

BSL-2 Practices for Administration of Modified RG1 Agents and Modified or Unmodified RG2 Agents in Insects

This document outlines the practices to be used for insect manipulations that fall into the requirements for biosafety level 2 (BSL-2) containment as determined by the Institutional Biosafety Committee (IBC) and/or the National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIHG). The practices within this document will be activated when laboratory personnel administer genetically modified risk group 1 (RG1) agents or genetically modified or unmodified risk group 2 (RG2) agents into living insects. These materials, and subsequently the insects, should be treated as biohazardous. Therefore, care must be taken to ensure proper BSL-2 containment during manipulation, transportation, and disposal of materials. Personnel who perform these experiments must be current with all BSL-2 biosafety training requirements and wear appropriate personal protective equipment (PPE) during experiments.

SPECIFIC SAFETY AND CONTAINMENT CONSIDERATIONS FOR BSL-2 INSECT ACTIVITIES

Biosafety Level 2 (BSL-2) containment laboratory requirements

All laboratory spaces where work at BSL-2 is proposed must meet the minimum requirements for BSL-2 laboratories as noted in the [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#). This includes features such as: non-porous laboratory furniture, easily cleanable laboratory surfaces (no fabric present), a hand washing sink, the use of laboratory coats and gloves, no eating, drinking, or food storage in the lab, and no animals or plants not associated with the lab work. For a full list of requirements see the "Laboratory Biosafety Level Criteria" section of the BMBL.

Arthropod Containment Level 2 (ACL-2) requirements

In addition to standard BSL-2 containment practices, work with insects requires additional considerations including primary container construction, insect disposal, management of escaped insects and release reporting, and transportation. For full ACL-2 criteria see the American Committee of Medical Entomology's [Arthropod Containment Guidelines](#).

Persons approved to work on BSL-2 insect activities

Only personnel who meet the following criteria may be approved to work on BSL-2 insect activities:

- Must be current with biosafety training requirements including Biosafety 101: Standard Microbiological Practices, Principles and Practices of Biosafety, and Annual Biosafety Refresher Training;
- Must wear appropriate laboratory body covering (lab coat, gown, etc.) and fluid-resistant gloves during these activities. Because most activities will be performed in the open lab, safety glasses should be worn when technically feasible.
- Must follow the PI approved procedures for rearing and feeding of experimental insects. They do not deviate from those procedures without approval from their PI.

Insect and materials transport

When biological materials are transported from one lab location to another, it is important to apply the same containment principles as when they are moved within the lab. Only lab staff are authorized to handle and transport insects and inocula that fall under this procedure. Personal protective equipment required for work at BSL-2 should be worn. For more information see [Transporting Biological Research Materials on Campus](#).

1. **Biohazardous materials transportation-** All modified RG1 agents and modified or unmodified RG2 agents should be transported in a closed primary container within a secondary container. This secondary container should be:
 - Made of materials that can be effectively cleaned and disinfected;
 - Securely closed in a way that the container will not open if dropped;

- Labeled with the biohazard symbol and lab identification information.
2. **Insect transportation-** Insects should be maintained in primary containers that do not allow for their escape. When transporting several containers of insects, these should at least be placed in a tray or on a cart to reduce the chance of dropping the primary containers. Wherever possible, a secondary container should be used that meets the criteria outlined above. Allowances for ventilation openings are acceptable provided that meshing will prevent escape.
 3. **Sharps transportation-** Specially generated sharps for insect injections should be transported within an unbreakable container that safely secures the sharps hazard.

Spills, Releases, and Exposures

1. In the event of suspected exposure incident (i.e. puncture, cut or abrasion with contaminated item, splash to the eyes, nose or mouth), follow the [Responding to Personnel Exposures and Spills Involving Biological Materials](#) protocol.
2. In the event of a spill, retrieve the biological spill kit and follow the procedure provided in the kit.
3. In the event of a release of insects, if possible, attempt to capture the escapee(s). Contact VEHS Biosafety immediately (322-2057) to determine appropriate next steps.

Please note that spills are most effectively remediated by a team. If a spill occurs and you are not prepared to clean it up (i.e. insufficient supplies or training), isolate the area and call another trained person in the laboratory or VEHS Biosafety to assist if possible to achieve the safest and most effective cleanup.

Preparing for the procedure

1. Ensure all materials needed (insects, microscope, microscope slides, needles, sharps container, etc.) are placed on the bench that will be used for the procedure. Remove all unnecessary items from within arm's reach of the area.
2. Set up the procedure area such that there is minimal disruption during the activity. Also, inform other lab members that the procedure will be occurring so there is minimal interruption during this time.
3. Don personal protective equipment. Required PPE includes a laboratory coat or gown and a pair of fluid-resistant disposable gloves. Because most activities will be performed in the open lab, safety glasses should be worn when technically feasible.

Sharps Maintenance

Sharps (needles, scalpels, etc.) should be handled carefully to avoid accidental exposure at all times.

1. Sharps should transported in a secure fashion such that there is no potential for exposure to sharp edges or the shattering of glass objects.
2. When utilizing sharps, insects to be manipulated should be held with forceps, other mechanical device, or placed on unmovable surfaces to remove the non-dominant hand from potential sharps exposure area.
3. Following the completion of use of sharps, the sharp should be immediately disposed of in a biohazardous sharps container located within arm's reach of procedure space.
4. For more information about sharps handling see [Using Sharps Safely in the Lab](#).

Decontamination after the procedure

1. At conclusion of procedures, collect any waste that has come in contact with the insect, transport containers, and any contaminated items and dispose of as biohazardous waste.
2. Disinfect all lab equipment and surfaces that have come in contact with the insect or inocula and all items in the immediate vicinity with disinfectant appropriate for the organisms present (use a paper towel wetted with disinfectant, do not spray directly onto surfaces).
3. Remove PPE and dispose of in the biohazardous waste receptacle.

4. Wash hands after glove removal.

Disposal of insects

1. All insects must be terminated prior to final disposal. The preferred method is to place them into a -20°C freezer until the insects are no longer viable.
2. Insects should be collected, terminated, and disposed of in accordance with [Insects Used in Research: Biohazardous Waste Collection, Termination & Disposal Guide for Vanderbilt Researchers](#).

NOTE: This document is intended to be a foundational document for development of sound and uniform containment practices, and will be reviewed and updated with refinements as these are recognized. It is expected that unique procedural requirements may challenge the feasibility of some elements of the document as written. In this circumstance, you are expected to contact the BSO in order for an alternative method to be identified for your study needs.

For more information or assistance with biosafety practice-related questions that are not addressed in this document, please contact Robin Trundy, Biosafety Officer at 322-0927.