

Document Number: ANP047	Title: SUBRETINAL AND INTRAVITREAL INJECTION INTO EYES OF RATS AND MICE <i>CONFIDENTIAL INFORMATION</i> MOLECULAR MEDICINE RESEARCH INSTITUTE	Effective Date: May 2014
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1.0 OBJECTIVE

- 1.1 The objective of this procedure is to describe the procedures for subretinal (SRI) and intravitreal (IVT) injection of substances in rats and mice

2.0 SCOPE

- 2.1 This procedure applies to animals used for experimentation.

3.0 POLICY

- 3.1 It is the policy of MMRI to establish written and approved procedures to ensure that the health and well being of employees is protected, and that potentially hazardous procedures are performed in a safe manner.

4.0 RESPONSIBILITIES

- 4.1 It is the responsibility of Manager of Animal Research or designated alternate to implement this procedure and revise it when necessary.

5.0 REFERENCES

- 5.1 Aditya Venkatesh,1,2 Shan Ma et al: Retinal Gene Delivery by rAAV and DNA Electroporation. *Curr. Protoc. Microbiol.* 28:14D.4.1-14D.4.32.

- 5.2 ANP016 Animal Anesthesia

6.0 SAFETY PRECAUTIONS

- 6.1 It is the responsibility of all personnel to use good judgment and safe practices in the laboratories. Protective clothing (e.g., laboratory coats, coveralls, boots, face masks, aprons, rubber gloves and safety glasses) are provided by the company.
- 6.2 Sterile rubber gloves, facemasks, bouffants and safety glasses are worn when performing surgery.
- 6.3 Used disposable scalpel blades, hypodermic needles and syringes are placed in a disposable sharps container located in the procedure room. When full, the container is disposed of safely.
- 6.4 All injury accidents are promptly reported to the appropriate supervisor.

7.0 EQUIPMENT AND MATERIALS

Buprenorphine
Betadine
70% ethanol
Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic

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1%Aptopine Ointment
 0.5% Proparacaine HCl Ophthalmic Solution Sterile
 Saline
 Insulin injection needles
 Weight trays appropriate for mice
 Heating plate/mat
 Rubber gloves
 Ice bucket
 33 -36 gauge disposable/reusable needle
 Dissecting microscope with appropriate light source or surgical microscope
 Cotton swabs
 Forceps (student Dumont no. 5)
 Microcentrifuge
 Intraocular injection kit –WPI
 Micro injection pump-WPI

Test articles

8.0 PROCEDURE

8.1 Preparation

- 8.1.1 Preparation of rat procedure will start from step 8.1.3
- 8.1.2 Remove animal from cage and inject each animal subcutaneously with buprenorphine (0.1 mg/kg) using an insulin injection needle. Place animal in a tray on a warm (37°C) heating mat for one hour.
- 8.1.3 Set up the injection station.
- 8.1.4 Prepare test articles
- 8.1.5 Animals will be anesthetized by an intraperitoneal or intramuscular injection with a mixture of ketamine/xylazine (up to 100 mg/kg and 30 mg/kg). Topical cornea anesthetic eye drop (0.5% Proparacaine HCl Ophthalmic Solution Sterile) may be used if the eye blinking during the period of intravitreal or subretinal injection procedure.
- 8.1.6 Meanwhile, load test article to a 10-100 µl Hamilton syringe with 33 - 36 gauge needle.
- 8.1.7 Once the animal is anesthetized observed by toe pinch, Clean the skin over the eye with cotton swabs soaked with Betadine, followed by 70% ethanol swabs carefully. Avoid any of betadine and Ethanol to flow into eye.
- 8.1.8 Move it onto a paper towel under the dissecting microscope, placing it on its side with one eye facing up.

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8.1.9. It is not necessary to routinely use the ophthalmic local anesthetic for the procedure of intravitreal or subretinal injection. However, one drop of 1% Proparacaine will be applied into the injected eye if eye is blinking during the operation.

8.2 Injections

8.2.1 Injections after proptosis of the eyeball (recommended)

- 8.2.1.1 Push the skin to the side with thumb and index finger to expose the eyeball. The conjunctiva at the limbus area will be gripped and eyeball will be gripped out by using sterile forceps with left hand. Once the eyeball is out, hold the skin pushed down with thumb and index finger.
- 8.2.1.2 With other hand, hold the needle tip perpendicular to the sclera to optimize the force at the tip of the needle. Gently push the beveled 33 gauge disposable needle through the sclera.
- 8.2.1.3 Slowly inject up to 3-5 ul of test solution into vitreal cavity for IVT (Fig 1A, 2A) or 0.5-2.0 µl into subretinal space for SRI (Fig 1B, 2B). And then keep the needle tip in the injected eye at the injected position for 10 seconds to allow all material to flow into injection site.
- 8.2.1.4 After pulling back the needle, close the eyelid gently with a cotton swab soaked with Betadine. Then, the Neomycin and Polymyxin B Sulfates and Bacitracin Zinc ophthalmic ointment and 1% Atropine ophthalmic ointment. (See 8.3.1)
- 8.2.1.5 Place the animal back onto a tray on a heating mat at 37°C.
- 8.2.1.6 When the animal starts to have blink response and move in cage, they will be returned to the cage.

