



**Dartmouth College**

**Institutional Biosafety Committee**

**Biosafety Level Determination Guidelines**  
IBC Approved: 10/3/18



**I. DEFINITION OF BIOSAFETY LEVEL:**

Biosafety Levels (BSLs) are containment levels ranked from one to four, with BSL1 being the lowest level of containment and BSL4 being the highest level of containment. All BSLs require following standard microbial practices. Each BSL builds upon the requirements of the previous BSL. At Dartmouth, the highest permitted BSL is Enhanced BSL2 (BSL2+), as defined below. Each level has specific containment and best practices requirements dependent upon the microbes and biological agents used, the facilities available, and the manipulations being conducted. The risks that determine levels of containment include infectivity, severity of disease, transmissibility, attenuation, route of exposure and the nature of the work conducted.

**II. RESPONSIBILITIES:**

- a. **Principal Investigator (PI):** It is the responsibility of the PI to conduct a risk assessment and suggest a containment level for his/her laboratory. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary safety equipment is available to prevent exposures, and exposure evaluation procedures are implemented.
  
- b. **Institutional Biosafety Committee (IBC):** The IBC will make the final determination of Biosafety Level upon IBC review of the proposed work. The IBC has the authority to raise or lower containment levels based on risk assessment.
  
- c. **All researchers:** All researchers are responsible for abiding by best practices for working at a given BSL. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations and response to prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age and immunocompromised individuals should be provided with information regarding immune competence and conditions that may predispose them to infection.

**III. BIOSAFETY LEVEL 1 (BSL1):**

- a. **Description:** Biosafety Level 1 (BSL1) is the lowest containment level. BSL1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans and which present minimal potential hazard to laboratory personnel and the environment. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not typically required other than a sink for handwashing but may be required 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 a related science.
  
- b. **BSL1 Practices:**
  - 1. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
  - 2. Eating, drinking, handling contact lenses, applying cosmetics, and storing food for human consumption is not permitted in laboratory areas.
  - 3. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes or otherwise manipulated by hand before disposal into puncture resistant biohazard containers.



4. Perform all procedures carefully to minimize the creation of splashes and/or aerosols.
5. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant according to the [Dartmouth Biohazard Waste Guide](#).
6. Decontaminate all cultures, stocks and other potentially infectious materials before disposal or transport using an appropriate method according to the [Biosafety in Microbiology and Biomedical Laboratories](#) (BMBL) and the [Dartmouth Biohazard Waste Guide](#).
7. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport. These items must be labeled with a name, date and identity of the material.
8. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
9. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials.
10. Gloves must be worn to protect hands from exposure to hazardous materials. Gloves should be changed when contaminated or compromised. Dispose of used gloves with other contaminated laboratory waste. Wash hands after removing gloves.

**c. BSL1 Facility Requirements:**

1. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present.
2. Special containment devices or equipment, such as biological safety cabinets (BSCs), are not generally required at BSL1.
3. Laboratories should have doors for access control.
4. Laboratories must have a sink for hand washing.
5. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not allowed.
6. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
7. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

**IV. BIOSAFETY LEVEL 2 (BSL2):**

- a. Description:** Biosafety Level 2 (BSL2) is the next highest containment level and includes all of the requirements of BSL1 (see above). BSL2 is suitable for work involving agents that are moderately pathogenic or infectious to healthy adult humans and for which treatments or vaccines are often available or those agents that may pose a moderate hazard to the environment if released. Most labs at Dartmouth are BSL2 labs. Materials commonly used at BSL2 include all human cell lines, human primary cells, human tissues/fluids, and Risk Group 2 agents as listed by the [NIH Guidelines Appendix B](#) (*Classification of Human Etiologic Agents on the Basis of Hazard*). Certain replication-defective viral vectors may also require handling at BSL2 or above. Examples include lentiviral, retroviral, adenoviral, rabies, and vaccinia vectors.

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**b. BSL2 Practices (in addition to all BSL1 practices above):**

1. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a biological safety cabinet (BSC) or other physical containment device. **See below for a list of aerosol transmissible agents and common techniques that generate aerosols.**
2. All procedures involving high concentrations or large volumes of infectious agents must be conducted in a BSC. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cup.
3. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
4. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL2 agents.
5. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Protective clothing must be removed before going to non-laboratory areas including administrative offices, elevators and common hallways/stairways.
6. Eye and face protection (such as goggles, mask or face shield) must be used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device.
7. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.
8. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
9. Laboratory equipment and work surfaces must be decontaminated on a routine basis as well as after spills, splashes, or other contamination. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
10. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to the ***Emergency Response and Biohazard Exposure Control Plan***. All such incidents must be reported to the laboratory supervisor and the Biosafety Officer. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

**c. BSL2 Facility Requirements (in addition to BSL1):**

1. Laboratory doors must be self-closing and must have the ability to be locked/secured. Access to lab is restricted when work is being conducted. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. An eyewash station must be readily available.



3. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
4. HEPA filtered exhaust air from a Class II BSC can be safely recirculation back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
5. All vacuum lines must be protected with inline HEPA filters for liquid disinfectant traps.
6. Mechanical ventilation systems should provide an inward flow of air without recirculation to spaces outside of the laboratory.
7. A method for decontaminating all laboratory wastes must be available in the facility (autoclave, chemical disinfection, incineration, or other validated decontamination method).

**V. ENHANCED BIOSAFETY LEVEL 2 (BSL2+):**

**a. Description:** Enhanced Biosafety Level 2 (BSL2+) is a higher containment level than BSL2. It is used to contain certain agents that may be handled in a BSL2 facility, but require higher precautions, such as those used at BSL3. These added precautions are designed to minimize the potential for exposure. The IBC will determine, based on risk assessment, the need for BSL2+ practices. Examples of work that would be considered for BSL2+ include use of lentiviral or retroviral vectors expressing genes with oncogenic potential or which inhibit tumor suppressor function, use of certain Risk Group 3 agents that have been attenuated, the expression of certain biotoxins, or working with an agent or procedures for which an inhalation exposure presents a significant risk. The scale of an experiment will also be considered when determining risk and containment level.

**b. BSL2+ Practices (in addition to BSL2):**

1. All labs working at BSL2+ must read and sign the **Dartmouth IBC Enhanced BSL2 (BSL2+) Policy**.
2. All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.
3. No work with open vessels is permitted on the bench.
4. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL2+ agents.
5. In addition to standard PPE at BSL2, workers must wear back closing disposable gowns. All disposable gowns must be disposed of with BSL2+ waste. Disposable gowns must not be worn outside of the lab. Reusable clothing is decontaminated by soaking in bleach or autoclaving before being laundered. Gowns must be changed when contaminated.
6. Two pairs of gloves must be worn while working with Enhanced BSL2 material. The outer pair should be removed inside the BSC and disposed of in the biohazard bag inside the cabinet. The inner pair is then removed outside the cabinet and also disposed of as biohazardous waste.



7. All biohazard waste generated in the hood is collected inside of the BSC. Details for collection and decontamination can be found in the Enhanced BSL2 Lab Manual.

**c. BSL2+ Facility Requirements (in addition to BSL2):**

1. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted.
2. A faucet that allows hands-free operation is recommended.
3. Decontamination of the entire laboratory must be done when there has been gross contamination of the space, significant changes in laboratory usage, and before major renovations or maintenance shut downs.
4. Equipment and protocols that may produce infectious aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory (BSC). These HEPA filters should be tested and/or replaced at least annually.

**VI. SPECIAL CONSIDERATIONS:**

- a. **Description:** Special considerations may be made when working with agents that could be transmitted through the aerosol route by inhalation (airborne) or mucous membrane (droplet) exposure. Examples are listed in Appendix A below. Containment, facility, or personal protective equipment requirements may be modified from the above based on risk assessment by the IBC and Biosafety Officer. An example of when special considerations may be made include work conducted in an open lab concept building where multiple labs may share space and equipment.
- b. Please see **Appendix A** for a representative list of **Aerosol Transmissible Organisms**
- c. Please see **Appendix B** for a representative list of common **Aerosol Generating Procedures**

**VII. RESOURCES**

- a. CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL, 5<sup>th</sup> Ed., 2009), Section IV – *Laboratory Biosafety Level Criteria*
- b. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (April 2016), Appendix B - *Classification of Human Etiologic Agents on the Basis of Hazard*
- c. Dartmouth Biosafety website: [http://www.dartmouth.edu/~ehs/biological/policies\\_sops.html](http://www.dartmouth.edu/~ehs/biological/policies_sops.html)
  - Dartmouth IBC Emergency Response and Exposure Control Plan
  - Dartmouth IBC Enhanced BSL2 Manual
- d. UC San Diego Biosafety Containment website: <http://blink.ucsd.edu/safety/research-lab/biosafety/containment/aerosol.html#ATD-Biosafety-Plan>



