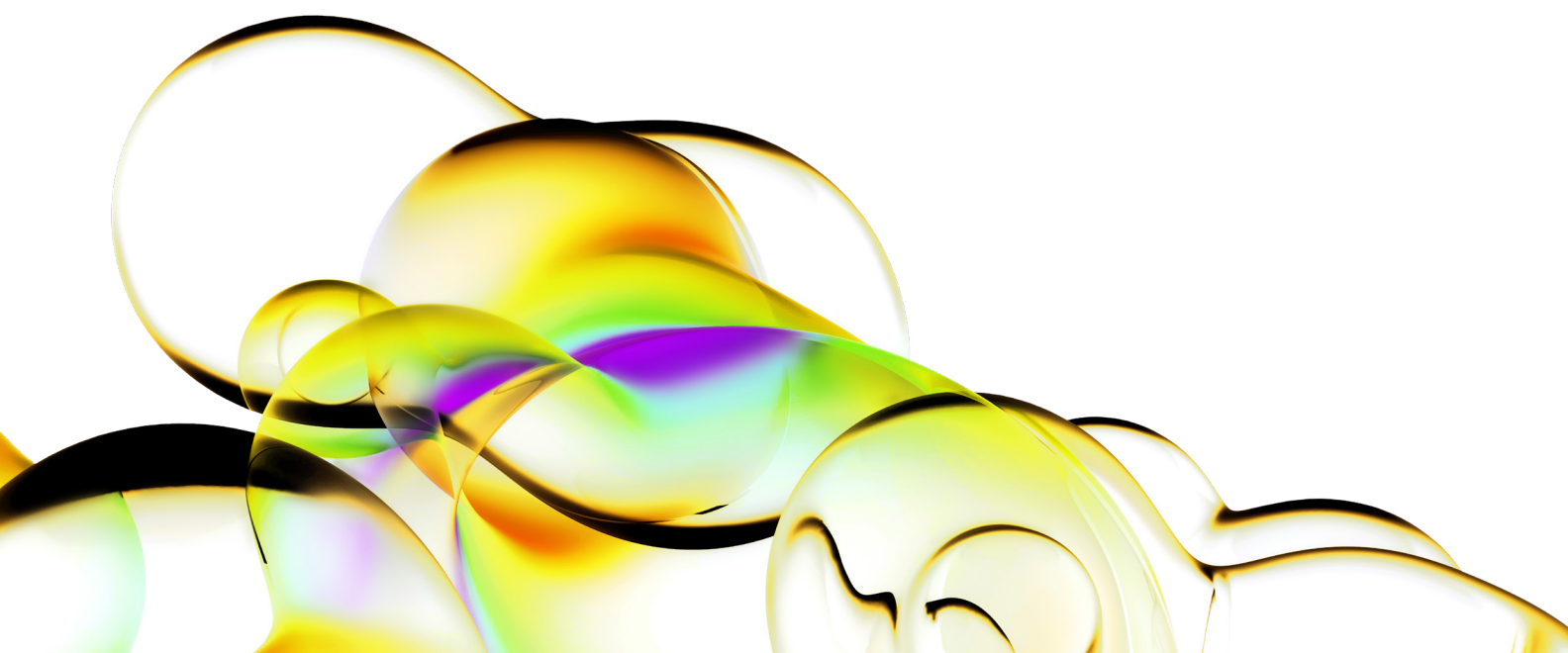


Establishing and utilizing a continuous infusion line for preclinical studies.

Introduction

The purpose of this protocol is to maintain a continuous flow of contrast agents such as microbubbles into the bloodstream to achieve a consistent concentration. Administering agents as a bolus injection provides only a brief window (~10 minutes) for vascular or perfusion imaging, as the agents are rapidly cleared from the bloodstream. Therefore, establishing a stable agent concentration via a tail-vein infusion line is essential for accurately assessing blood perfusion in target tissues or organs. Additionally, agent infusion allows for the imaging of larger regions of the animal, such as the entire ventral side—from the chest to the lower abdomen.

To achieve this, it is recommended to set up a lateral tail-vein intravenous injection while imaging, which involves creating a reliable infusion line for mice or rats. This procedure outlines the steps to assemble and utilize the infusion line. The same approach can be adapted for larger rodents, using a larger needle and infusion tubing to accommodate their increased size and weight, along with the higher infusion volumes required.



