

Requirements for Research Classified as BL2-N in *The NIH Guidelines Appendix M*

The BL-N classification refers to research involving animals that are not housed in standard animal containment housing (i.e., cages) and are introduced to genetically modified agents and/or are themselves genetically modified. Some animals that typically fall into this classification are, but not limited to, cows, sheep, horses and poultry.

Research classified as BL2-N must adhere to the listed requirements. Failure to follow these requirements would result in an incident report submitted to the Virginia Tech IBC and NIH office of Science Policy.

BL2-N Standard Practice Requirements:

- Follow all BSL-2/ABSL-2 facility and containment requirements and facility-specific manuals, including appropriate PPE. All personnel must wash their hands prior to exiting the containment area.
- IBC approval must be obtained prior to the start of the experimental procedures and/or procurement of genetically modified animals.
- A **permanent** record must be maintained of the experimental use and disposal of each animal or group of animals.
 - At the end of the experiment, if you wish to have the IBC maintain the permanent record of the procedures use and disposal, please contact the IBC Program Director (ibc@vt.edu).
- All carcasses must be disposed of as RMW or RMW for incineration (i.e., animals treated with chemicals); seek guidance from EHS regarding your specific disposal requirements.
 - Animals in this classification may **NOT** be disposed of through rendering, landfill and/or other methods that may result in use of the carcass or associated parts being used as a human and/or animal food source.
- Contaminated materials that are decontaminated at a site away from the laboratory shall be placed in a closed durable leak-proof container prior to removal from the laboratory.
- Needles and syringes:
 - Shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
 - Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe.
 - To be 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) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant or synthetic nucleic acid molecules.
- Any incident involving spills and accidents that result in environmental release or exposures of animals or laboratory workers to organisms containing recombinant or synthetic nucleic acid molecules shall be reported immediately to the Animal Facility Director/Manager, Biosafety Officer, Institutional Biosafety Committee, NIH/OSP, and other appropriate authorities (if applicable).
 - Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.
- For the transfer of any biological materials/samples out of the animal area:
 - All materials/samples must be sealed in a leak-proof primary tube/flask or other container and disinfected on the outside before being placed into a durable, leak proof secondary transport container. A biohazard symbol will be placed on the primary container.
 - The primary and secondary containers must be disinfected and secured prior to removal from the facility.
 - For added safety, containers should be transported on a cart to further minimize spill hazards.
 - The animal facility Director/Manager must give advanced approval for the materials/samples to be removed.
 - Viable materials/samples can only be transferred to a location having prior approval for BSL-2 containment.

BL2-N Facility Requirements:

- The Animal Facility Director/Manager shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal rooms.
- A facility biosafety manual must be prepared and adopted.
 - All personnel and researchers are required to review and follow procedures in the manual.
- The animals shall be confined within an enclosed space (animal room or equivalent) to minimize the possibility of theft or unintentional release.
 - If the agent in use can be transmitted by arthropods, the room must be set-up/designed to avoid arthropod access.
- The containment area where the animals are housed must be locked.
- The containment area must be patrolled/monitored at frequent intervals.
- The containment building must be controlled and have a locking access.
- Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.
- The containment area shall be in accordance with state and Federal laws and IACUC requirements.
- If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, interior work areas shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening of access doors or the equivalent.

BL2-N Signage Requirements:

- When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory/animal area.

BL2-N requirements specific to genetically modified animals:

- All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.
- A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.