**APPENDIX A. Representative List of Aerosol Transmissible Organisms\***

Adenovirus	Helicobacter pylori	Parainfluenza viruses
Arboviruses	Hemorrhagic fever viruses	Parvovirus B19
Arenaviruses	Hendra virus	Pneumococcus
Aspergillus fumigatus; spp	Hepatitis B Virus	Prions
Bacillus anthracis	Hepatitis C Virus	Pseudomonas aeruginosa
Blastomyces dermatitidis	Hepatitis D virus	Rabies virus
Bordetella pertussis	Herpes Simplex Virus 1	Respiratory Syncytial Virus
Brucella abortus	Herpes Simplex Virus 2	Retroviruses
Brucella canis	Herpesvirus simiae (B-virus)	Rhinoviruses
Brucella maris	Histoplasma capsulatum	Rickettsia akari
Brucella melitensis	Human Herpesvirus 6A	Rickettsia australis
Brucella suis	Human Herpesvirus 6B	Rickettsia conorii
Burkholderia cepacia	Human Herpesvirus 7	Rickettsia japonicum
Burkholderia mallei	Human Herpesvirus 8	Rickettsia prowazekii
Burkholderia pseudomallei	Human Immunodeficiency Virus	Rickettsia rickettsii
Candida albicans	Influenza Viruses	Rickettsia siberica
Chlamydia pneumoniae	Junin virus	Rickettsia typhi
Chlamydia psittaci	Klebsiella pneumoniae	Rickettsia tsutsuagmushi
Chlamydia trachomatis	Kyasanur forest disease virus	Rift Valley fever virus
Clostridium botulinum	Lassa fever virus	Rubella Virus
Coccidioides immitis	Legionella spp	Sabia virus
Coccidioides posadasii	Lymphocytic Choriomeningitis Virus	Salmonella species
Coronaviruses	Machupo virus	Salmonella typhi
Corynebacterium diphtheriae	Marburg virus	SARS coronavirus
Coxsackieviruses A	Measles Virus	Shigella species
Coxiella burnetti	MERS coronavirus	Staphylococcus aureus
Crimean-Congo haemorrhagic fever virus	Metapneumovirus	Streptococcus pneumoniae
Cytomegalovirus (human)	Monkeypox virus	Streptococcus pyogenes
Eastern equine encephalomyelitis virus	Moraxella catarrhalis	Streptococcus species, group A
Ebola virus	Mumps Virus	Tick-borne encephalitis viruses
Epstein-Barr virus	Mycobacterium tuberculosis complex	Vaccinia Virus
Enterovirus	Mycobacterium species	Varicella-Zoster Virus
Escherichia coli (shiga toxin producing)	Mycoplasma hominis (type 1)	Variola major (Smallpox virus)
Filobasidiella neoformans	Mycoplasma pneumoniae	Variola minor (Alastrim)
Flexal virus	Neisseria gonorrhoeae	Venezuelan equine encephalitis virus
Francisella tularensis	Neisseria meningitidis	West Nile Virus
Guanarito virus	Nipah virus	Western equine encephalitis virus
Haemophilus influenzae	Omsk hemorrhagic fever virus	Yersinia pestis
Hantaviruses	Paracoccidioides brasiliensis	



\* This is a representative list modified from:

- [Cal/OSHA Aerosol Transmissible Diseases Standard Title 8 CCR; Section 5199](#)
- Medical Microbiology, 4<sup>th</sup> Ed, Chapter 93: Infections of the Respiratory System Baron S, Ed., 1996.

Any respiratory pathogen should be considered transmissible by the aerosol route. Other organisms will be considered as needed.





**APPENDIX B: Representative List of Common Aerosol-Generating Procedures**

- Blending
- Cage cleaning/changing animal bedding
- Centrifugation
- Dissections or necropsy
- Electroporation
- Flaming inoculation needles, slides or loops
- Flow Cytometry
- Grinding
- Homogenization
- Infection of animals by gavage
- Infection of animals by inhalation
- Injection
- Inserting a hot loop into a culture
- Lyophilization
- Opening containers with high internal pressures (lyophilized cultures, ampoules, tubes, etc.)
- Pipetting
- Pouring liquids
- Pumping culture in closed system
- Removing contaminated gloves
- Removing stoppers
- Shaking culture
- Sonication
- Streaking inoculum
- Trituration
- Use of needles/syringes
- Vacuum aspiration
- Vigorous shaking, stirring, or mixing
- Vortexing

The listed procedures should be performed in the BSC whenever possible. If not possible, personnel should wear additional PPE as outlined in the Practices sections of this document and also label equipment when being used to alert other lab members. Decontamination of this equipment must be done immediately after use.