Materials required

- Tubing: PE10

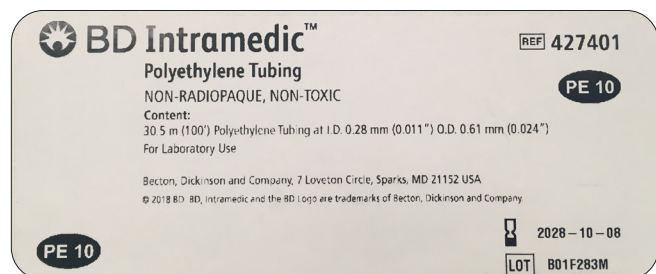


Figure 1: Tubing

- Syringe: 1 ml
- Needles: 30G ½ inch

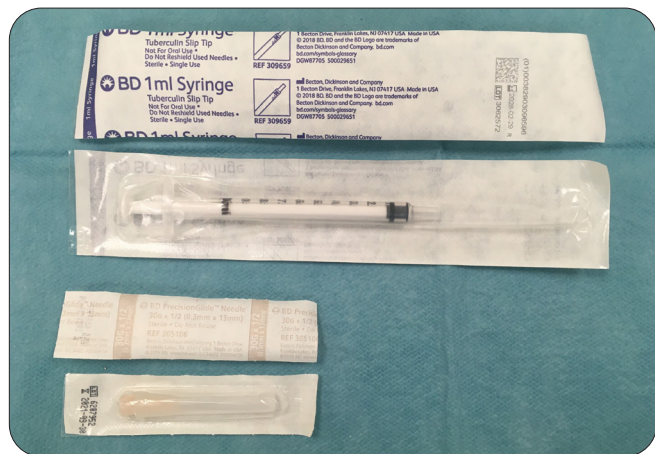


Figure 2: Syringe and Needle

- Needle holder



Figure 3: Needle Holder

- Surgical tape



Figure 4: Surgical tape

- Tweezers (curved, with smooth tips)



Figure 5: Tweezers, smooth with curved tips

- Alcohol wipe

Instrument layout example

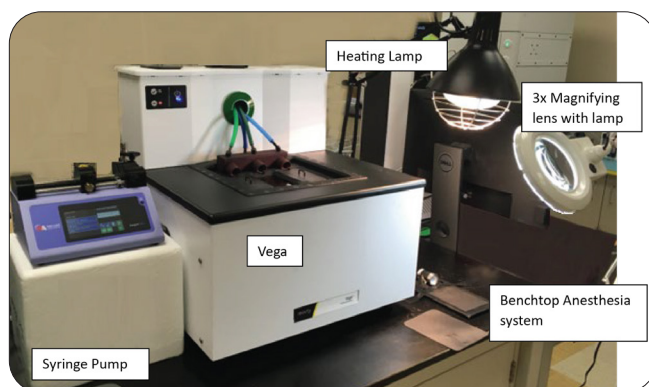


Figure 6: Vega instrument setup example

Modules explained

Syringe Pump - Capable of deliver constant flow of MB. With a programable interface to setup various flow rate by syringe types. This picture shows a 1 mL syringe with pre-loaded PBS (Phosphate Buffered Saline) for example.

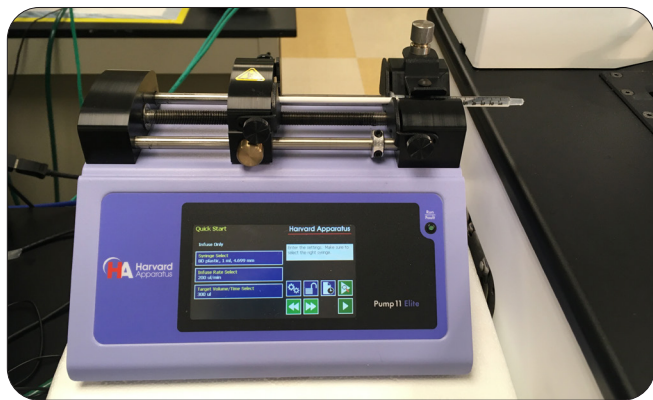


Figure 7: Syringe Pump loaded with the desired infusion fluid

Heating lamp - Keeps the mouse warm and the tail vein dilated

Magnifying Lens with lamp - Helps visualize the vein and injection.



