therefore, be justified by the research to be conducted.

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

13.2.5 Special Applications

Class II BSCs can be modified to accommodate particular tasks. For example, the front sash can be modified by the manufacturer to accommodate the eyepieces of a microscope, or the work surface can be designed to accept a carboy, a centrifuge, or other equipment that may require containment. A rigid plate with openings for the arms can be added if needed.

Good cabinet design, microbiological aerosol tracer testing of the modification, and appropriate certification are required to ensure that the basic systems operate properly after modification.

13.3 CLASS III BSC

The Class III BSC was designed for work with highly infectious microbiological agents and the conduct of hazardous operations and provides maximum protection for the environment and the worker. It is a gas-tight enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank, that is accessible through the cabinet floor, or double-door pass-through box (e.g., an autoclave) that can be decontaminated between uses. Reversing that process allows materials to be removed from the Class III BSC safely.

Both supply and exhaust air are HEPA-filtered on a Class III cabinet. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge to the outdoors. Airflow is maintained by an exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure (minimum of 0.5 inches of water gauge.) "The exhaust fan for the Class III cabinet is generally separate from the exhaust fans of the facility ventilation system."

Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow direct manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials, so the trade-off is clearly on the side of maximizing personal safety. Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment. Class III BSCs do not have laminar airflow.

13.4 HORIZONTAL / VERTICAL LAMINAR FLOW "CLEAN BENCH"

Horizontal laminar flow "clean benches" are not BSCs. These devices discharge HEPA-filtered air from the back of the cabinet across the work surface and toward the user. They only provide product protection and can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices.

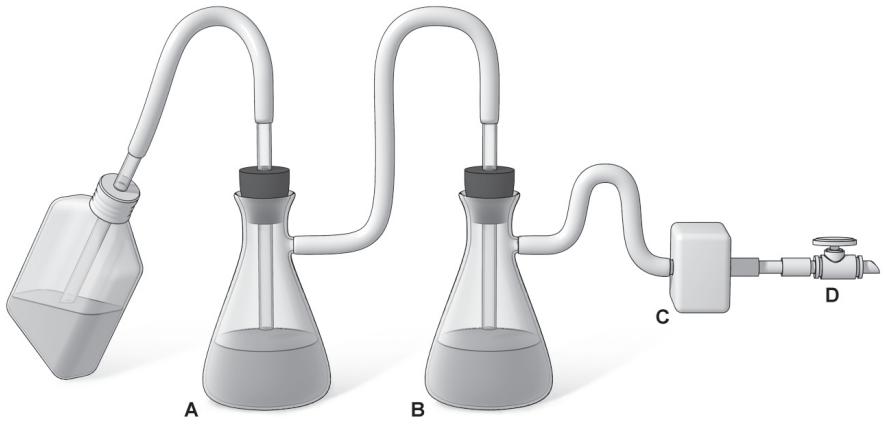
Clean benches should never be used when handling cell culture material, drug formulations, or when manipulating potentially infectious materials. The worker will be exposed to the materials being manipulated on the clean bench potentially resulting in hypersensitivity, toxicity, or infection; depending on the materials being handled.

Horizontal/Vertical laminar flow "clean benches" must never be used as a substitute for a biological safety cabinet. Users must be aware of the differences between these devices.

13.5 RECOMMENDATIONS FOR EFFECTIVE USE OF BSC'S

- Even though they are tested prior to shipping by the manufacturer, **biosafety cabinets must be tested upon arrival in the lab and certified** to ensure that no damage to the filter system resulted in shipment.
- **BSC overall integrity and function must be checked on a yearly basis.**
- **Adequate space use of the cabinet should be planned** to prevent over-crowding or restriction of movement in the cabinet.