8.2.2 Alternate injection technique

- 8.2.2.1 Push the skin to the side with thumb and index finger of one hand to expose the cornea and part of the sclera.
- 8.2.2.2 To inject into the vitreal cavity, insert the beveled 33-36 gauge disposable or reusable needle with other hand at the margin of the cornea and the sclera (Fig. 1C, 2A). Hold the needle such that it passes by the lens without damaging the lens. Slowly inject up to 3-5 ul of test solution into vitreal cavity. After injecting, keep needle in the injected eye at the injected position for 10 seconds

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to allow all material to flow into injection site, and then remove the needle and follow steps 8.2.1.4 to 8.2.1.6 above.

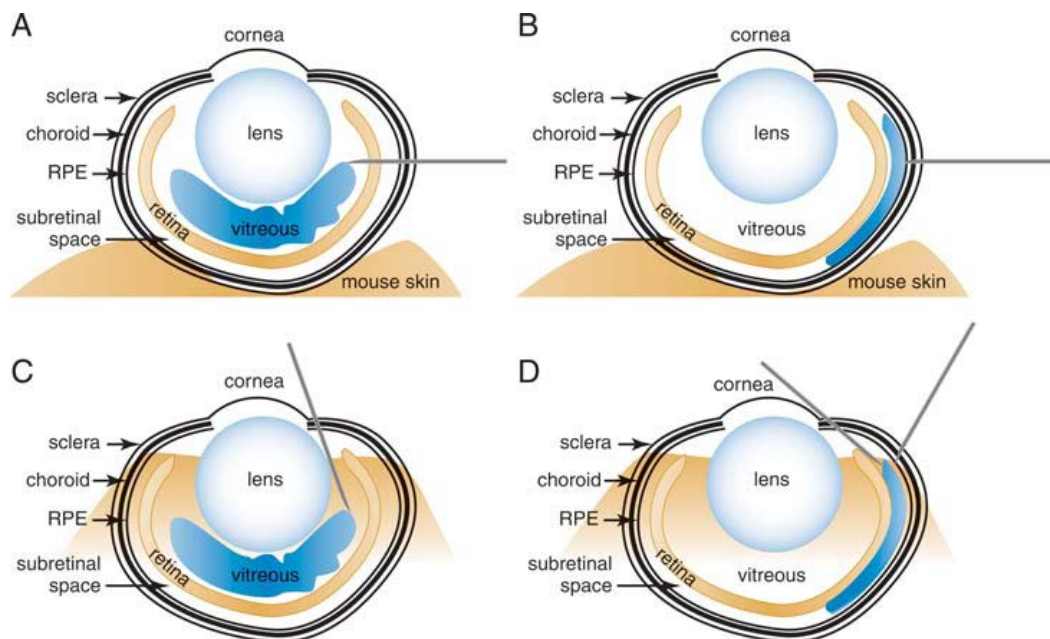
8.2.2.3 To inject into the subretinal space, either target the visible area of the sclera or the margin between the cornea and the sclera (Fig. 1D, 2B). Hold the beveled 33-36 gauge disposable or reusable needle at the appropriate angle to target the subretinal space without damaging the lens (Fig. 1D, 2B). Slowly inject 0.5-2.0 μ l into subretinal space. After injecting remove the needle and follow steps 8.2.1.4 to 8.2.1.6 above.

8.3 Post injection

8.3.1 Injected eye will be topically administrated with 1% Atropine Ophthalmic Ointment and Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment

8.3.2 Place animal in a tray on a warm (37°C) heating mat until the animal starts to have blink response and move in cage.

8.3.3 Return animal back to cage. Monitor all animals at end of working day to assess need for additional analgesic administration



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Fig 1: Schematic of injection routes for mouse using 33 - 36 gauge disposable needles. **(A-D)**. Cartoons of mouse eyes showing sclera, choroid, RPE, cornea, lens, retina, subretinal space, and injected solution (blue). (A, B) Example of injection routes for vitreal (A) and subretinal (B) injections into eyes that are popped out. (C,D) Injection routes for vitreal and subretinal injection if eyes are not popped out.

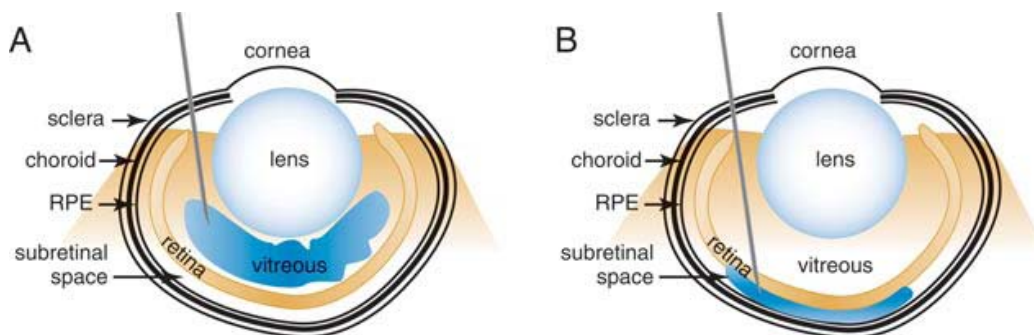


Fig 2: Schematic of vitreal and subretinal injection routes into mouse using a Hamilton syringe with a blunt needle. **(A, B)**. Cartoons of mouse eyes shown in Figure 2A is example of injection route for vitreal injection and (B) Injection route for subretinal injection. The blunt Hamilton needle is pushed through the retina. The needle stops at the sclera if not pushed too hard due to the tougher composition of the sclera.