Figure 8: Heating Lamp and Magnifying Lens with Light

Benchtop Anesthesia System - Supplies anesthesia to keep the mouse anesthetized and provides a clean surface for holding the mouse's tail down

Making the infusion tubing set

This section explains how to build the infusion tubing set.

1. Cut the tubing to 16 inches.

NOTE: Cutting the tubing longer than 16 inches will increase unnecessary resistance.

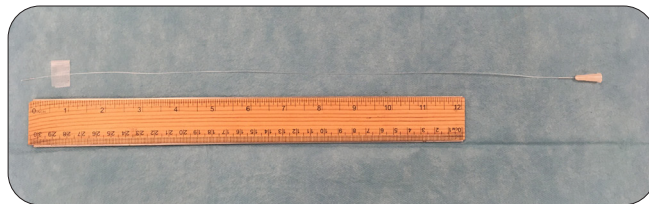


Figure 9: Tubing cut to 16 inches

2. To prepare the needle for tail injection (the left end of Fig. 9), use the needle holder to break off a 30G ½ inch needle from its hub. Hold the needle firmly close to the base of the needle hub and bend back and forth until the needle breaks off the base (for a ½ inch needle [12.5 mm], 1-2 mm left on the needle hub is okay as it will give you a ~10 mm needle.).

WARNING!

The needle is sharp and could pose a puncture hazard or worse (depending on the payload). Always use extreme caution when handling any needles.

3. Verify the break points on the ends of the needle are clean with no obstructions.
4. Use the curved tweezers with smooth contact (no serrated) to hold one end of the tubing.
5. Use the needle holder to hold the needle point.



Figure 10: Tweezers and Needle Holders

6. Insert the cut end of the needle point 3mm into the tubing.

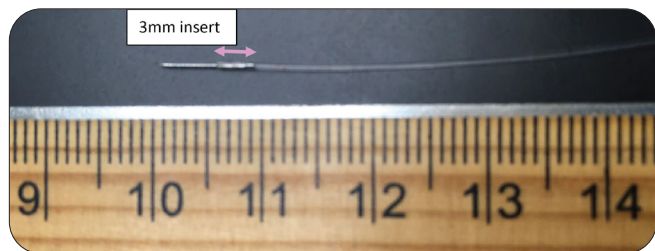


Figure 11: Needle inserted 3mm into Tubing

7. Insert the Needle Hub 10mm into the other end of the tubing.

NOTE: Verify the needle is at least 10mm into the tubing, this joint will experience the highest pressure as it is closest point to the pump.

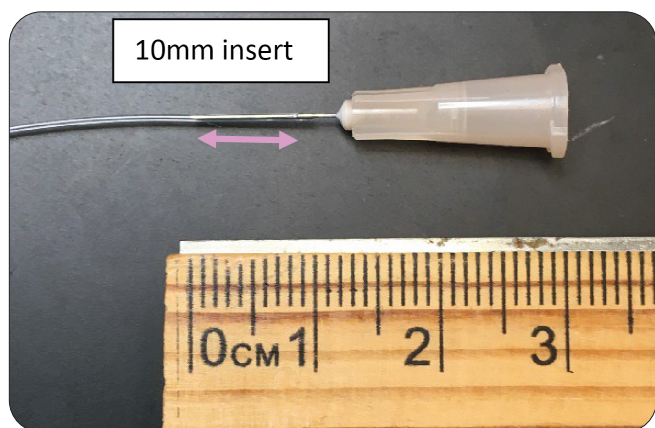


Figure 12: Needle hub to tubing connection

Testing the infusion tubing set

Use a 1 ml syringe loaded with 1 ml PBS. Press with steady pressure on the plunger and verify the flow is smooth and there are no leaks at both needle/tubing junctions.

