

## Rutgers University

### Standard Operating Procedure for Stereotactic Injection

#### Stereotactic Injection

Stereotactic injection creates a small risk of aerosol production while the greatest risk to the researcher is accidental inoculation, either during set-up or during the injection procedure. Other parameters that may affect the degree of risk with this technique are the number of separate plasmids used to package the vector (2nd generation systems are not recommended for procedures outside the biosafety cabinet), the nature of the transgene, and whether the experiment involves the use of human cells. The use of an oncogenic transgene (e.g., a known oncogene or a shRNA for a tumor suppressor), might necessitate stricter adherence to lab-specific standard operating procedures, whereas the use of human cells (e.g., injection of lentivirally transduced human cells into mouse brain) requires ABSL2 housing. Labs using stereotactic systems for pathogen and viral vector delivery must register their projects with the Institutional Biosafety Committee (IBC) and Institutional Animal Care and Use Committee (IACUC).

#### List of Personnel Trained in Stereotactic Injection:

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#### Description of Each Construct, Viral Vector or Pathogen:

*(Please provide a brief description of each construct, viral vector or pathogen -- include the strain or serotype, associated illnesses/diseases, pathogenicity, resistance to therapeutics and replication competency/deficiency information. Also, please comment on the potential hazards of the transgene [e.g. toxicity, oncogenicity, human growth factor, etc.], and include any relevant references.)*

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List of Personnel Trained to Handle Pathogen(s):

Description of Procedure: <i>(Please outline the procedure including the preparation of equipment and particle suspensions. How and where will the pathogen be drawn into the pipette or needle? How will the animal be stabilized or restrained during the injection? If possible, the needle/pipette should be loaded inside a biosafety cabinet.)</i>

**Safety Tips**

1. Locate the stereotactic injection apparatus away from high traffic areas.
2. Post signage when procedures are being conducted and pathogens are being used. Signage should state the name of the pathogen, and only personnel involved in the experiment should be allowed in the vicinity.
3. Wear lab coat, gloves and safety glasses.
4. Clean and disinfect the equipment and surfaces with an appropriate disinfectant.
5. Cover area with absorbent lab bench paper.
6. Maintain a waste container with 1:10 bleach solution for tips, and add an additional amount of solution to the container for 10 minutes prior to disposal.
7. Anesthetize the animal prior to injection.
8. Prepare the virus particle suspension in a biosafety cabinet (e.g., aliquots and filling the syringe). Remove the particle suspension exiting from the tip with a cotton swab when lowering the plunger.

9. Wait 2 minutes after injection before slowly retracting the pipette. This will prevent efflux of material.
10. Before the operator leaves the room, he/she must wash his/her hands.

### **Disinfection and Return of Animals**

When the operation is finished, lab members must disinfect work surface, including surgical light and microscope, using a 1:10 dilution of bleach to water (10% bleach), followed by 70% ethanol. Place paper towels and other disinfection/clean-up materials into a red biohazard bag. All paper liners and disposable diapers will be discarded in the red biohazard bag. Pathogen-infected animals should be returned to the post-operating room. Notify the vivarium supervisor before returning animals. Label the cage card with the type of pathogen injection, the injection date and a biohazard symbol. Animals may need to be in the negative pressure cages based on the pathogen/material being administered. The first cage change should be treated as biohazardous, and the soiled cages and bedding need to be autoclaved or chemically disinfected, per facility guidelines.

*NOTE: The disinfection and cage change procedures may differ based on the pathogen/vector being used.*

### **Contaminated Waste**

All contaminated items must be decontaminated by autoclaving or chemical disinfection prior to disposal. Details can be found in the Rutgers Biological and Medical Waste Disposal Policy published at the REHS website (<http://rehs.rutgers.edu>), and a summary of waste handling and disposal is as follows:

1. Non-Sharp Waste such as viral stock vials and the PPE must be immersed in 10% bleach for 30 minutes or autoclaved for one hour (at 121 degrees centigrade and 15 psi), **prior to** disposal in a red bag-lined medical waste box.
2. Sharp Waste such as needles, syringes and razors and pipette tips must be disposed in an approved resistant sharps container. Sharps containers must not be filled more than 2/3 of their capacity.
3. All surgical tools used for the pathogen injection to the animal must be disinfected with a 1:10 dilution of bleach to water or by autoclaving. After 30 minutes in bleach, the tools will be washed (to remove bleach residue) and autoclaved. Remaining bleach can be discarded down the drain.
4. Liquid waste should be disinfected with bleach or another disinfectant approved in the laboratory's IBC protocol. Add bleach until the final volume of bleach is 10% of the total volume of liquid waste. Drain disposal is only allowed if the liquid waste meets the requirements found in the Chemical Hygiene Guide / Laboratory Safety Plan ([http://rehs.rutgers.edu/lslab\\_lschem.html](http://rehs.rutgers.edu/lslab_lschem.html)).

