

**Biosafety Level 2 (BL2)**  
***NIH Guidelines for Research Involving Recombinant or  
Synthetic Nucleic Acid Molecules (Appendix G-II-B) -  
April 2024***



**Biosafety Level 2 (BL2)**

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that: (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment.

**Appendix G-II-B-1. Standard Microbiological Practices (BL2)**

- a. Access to the laboratory is limited or restricted by the Principal Investigator when work with organisms containing recombinant or synthetic nucleic acid molecules is in progress.
- b. Work surfaces are decontaminated at least once a day and after any spill of viable material.
- c. All contaminated liquid or solid wastes are decontaminated before disposal.
- d. Mechanical pipetting devices are used; mouth pipetting is prohibited.
- e. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.
- f. Persons wash their hands: (i) after handling materials involving organisms containing recombinant or synthetic nucleic acid molecules and animals, and (ii) when exiting the laboratory.
- g. All procedures are performed carefully to minimize the creation of aerosols.
- h. Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

**Appendix G-II-B-2. Special Practices (BL2)**

- a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.
- b. The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

- c. The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.
- d. When the organisms containing recombinant or synthetic nucleic acid molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.
- e. An insect and rodent control program is in effect.
- f. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.
- g. Animals not involved in the work being performed are not permitted in the laboratory.
- h. Special care is taken to avoid skin contamination with organisms containing recombinant or synthetic nucleic acid molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.
- i. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.
- j. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant or synthetic nucleic acid molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, before discard or reuse.
- k. Spills and accidents which result in overt exposures to organisms containing recombinant or synthetic nucleic acid molecules are immediately reported to the Institutional Biosafety Committee and NIH OSP. Reports to NIH OSP shall be sent to the Office of Science Policy, National Institutes of Health, preferably by e-mail to: [NIHGuidelines@od.nih.gov](mailto:NIHGuidelines@od.nih.gov) (Contact information: 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax)). Contact information is also available on the OSP website ([www.osp.od.nih.gov](http://www.osp.od.nih.gov)). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- l. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

- m. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

#### **Appendix G-II-B-3. Containment Equipment (BL2)**

Biological safety cabinets (Class I or II) (see [Appendix G-III-L](#), *Footnotes and References of Appendix G*) or other appropriate personal protective or physical containment devices are used whenever:

- 1) Procedures with a high potential for creating aerosols are conducted (see [Appendix G-III-O](#), *Footnotes and References of Appendix G*). These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.
- 2) High concentrations or large volumes of organisms containing recombinant or synthetic nucleic acid molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

#### **Appendix G-II-B-4. Laboratory Facilities (BL2)**

- a. The laboratory is designed so that it can be easily cleaned.
- b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- c. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.
- d. Each laboratory contains a sink for hand washing.
- e. If the laboratory has windows that open, they are fitted with fly screens.
- f. An autoclave for decontaminating laboratory wastes is available.