

INJECTION OF REPLICATION COMPETENT VIRAL AGENTS (SOP-02)

This applies to N2C Rabies and H129 HSV

A. Background

- Precautions must be taken not to spill the viral solution to be injected.
- Personnel protection equipment includes goggles while handling N2C and H129 (safety glasses when work does not involve prior) hair cover, double nitrile gloves, disposable gown, and shoe covers over closed shoes.
- All procedures are done in a Biosafety cabinet
- All procedures are started only after the animal is anesthetized and properly fixed in the stereotaxic device.
- While working in BSB RIU anesthesia will be by injectables.
- Handle injection vials and sterile needles appropriately to avoid contamination.
- Examine the label on the virus vial to ensure that you have the proper solution for injection.
- Confirm the animal identification number by comparing: cage card, animal's ear notches, and the dosage record.
- Open the log book and record the IDs for all animals (typically four) that are to be injected.

B1. Procedure - Nanoinjector

- Mount the Nanoinjector on the manipulator.
- Fill a glass micropipette with mineral oil and attach to the Nanoinjector.
- Break-open the viral container.
- Place a small piece of Parafilm on the flat of the ear bar of the stereotaxic and, using a Pipetman with a disposable pipet tip, place a drop of the virus onto the Parafilm; typical volumes are 0.5-2.0 μL .
- Align the tip of the glass micropipette to the drop and backfill the virus into the Nanoinjector.
- Align the Nanoinjector to the muscle or brain area of interest and perform one to two test injections onto a small piece of filter paper to confirm that virus is ejected from the tip of the pipette.
- Discard the filter paper and the Parafilm in a beaker of 1:200 Amphyl.
- Lower the Nanoinjector to the muscle or brain area of interest and inject.
- Wait about five minutes for the viruses to permeate the tissue
- Raise the Nanoinjector and rotate and slide the unit along the stereotaxic rail to the side.
- Remove the injection micropipette from the Nanoinjector and discard in a sharps container containing 1:200 Amphyl.
- Remove the animal and place in cage to recover.
- Place a second animal in the stereotaxic apparatus and repeat the injection procedure.

B2. Procedure - Timed pressure injector

- Mount the micropipette holder on the manipulator. Ensure that the pressure port is connected.
- Use a plastic loading needle and disposable syringe to load an omega-dot micropipette. Ensure that there are no bubbles
- Connect the omega-dot micropipette to the micropipette holder.

- Align the micropipette holder to the muscle or brain area of interest and perform one to two test injections - using 100 ms pulses and approximately 20 PSI of pressure - onto a small piece of filter paper to confirm that virus is ejected from the tip of the pipette. Visually monitor the meniscus level to determine the speed and injection volume.

- Discard the filter paper and the Parafilm in a beaker of 1:200 Amphyl.

- Lower the micropipette holder to the muscle or brain area of interest and inject.

- Visually monitor the meniscus level to confirm the injection volume.

- Wait about five minutes for the viruses to permeate the tissue

- Raise the micropipette holder and rotate and slide the unit along the stereotaxic rail to the side.

- Remove the injection micropipette from the holder and discard in a sharps container containing 1:200 Amphyl.

- Remove the animal and place in cage to recover.

- Place a second animal in the stereotaxic apparatus and repeat the injection procedure.

- C. Clean-up**

- Wipe down all surfaces on the stereotaxic and manipulator, as well as the biosafety cabinet with 1:200 Amphyl.