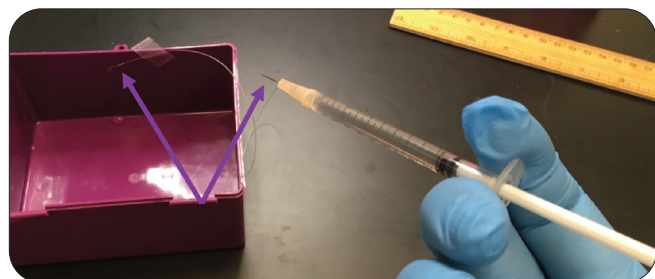


Figure 13: Needle/tubing junctions

Some resistance is expected but there should be no back pressure when released. A good flow from the needle is a sign that the line is acceptable.



Figure 14: Infusion Line flow

Establishing the canulation/infusion line

1. Place the black plastic Lexan sheet on the board.
2. Place the mouse into the anesthesia nose cone. Position the animal on its side so its lateral vein facing up (in this example, the right-side vein is facing up).
3. Use an alcohol wipe to reveal the tail vein (on sides)

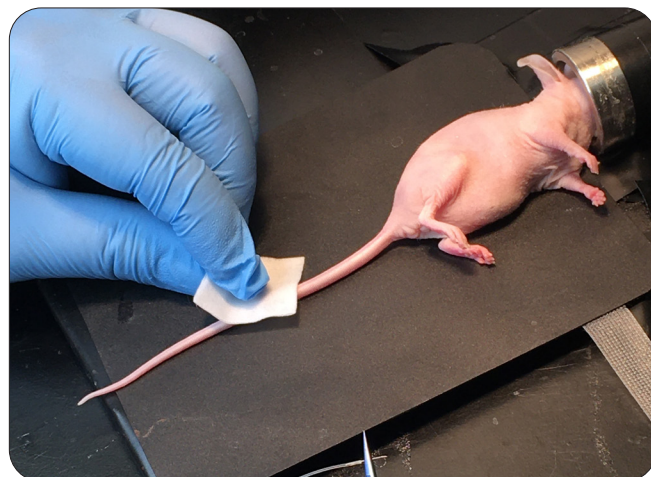


Figure 15: alcohol wipe to reveal the tail vein

- Secure the needle with the needle holder. Use the base of the needle holder to secure the needle tightly.

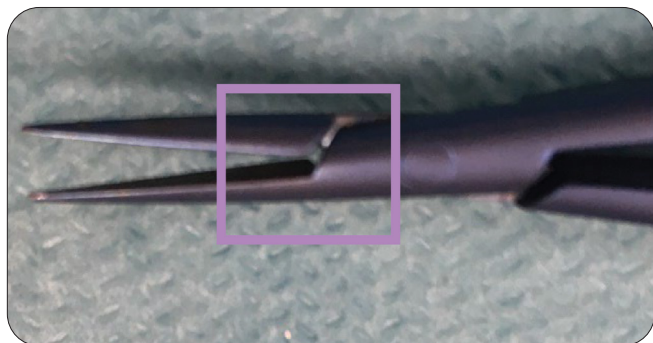


Figure 16: Needle holder

WARNING!

The needle is sharp and could pose a puncture hazard or worse (depending on the payload). Always use extreme caution when handling any needles.

- Secure the mouse's tail, ensuring the risk of user puncture is minimized.
- Place the tubing along the tail and guide it with the side of tail-holding thumb to keep the line straight.

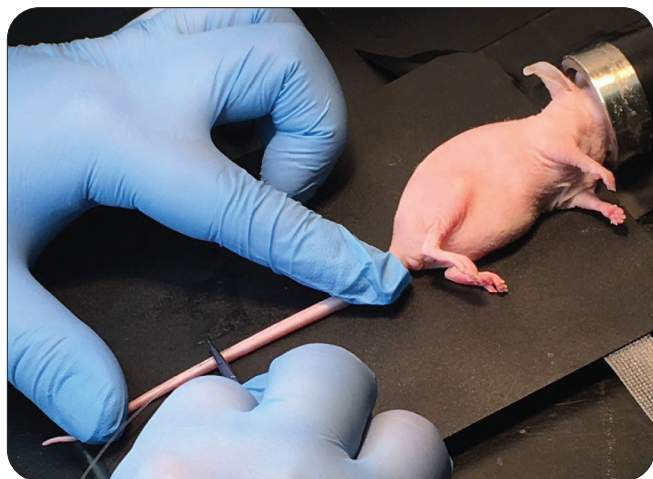


Figure 17: Tubing to tail and thumb guide

- Keep the needle as parallel to the vein as possible.



