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# Pseudorabies Virus Injection (PRV) into Spleen and Mesenteric Lymph Nodes (MLN)

Kavi Rude<sup>1</sup>, Jessica Sladek<sup>1</sup>, Colin Reardon<sup>1</sup><sup>1</sup>UC Davis**1** Works for me [dx.doi.org/10.17504/protocols.io.bfhfj3n](https://dx.doi.org/10.17504/protocols.io.bfhfj3n)Kaitlin Murray  
UC Davis

## PRV Injection and Surgery

- 1 Place the mouse into the induction chamber and slide the lid all the way closed. Turn the appropriate stopcock valves to deliver the appropriate amount of isoflurane and O<sub>2</sub>.
- 2 As the mouse transitions from a state of awakesness to anesthetized, you should observe a slower respiratory rate and rhythm, alongside a loss of balance and slowed movement. After around 3 minutes, the mouse will become recumbent and non-responsive, at which point you may remove the mouse from the induction chamber.
- 3 Place the mouse in dorsal recumbency on the shaving station pad. Place the nose cone over the mouse's nose, and redirect the flow of Isoflurane and O<sub>2</sub> by adjusting the stopcock valves. Access to the induction chamber should be closed and access to the nose cone opened. Note: Mice are obligate nasal breathers and cannot breathe through the mouth. Therefore, it is not essential to include the mouth in the nose cone. If necessary, you may secure the position of the nose by creating a "strap" out of suture that runs just under the mouse's front central incisors and ties to the nose cone.
- 4 Apply ample amounts of sterile eye lubricant to each of the mouse's eyes. Take care not to wipe off all the lube upon inserting the mouse's head back into the nose cone.
- 5 Using a charged shaver, remove the hair on the mouse's left side starting just above the hip and the insertion of the left forelimb. For splenic injections, the width of the shaved area should extend over the margins of the latissimus dorsi muscle whereas for mesenteric lymph node (MLN) injections, the placement should be lower, roughly over the caudal external abdominal oblique muscles. Tip for spleen visualization: When the mouse is lying in right lateral recumbency, mentally draw a line representing the coronal plane running rostral to caudal oriented from the mouse's ear. Mentally draw a second line representing the transverse plane running ventral to dorsal oriented from the bottom of the mouse's rib cage (vertebrochondral "false" ribs). The intersection of those lines typically lies right over the spleen.
- 6 Monitor the anesthesia to ensure depth of anesthesia is appropriate, then clean the incision site first with alcohol and then chlorhexidine. Always prep from "clean to dirty" areas, starting in the center of the animal and wiping in a spiral to the outside. Be careful not to accidentally transfer microorganisms from the periphery back to your target incision site. Never "double dip" a contaminated cloth wipe back into the antiseptic solution and do not "back track" over an area that has already been prepped.
- 7 Using a pair of forceps, grasp the mouse's skin along the coronal midline and using a sharp pair of scissors, make a small incision through the skin roughly 7mm in length over the appropriate area corresponding with the intended target organ (spleen or MLN). The first incision should pass through the skin, subcutaneous tissue and fascia. Further separate the skin from the abdominal cavity lining by placing the closed tip of the scissors between the layers and gently opening up the blades. Be sure to angle the scissors up and away from the body cavity.

- 8 Make a second incision, underneath the first, passing through the linea alba, transversalis fascia and peritoneum lining. Angle the scissors up and away from the viscera as you do this. The spleen can usually be visualized through the body cavity lining once the skin is peeled back. Rather than make the incision directly over the spleen, cut caudal to it.
- 9 If the target organ is the MLN, dip your closed forceps into the body cavity and gently pull up the intestinal mesentery until you expose the abdominal aorta. Spread the “fan” of the mesentery out carefully and look along the abdominal aorta for the chain of lymph nodes. If you have to flip the mesentery over to visualize the lymph nodes properly, pay close attention to which direction the tissue is twisting (usually counterclockwise, but not always) so you can be sure to restore the tissue to its original orientation before closure.
- 10 Use your forceps to gently hold the body cavity lining out of the way and have an assistant with the prepared needle inject the target organ. For spleen, make three injections spread out along the length of the organ. For MLN, make one single injection.
- 11 Briefly examine the mouse’s internal organ orientation and check that all organs are intact and in their correct place.
- 12 Begin closure. If your incision is 1 cm or less, you may use one cruciate suture if the closure is deemed secure and appropriately placed. If the incision is greater than 1 cm, perform a line of simple interrupted sutures spaced approximately 2-3mm apart.
- 13 Once the linea alba is securely closed, use the scissors to free the skin from any remaining fascia. You will want the skin quite loose for staple closure. Taking forceps in both hands, grab both seams of skin and bring them together, raising the skin upward like a tent. Have an assistant wield the stapler and place a staple between where your forceps are holding the skin. There should be a space about half a staple wide between each staple. You may need to use a forceps or needle driver to tighten the staples. If there is skin left over after the last staple, consider passing a line of suture over the pucker to keep the skin sealed together.

#### Anesthesia Recovery

- 14 Once the animal is able to walk around the cage freely and appears to have recovered and an appropriate level of alertness and responsivity, it may be returned to its home cage.
- 15 Prior to placing the animal in the recovery cage, check the temperature of the heating pad underneath. The animal should be provided a means to move away from the heat source once awake, and this can be accomplished by supplying heat to one half of the cage only. Use a clean paper towel for a small barrier of material between the animal and the heated part of the cage. Bedding should never be used.
- 16 Place the animal in the recovery cage either in ventral or lateral recumbency. Monitor how long it takes for the mouse to awaken. While the mouse is recovering from anesthesia, someone must stay in the procedure room to monitor the mouse until it has completely recovered.



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