### **Operator exposure**

1. Exposure from Splash or Aerosols – INHALATION – Report the incident to your supervisor and refer to the Rutgers Emergency Action Plan (<http://emergency.rutgers.edu>) for further instructions.
2. Exposure from Splash or Aerosols – EYE CONTACT, SKIN AND/OR MUCOUS MEMBRANE – Rinse a minimum of 15 minutes in eye wash or flush area with water,

report the incident to your supervisor and refer to the Rutgers Emergency Action Plan (<http://emergency.rutgers.edu>) for further instructions.

3. Needle stick and/or Sharps Exposure – Contaminated skin should be thoroughly rinsed with copious amounts of water. Report the incident to your supervisor and REHS immediately after scrub. Seek medical attention at Campus Employee Health Services/Occupational Medicine Services. Refer to the Rutgers Emergency Action Plan (<http://emergency.rutgers.edu>) for after-hours exposure.
4. Medical Emergencies – For situations in which exposure to pathogen/material occurred and medical treatment is an emergency, personnel should report to the nearest hospital emergency room.

**For all exposure incidents (including needle sticks):**

Notify the lab manager or principal investigator. The injured person's supervisor must submit the initial accident report by the end of the work shift and fill out the investigation report within 5 days of the accident. The Accident Database can be found at <http://myrehs.rutgers.edu> (*Paper copies are no longer accepted*).

**Spill Clean-Up**

1. Notify other workers in the area of the spill and control traffic through area (i.e., close door to lab and/or post notice of spill, etc.).
2. Remove any contaminated clothing and collect it in an autoclavable biohazard bag.
3. Prepare a fresh solution of 10% bleach.
4. Wear shoe covers if the spill is on a floor. Wear gloves and cover spilled material with paper towels saturated with 10% bleach.
5. Decontaminate all objects in spill area, allowing 20-30 minutes of contact time with the liquid disinfectant (10% bleach).
6. Use forceps or tongs to pick up any sharps, including broken glass, and place these items in sharps container.
7. Remove shoe covers and then gloves before leaving area of the spill, and place spill clean-up waste into an autoclave-safe biohazard bag.
8. Autoclave the bags of waste.
9. Wash your hands thoroughly with soap and running water.

**Emergency Contact Information**

**Principal Investigator Contact Information:**

- **PI Name**
- **PI Emergency Phone Number(s)**

Serious injury or illness & major spills:

- **Immediately obtain an outside line and dial 9-1-1.**
- The alternate RUPD phone numbers are: New Brunswick 732-932-7111, Newark 973-353-5111 or 973-972-4491, Camden 856-225-6111.

**For More Information:**

More information can be obtained by contacting Rutgers Environmental Health & Safety, 973-972-4812 or 848-445-2550 (and request a member of the biosafety group).

**References**

1. “OHSU IBC Standards for Stereotactic Injection of Lentiviral Particles”. Oregon Health and Safety University. May 4, 2011.  
<http://www.ohsu.edu/xd/about/services/integrity/policies/upload/Standards-for-Stereotactic-Injection-of-Lentiviral-Particles.pdf>
2. “Stereotaxic Injection of a Viral Vector for Conditional Gene Manipulation in the Mouse Spinal Cord”. Journal of Visualized Experiments (JoVE). March 18, 2013.  
<http://www.jove.com/video/50313/stereotaxic-injection-viral-vector-for-conditional-gene-manipulation>
3. “Stereotaxic Microinjection of Viral Vectors Expressing Cre Recombinase to Study the Role of Target Genes in Cocaine Conditioned Place Preference”. Journal of Visualized Experiments (JoVE). July 30, 2013. <http://www.jove.com/video/50600/stereotaxic-microinjection-viral-vectors-expressing-cre-recombinase>
4. “Standard Operating Procedures for Working with Adenovirus in Mice”. Rutgers University. July 12, 2013.