Figure 18: Needle to tail and vein example

- Gently insert the needle into the lower end of the tail toward the upper portion of the tail.

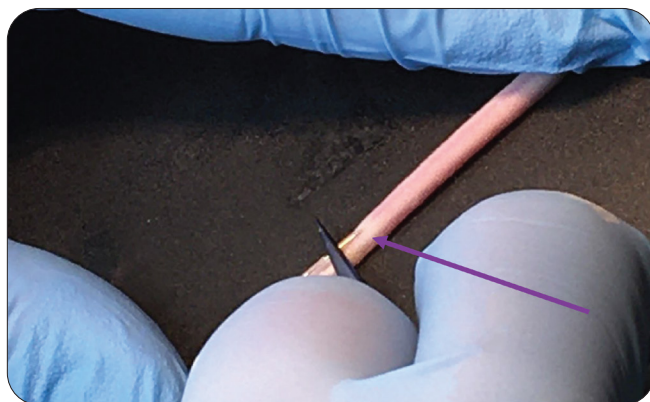


Figure 19: Needle inserting toward upper portion of tail

- Slowly insert the needle until the vein is pierced. Blood will reflux into the tubing when the vein has been punctured.

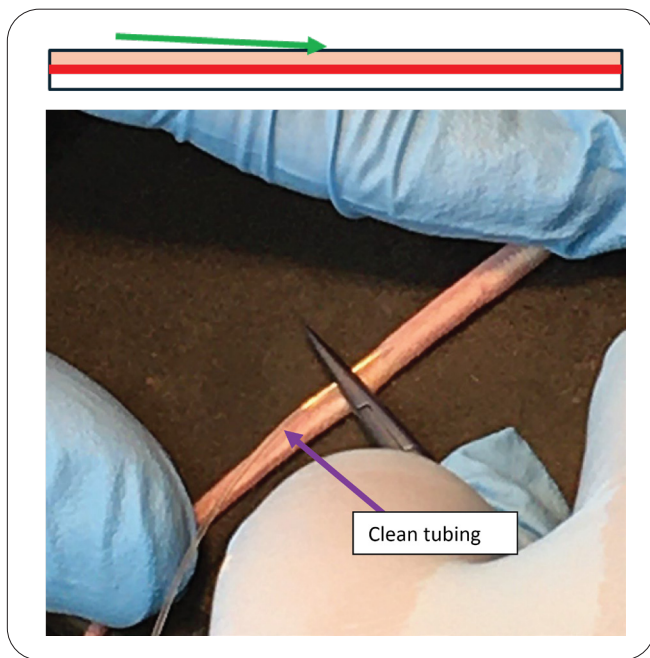


Figure 20: Clean tubing

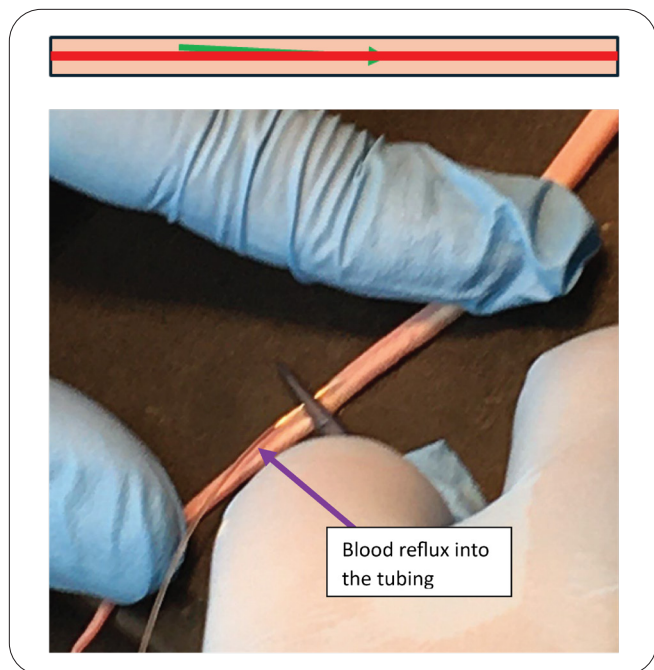


Figure 21: Blood reflux into the tubing

10. Gently release the needle holder.
11. Test the line by pressing the plunger gently. The blood in the tubing should be flushed back into the vein, and after releasing the plunger there should be no back pressure. The upper tail will be a bit paler.