- **The biological safety cabinet should be allowed to operate five minutes before manipulations are initiated.** This step allows removal of any possible contaminated air that may have entered while breaching the air-barrier. Insertion of hands and equipment causes turbulence at the point of entry, mixing clean air with dirty air.
- **Interior surfaces must be wiped down with 70% alcohol at the beginning and at the end of the day.**
- **All equipment to be used should be brought inside the cabinet before starting up the cabinet's internal air barrier.**
- **Do not place anything over the front grill, especially if sterility is necessary.** A substantial portion of the air is contaminated (make-up air for the exhaust), therefore this practice defeats one of the key features of the cabinet.
- **Learn to work deep in the interior of the cabinet, at least four inches from the intake grill.** This prevents contamination of the work area and eliminates spillage of liquids into interior surfaces of the cabinet through the grills.
- **Personnel movement near the cabinet front should be kept to a minimum.** Ideally, a separate room with a door will reduce the chances of disturbing air-barrier flow. Movement at the hood face should be minimal, with all movements made slowly so that the airflow at the face of the BSC is not disturbed.
- **The use of centrifuges and shakers must be performed with care** since these activities disturb air-flow in the cabinet and can breach the air-barrier.
- **Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant and to an in-line HEPA or equivalent filter (see figure below)**. This combination will protect the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution (bleach, 1/10 of volume) into the flask to inactivate the microorganisms as they are collected.
- **Biosafety cabinet users should refrain from using natural gas and other flammable substances within a recirculating BSC.** Open flames are not required in the near microbe-free environment of a biological safety cabinet.
- **Ultraviolet (UV) lamps are not recommended in BSCs, nor are they necessary**. UV lamps should be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer. If the cabinet has a sliding sash, close the sash when operating the UV lamp.
- **When finished working within the BSC, it should be surface decontaminated.** With the cabinet blower running, all containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. At the end of the workday, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back and the interior of the glass.
- **The biological safety cabinet is not a substitute for good microbiological technique.**



Protection of the house vacuum system during aspiration; The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.

14.0 Biological Spill Response

The procedures for containing, mitigating, reporting, and treatment following accidental spills or personnel contamination with biological materials are detailed in the Biological Spill Planning and Response Manual.

Biological spills that do not involve injury, are contained, or pose little hazard to personnel, can be cleaned up so long as the person cleaning the spill:

- Has received the proper training on how to clean up the spill,
- Is wearing the proper protective equipment to do the cleanup, and
- Performs the cleanup by following the procedure described within the Biological Spill Planning and Response Manual.

For other types of spills, i.e., larger spills or spills of a biological agent that may cause injury or illness, personnel should hold their breath and leave the lab immediately and notify Environmental Health and Safety (ext. 2-7233). In reporting of a biological spill or incident, principal investigators and laboratory personnel should follow the notification procedures posted within the lab on the Exposure and Spill Response Guide.

15.0 Biological Waste Disposal

The management of biological waste must be as required by applicable local, state, and federal regulations. Prior to disposal of your biological waste, please refer to Section 7 of the EHS Waste Disposal Procedure Manual; which details the appropriate procedures for all biological waste streams encountered in the research laboratory.

16.0 References

- Disinfection, Sterilization and Preservation, 5th Edition, Block, S.S.; (2001) Lippincott Williams & Wilkins, Philadelphia.
- Class II (laminar flow) biosafety cabinetry, NSF/ANSI 49 – (2002), NSF International Standard/American National Standard
- Manual of Clinical Microbiology; 6th Edition, Murray, P.R., et al.; (1995) American Society for Microbiology, Washington DC.
- Biological Safety Principals and Practices, 3rd edition, (2000), Fleming, D.O., Hunt, D.L., American Society for Microbiology, Washington DC.
- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), April 15, 2016, Federal Register, April 15, 2016 (81 FR 22286)
- Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, U.S. Department of Health and Human Services Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health, HHS Publication No. (CDC) 21-1112, (2009)
- Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, 3rd Edition, DEPARTMENT OF HEALTH & HUMAN SERVICES, Centers for Disease Control and Prevention and National Institutes of Health, (September 2007)
- Arthropod Containment Guidelines (Version 3.1), A project of The American Committee of Medical Entomology of the American Society of Tropical Medicine and Hygiene

Appendix A: Biological Material Shipments- Transportation of Hazardous Materials

Shipping of biological materials must be in compliance with the Biological Materials and Dry Ice Shipments Manual available on the EHS website. Additional information on the transportation of dangerous goods, importation, and interstate shipment of etiologic agents of human disease, diagnostic specimens, and other related materials may be obtained by contacting Environmental Health and Safety.

Transportation of infectious substances and materials that are known or suspected to contain them, as well as the shipping of recombinant DNA molecules, are regulated as hazardous materials by the United States Department of Transportation (DOT), foreign governments, and the International Civil Aviation Organization. For this reason, their transportation is subject to regulatory controls. For transport purposes, the term “infectious substance” is understood to include the term “etiologic agent.”

Further information on shipping etiologic agents is available from:

(i) The Centers for Disease Control and Prevention, ATTN: Biohazards Control Office, 1600 Clifton Road, Atlanta, Georgia 30333, (404) 639-3883, FTS 236-3883;

(ii) The U.S. Department of Transportation, ATTN: Office of Hazardous Materials Transportation, 400 7th Street, S.W., Washington, DC 20590, (202) 366-4545; or

(iii) (iii) U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service (APHIS), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, Maryland 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

Appendix B: Agricultural Pathogens

Some pathogens of livestock, poultry and fish may require special laboratory design, operation, and containment features. This may be BSL-3, BSL-3 plus enhancements or BSL-4 and for animals ABSL-2, ABSL-3 or BSL-3-Ag.

