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Title: A Contemporary Warming/Restraining Device for Efficient Tail Vein Injections in a Murine Fungal Sepsis Model

Authors and Affiliations:
Junko Yano[1], Elizabeth A. Lilly[2], Mairi C. Noverr[2], Paul L Fidel, Jr.[1]

[1]Center for Oral and Craniofacial Biology, Louisiana State University Health-School of Dentistry, New Orleans, LA, USA
[2]Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA, USA

Corresponding Authors:
Paul L. Fidel, Jr. (pfidel@lsuhsc.edu)

Email Addresses for All Authors:
jyano@lsuhsc.edu
elilly1@tulane.edu
mnoverr@tulane.edu
pfidel@lsuhsc.edu

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 17
Number of Shots: 29

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Paul Fidel:** The heating or restraining device offers a streamlined technique to successfully conduct intravenous tail vein injections. It provides heating of the animal in a controlled environment and effective handling for the tail vein injection.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL

1.2. **Paul Fidel:** Before attempting this technique, it is important to become familiar with the protocol and to practice with saline injections.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.3. **Paul Fidel:** Visual demonstration makes it possible to show caveats and tips for performing the injection.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Introduction of Demonstrator on Camera

1.4. **Paul Fidel:** Junko Yano, a postdoc from my laboratory, will set up the equipment. I will demonstrate the procedure.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. Procedures involving animal subjects were reviewed and approved by the local Institutional Animal Care and Use Committee (IACUC).

Protocol

2. Setting up of the warming and restraint device

2.1. To initialize the warming device, place the warming unit on a clean flat benchtop **[1]** and power it on, ensuring that the thermostat power indicator lamp is lit green **[2]**. Then, place bedding materials inside the warming chamber to keep the area dry and retain heat **[3]**.

2.1.1. Talent placing the warming unit on benchtop.

2.1.2. Talent turning on the power of the device.

2.1.3. Talent placing bedding material inside warming chamber.

2.2. For restraint device setup, place the restraint unit alongside the warming unit and determine the appropriate cone sizes for the animal **[1]**. Manually adjust the base widths of the pliable aluminum cone to provide adequate restraint for the animal **[2]**.

2.2.1. Talent placing the restraint unit alongside the warming unit.

2.2.2. Talent adjusting the base width of the aluminum cone.

2.3. Alternatively, replace the cone with custom-fitted models to accommodate animals of varying sizes **[1]**.

2.3.1. Talent replacing the cone.

3. Tail vein injection

3.1. Set the thermostat to the desired temperature using the control dial, ensuring that the heater indicator is lit red, and that the light bulb is on **[1]**. Monitor the internal temperature display carefully while the bulb is on. It will inactivate automatically once the target temperature has been reached **[2-TXT]**.

3.1.1. Talent setting the thermostat temperature.

3.1.2. Bulb illuminating and turning off when target temperature is reached. **TEXT: Never set above the body temperature (>37 °C)**

3.2. Position the restraint platform cone height to the optimum level for the user with the height adjustment knob **[1]**.

3.2.1. Talent adjusting the restraint platform cone height.

3.3. Once the target temperature has been reached, gently transfer the animals from the housing cage into the warming chamber. The warming chamber can safely contain 4 to 6 mice or 1 rat for a maximum of 20 to 30 minutes **[1]**.

3.3.1. Talent transferring the animals from the cage to the warming chamber.

3.4. Heat treatment for 5 to 10 minutes is sufficient to induce vasodilation and enhance the visibility of tail veins **[1]**. Monitor the animal for any signs of acute heat stress such as rapid respiration, lethargy, or jumping escape behavior **[2]**.

3.4.1. Animal undergoing heat treatment.

3.4.2. Talent monitoring the animal for signs of hyperthermia.

3.5. Remove the animal from the chamber by lifting it by the base of the tail **[1-TXT]** and place it onto the cone opening of the restraining unit **[2]**. *Videographer: This step is important!*

3.5.1. Talent removing the animal from the chamber by lifting it with tail. **TEXT: CAUTION: Never lift mice from the tail end**

3.5.2. Talent introducing the animal onto the restraining unit cone.

3.6. As the animal grasps on the far edge of the cone with its forelegs, gently pull the tail backward and pass the tail through the open slit **[1]**. Secure either of the hind legs protruding out from the cone **[2]** so the lateral vein is visible at a position of 12 o'clock **[3]**. *Videographer: This step is important!*