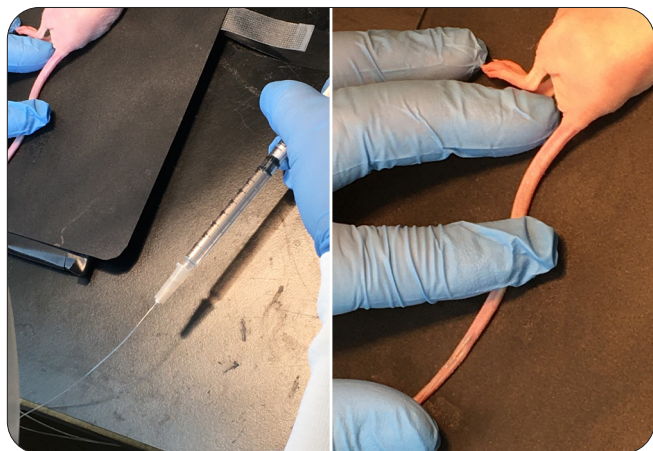


Figure 22: Blood flushed back into the vein

Securing the canulation/infusion line

1. Cut a segment of surgical tape, about 1.5 inches.
2. Fold both ends to make non-stick tabs.
3. Use the tweezers to lift the tail by inserting the tweezers under the base of the tail and slowly slide the tweezers down to the injection site.
4. Place the tape under the injection site.

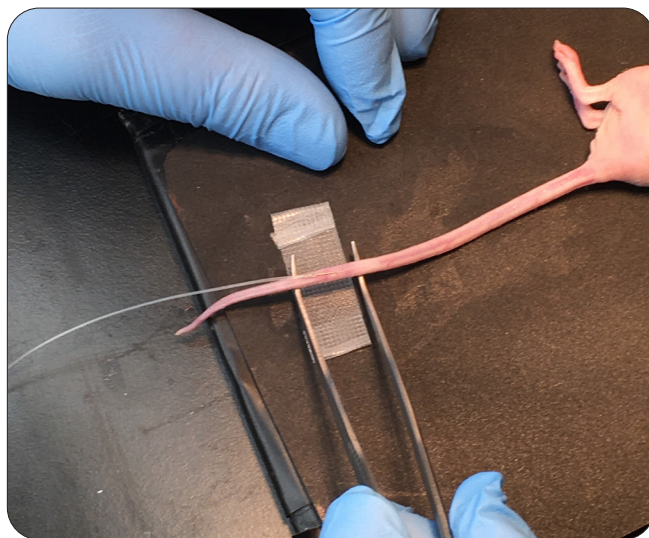


Figure 23: Tail lifted over surgical tape

5. Wrap the surgical tape around the tail and injection site.
6. Verify the shaft of the needle and tubing are secured along the tail by the tape.