The importation, possession, or use of the following agents is prohibited or restricted by law, USDA regulations, or administrative policies.

- African horse sickness virus
- African swine fever virus
- Akabane virus
- Avian influenza virus (highly pathogenic)
- Bacillus anthracis
- Besnoitia besnoiti
- Bluetongue virus (exotic)
- Borna disease virus
- Bovine infectious petechial fever agent
- Bovine spongiform encephalopathy prion
- Brucella abortus
- Brucella melitensis
- Brucella suis
- Burkholderia mallei/Pseudomonas mallei (Glanders)
- Burkholderia pseudomallei
- Camelpox virus
- Classical swine fever virus
- Coccidioides immitis
- Cochliomyia hominivorax (Screwworm)
- Coxiella burnetti (Q fever)
- Ephemeral fever virus
- Ehrlichia (Cowdria) ruminantium (heartwater)
- Eastern equine encephalitis virus
- Foot and mouth disease virus
- Francisella tularensis
- Goat pox
- Hemorrhagic disease of rabbits virus
- Hendra virus
- Histoplasma (Zymonema) farciminosum
- Infectious salmon anemia virus
- Japanese encephalitis virus
- Louping ill virus
- Lumpy skin disease virus
- Malignant catarrhal fever virus
- (exotic strains or alcelaphine herpesvirus type 1)
- Menangle virus b
- Mycobacterium bovis
- Mycoplasma agalactiae
- Mycoplasma mycoides subsp. mycoides (small colony type)
- Mycoplasma capricolum
- Nairobi sheep disease virus (Ganjam virus)

- Newcastle disease virus (velogenic strains)
- Nipah virus
- Peste des petits ruminants virus
- (plague of small ruminants)
- Rift Valley fever virus
- Rinderpest virus
- Sheep pox virus
- Spring Viremia of Carp virus
- Swine vesicular disease virus
- Teschen disease virus
- Theileria annulata
- Theileria lawrencei
- Theileria bovis
- Theileria hirci
- Trypanosoma brucei
- Trypanosoma congolense
- Trypanosoma equiperdum (dourine)
- Trypanosoma evansi
- Trypanosoma vivax
- Venezuelan equine encephalomyelitis virus
- Vesicular exanthema virus
- Vesicular stomatitis virus (exotic)
- Wesselsbron disease virus

For the agents listed above, an Export license may be required by the Department of Commerce, or they may be agents regulated as Select Agents under the Bioterrorism Act of 2002. Possession of these agents requires registration with either the CDC or APHIS, and a permit issued for interstate movement or importation by APHIS-VS.

The importation, possession, use, or interstate shipment of animal pathogens other than those listed above may also be subject to regulations of the U.S. Department of Agriculture.

Appendix C: Select Agents

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002, Subtitle A of Public Law 107-188 (42 U.S.C. 262a), requires DHHS to regulate the possession, use, and transfer of biological agents or toxins (i.e., select agents and toxins) that could pose a severe threat to public health and safety. The Agricultural Bioterrorism Protection Act of 2002, Subtitle B of Public Law 107-188 (7 U.S.C. 8401), requires the USDA to regulate the possession, use, and transfer of biological agents or toxins (i.e., select agents and toxins) that could pose a severe threat to animal or plant health, or animal or plant products.

The current list of select agents and toxins and additional permitting information is available on the CDC and USDA Federal Select Agent Program website.

In keeping with these Acts, DHHS and USDA promulgated regulations requiring entities to register with the CDC or the USDA if they possess, use, or transfer a select agent or toxin (42 CFR Part 73, 7 CFR Part 331, and 9 CFR Part 121). CDC and USDA coordinate regulatory activities for those agents that would be regulated by both agencies ("overlap" select agents).

The Attorney General has the authority and responsibility to conduct electronic database checks (i.e., the security risk assessments) on entities that apply to possession, use, or transfer of select agents, as well as personnel that requires access to select agents and toxins. The FBI, Criminal Justice Information Services Division (CJIS), has been the delegated authority for conducting these security risk assessments.

The regulations provide that, unless exempted, entities must register with CDC or USDA if they possess, use, or transfer select agents or toxins. The regulations set out a procedure for excluding an attenuated strain of a select agent or toxin and exemptions for certain products and select agents or toxins identified in specimens presented for diagnosis, verification, or proficiency testing.

All work with select agents must be approved by Environmental Health and Safety and the WCM Research Dean; and must conform to the WCM Select Agent policies. Contact EHS for more information.