3.6.1. Talent placing the animal inside the cone and pulling the tail.

3.6.2. Talent securing the hind leg.

3.6.3. Properly positioned vein.

3.7. Grasp the tail at the mid- to two-thirds-length with the non-dominant hand between the thumb and forefinger, putting slight tension on the lateral vein to maintain the tail positioning and vasodilation **[1]**. *Videographer: This step is important!*

3.7.1. Talent properly grasping the tail.

3.8. Wipe the skin of the injection site with a gauze sponge or pad moistened with 70% alcohol **[1]**. Hold the syringe with dominant hand and position the needle parallel to the tail **[2]**. *Videographer: This step is important!*

3.8.1. Talent wiping the tail with alcohol moistened gauze.

3.8.2. Talent placing the syringe needle at the injection site.

3.9. Insert the needle toward the direction of the blood flow at an angle of 10 to 25 degrees and advance further into the lumen of the vein by penetrating 2 to 4 millimeters. Slowly inject the solution **[1]**. *Videographer: This step is difficult and important!*

3.9.1. Talent inserting and advancing the needle, then injecting the solution.

3.10. In case of resistance or white blisters above the injection site, remove the needle and attempt a second injection at a site above the original needle placement **[1]**. If injection of one lateral vein is unsuccessful, reposition the animal to the opposite side and make more attempts on the contralateral vein **[2]**. *Videographer: This step is difficult and important!*

3.10.1. Talent changing the injection site.

3.10.2. Talent repositioning the animal.

3.11. Remove the needle **[1]** and firmly press the injection site with the thumb to prevent backflow of the injected solution or blood **[2]**. Continue applying gentle compression with a clean gauze or tissue wipe until bleeding is stopped **[3]**.

3.11.1. Talent removing the needle.

3.11.2. Talent pressing the injection site firmly with thumb.

3.11.3. Talent giving gentle compression with clean tissue or gauze.

3.12. Return the animal to its cage **[1]** and monitor it for 5 minutes, ensuring that the animal resumes normal activity without further bleeding **[2]**.

3.12.1. Talent returning the animal to cage.

3.12.2. Animal displaying normal activity.

Results

4. Sepsis mortality vs. vaccine-mediated protection following a lethal challenge with *Candida albicans*

4.1. Once the heater of the warming chamber was activated, heat emission by the light bulb rapidly raises the internal temperature during the first 5 to 15 minutes, depending on the target temperature **[1]**.

4.1.1. LAB MEDIA: Figure 7. *Video Editor: Focus on the initial yellow dot (0 min) then transition toward the first gray dot in each curve to display the linear increase.*

4.2. The heater inactivates the light bulb if the detected internal temperature exceeded the set temperature. Subsequently, the device automatically repeats the heat cycle to maintain the internal temperature **[1]**.

4.2.1. LAB MEDIA: Figure 7. *Video Editor: Focus on the first grey dots then transition toward the next yellow dots to illustrate the heat cycle.*

4.3. In a mouse model of bloodstream candidiasis resulting in sepsis, an intravenous challenge with *Candida albicans* in Swiss Webster mice caused a rapid onset of sepsis and dissemination of the organisms, leading to high mortality within 3 to 4 days **[1]**.

4.3.1. LAB MEDIA: Figure 8A. *Video Editor: Focus on the line graph with open dots and display the progressive decrease.*

4.4. In contrast, animals could be protected from sepsis by pre-immunization or vaccination with an avirulent yeast strain, *Candida dubliniensis*, achieving more then 95% survival following the lethal i.v. challenge with virulent *C. albicans* **[1]**.

4.4.1. LAB MEDIA: Figure 8B. *Video Editor: Focus on the line graph with solid dots and display the flat line.*

4.5. The unvaccinated animals with lethal infection had a significant increase in sepsis-induced morbidity **[1]**, whereas the vaccinated group exhibited minimal symptoms following the lethal challenge **[2]**.

4.5.1. LAB MEDIA: Figure 8B. *Video Editor: Emphasize the curve with squares.*

4.5.2. LAB MEDIA: Figure 8B. *Video Editor: Emphasize the curve with triangles.*

4.6. The results in progressive mortality versus vaccine-mediated protection were obtained reproducibly in four independent experiments **[1]**.

4.6.1. LAB MEDIA: Supplementary figure 1.

Conclusion

5. Conclusion Interview Statements

5.1. **Paul Fidel:** Be careful when positioning the animal in the cone, angling the needle to access the vein, adjusting the angle to move the needle into the vein, and injecting the vein.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.7 and 3.9*

5.2. **Paul Fidel:** This streamlined technique allows for more effective and efficient means for researchers successfully perform tail vein injections, which will ultimately enhance their scientific experimental outcomes.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.