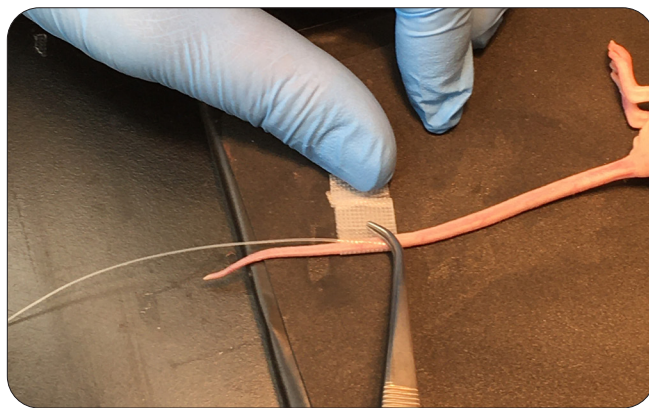


Figure 24: Taped injection site

Move the mouse and infusion set to the imaging bay

1. Tape the test syringe with PBS attached to the black sheet.

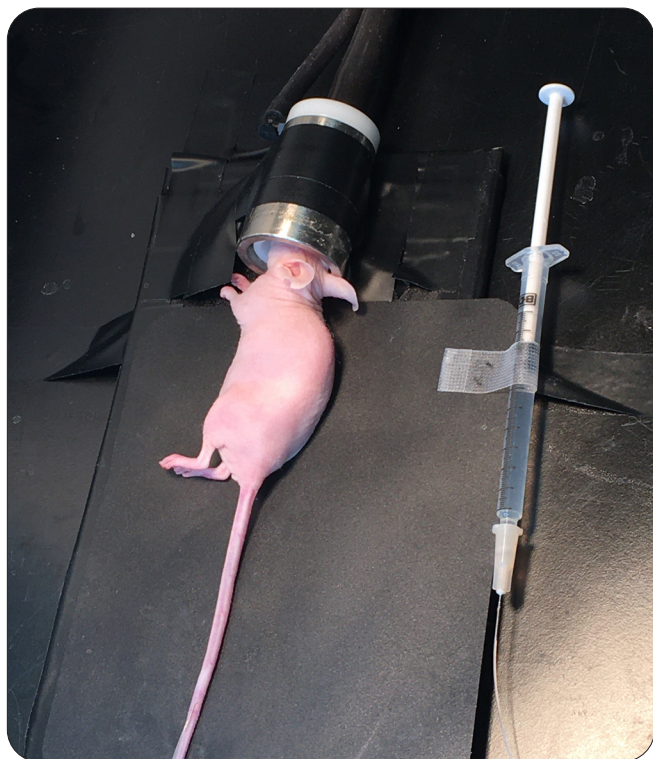


Figure 25: Pilot syringe taped to the black sheet

2. Move the whole set to the imaging bay.
3. Gently transfer the mouse from the black Lexan plastic sheet and place the mouse under the anesthesia nose cone. Note, please avoid pulling on the line as it may dislocate the needle placement in the vein.

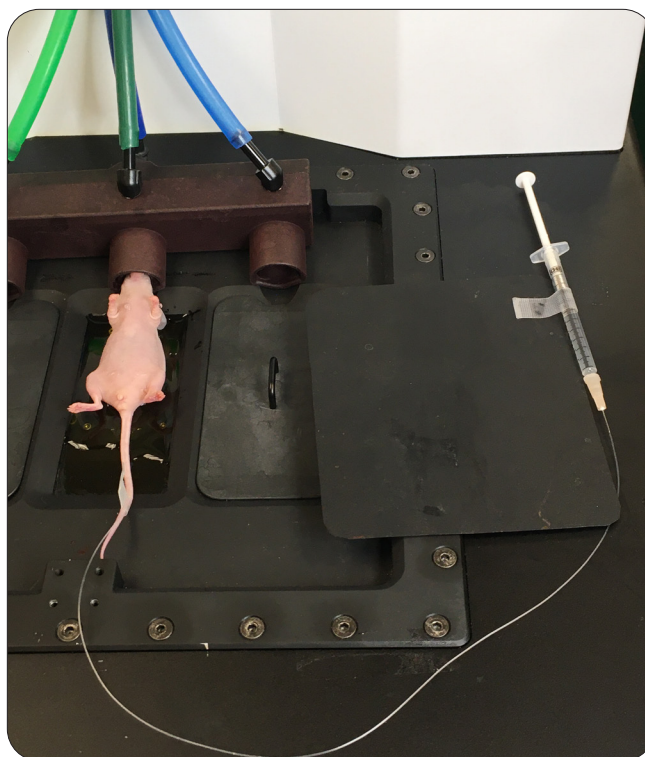


Figure 26: Mouse moved to the Vega Imaging Bay

4. Test the pilot plunger one more time to make sure the connection is still good.

Switch the connection to the pump

1. Detach the needle hub from the pilot syringe and connect the needle hub to the pre-loaded pump syringe.
2. Set the syringe pump's and start desired flow rate accordingly. Refer to the syringe pump manual for operating instructions.

NOTE: The dead volume for a 16-inch infusion set is about 50ul in this example (including the space of tubing and the needle hub on the pilot syringe). Using different build material (needles, tubing type and length) will affect the dead space. For example, if you want to deliver bolus injection of 100ul you should aim to deliver 150ul to compensate the extra 50ul needed for the dead volume.

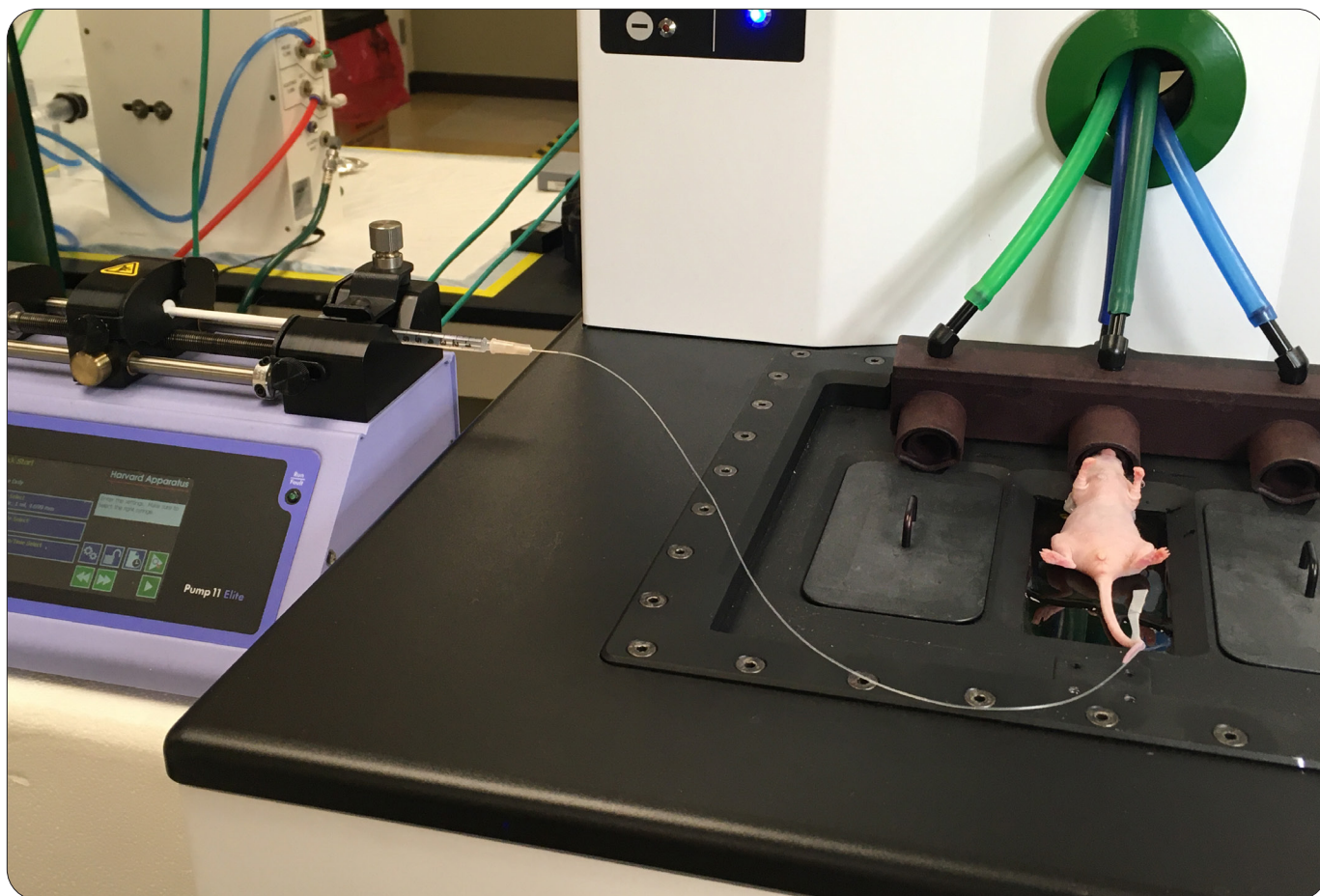


Figure 27: Pre-loaded syringe pump connected to the infusion line