Submission ID #: 68776
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=20976728

Title: Facial Vein Venipuncture for Murine Blood Collection

Authors and Affiliations:
Stephanie Kalinowski, Claudia V. Jakubzick

Department of Microbiology and Immunology, Dartmouth Geisel School of Medicine

Corresponding Authors:
Stephanie Kalinowski Steph.Kalinowski@dartmouth.edu

Email Addresses for All Authors:
Stephanie Kalinowski Steph.Kalinowski@dartmouth.edu
Claudia V. Jakubzick claudia.v.jakubzick@dartmouth.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. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 19
Number of Shots: 30

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Stephanie Kalinowski:** The goal of our research is to present an optimized blood collection method for murine subjects. This method reduces the time and skill needed for small-volume blood collection in mice while still prioritizing ethical and minimally invasive practices.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What research gap are you addressing with your protocol?

1.2. **Stephanie Kalinowski:** Blood collection methods are often overcomplicated. We wanted to simplify them all and show that you can achieve clear lysis with minimal time, effort, and materials.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.5.1*

What advantage does your protocol offer compared to other techniques?

1.3. **Stephanie Kalinowski:** The main advantages this protocol offers over other techniques is giving researchers their time back. This method is very quick and requires very few materials or specialized skill to execute.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.2*

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL): **NOTE: Did not shoot**

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.4. **Stephanie Kalinowski, Research Assistant**: (authors will present their testimonial statements live)

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.5. **Stephanie Kalinowski, Research Assistant**: (authors will present their testimonial statements live)

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

Ethics Title Card

This research has been approved by the Institutional Animal Care and Utilization Committee at the Dartmouth Geisel School of Medicine

Protocol

2. Mouse Handling and Sample Collection

Demonstrator: Stephanie Kalinowski

2.1. To begin, label each cage card and mouse with a phenotype identifier that matches the corresponding labels on the collection tubes **[1]**. Mark the tails of mice with a permanent marker to correspond with the markings on the tubes **[2]**.

2.1.1. WIDE: Talent labelling cage cards.

2.1.2. Close-up of talent marking the tail of a mouse with a permanent marker and then labelling the tube to correspond with the mouses tail color.

2.2. Open all lancets and gauze sponges required for the procedure **[1]** and add 1 to 2 milliliters of lysis buffer to each labelled tube **[2].** NOTE: The VO is edited for the deleted shot

2.2.1. Talent opening multiple lancet packages and arranging them neatly on the workstation.

Added shot: Talent adding 1-2 ml of lysis buffer to each tube

2.3. Now, using the dominant hand, grasp the mouse by the tail **[1]** and place it on a wire feeding rack or a flat surface **[2]**.

2.3.1. Talent holding the mouse by the tail with the dominant hand. NOTE: 2.3.1 and 2.3.2 are filmed together

2.3.2. Talent placing the animal on the wire rack.

2.4. With the non-dominant index finger and thumb, scruff the mouse by maneuvering from the tail toward the cervical region **[1]**.

2.4.1. Close-up of talent using non-dominant fingers to scruff the mouse near the cervical region.

2.5. Then, using the remaining fingers, secure the tail to minimize mouse movement **[1]**.

2.5.1. Close-up of talent securing the mouse tail with the remaining fingers while holding the scruff.

2.6. Next, use the dominant hand to retrieve an opened 3-millimeter lancet **[1]**.

2.6.1. Talent picking up an opened lancet from the workstation.

2.7. Locate the small hairless spot just beyond the mouth and trace a straight line toward the ear **[1]**. Stop where the line intersects with the outer corner of the eye **[2]**.

2.7.1. Close-up of talent locating the hairless spot beyond the mouth. NOTE: 2.7.1 and 2.7.2 are filmed in a single shot

2.7.2. Close-up of talent pointing to the line intersecting with the outer corner of the eye.

2.8. Now, insert the lancet at this intersection to ensure an ample blood supply **[1]** and use the flat lateral edge of the lancet to collect a single drop of blood **[2]**.

2.8.1. Close-up of the lancet being inserted at the intersection point near the eye. NOTE: Shots 2.8.1, 2.8.2, 2.10.1 and 2.10.2 were combined into one shot.

2.8.2. Close-up of the lancet edge capturing a drop of blood.

2.9. Then, deposit the entire lancet, with the collected blood, into the labelled tube containing red blood cell lysis buffer **[1]** and gently agitate the tube to mix the blood completely with the buffer **[2]**.

2.9.1. Talent placing the used lancet with blood into the labeled tube containing buffer. NOTE: Shot 2.9.1 and 2.9.2 were combined

2.9.2. Talent gently swirling the labeled tube to mix the blood with the buffer.

2.10. Using a gauze sponge, apply gentle pressure to the puncture site for 5 to 10 seconds **[1]** and return the mouse to its cage **[2]**.

2.10.1. Close-up of talent pressing a gauze sponge against the puncture site on the mouse.

2.10.2. Talent carefully returning the mouse into its cage.

3. Lysis, Preparation, and Analysis of the Sample

3.1. Add approximately 2 milliliters of Hank's Balanced Salt Solution with 0.5 millimolar EDTA to each sample tube **[1]**.

3.1.1. Talent pipetting Hank's Balanced Salt Solution with EDTA into the sample tube.

3.2. Place the tubes into the centrifuge and spin at 400 *g* for 5 minutes **[1]**. During this time, prepare the antibody cocktails **[2]**.

3.2.1. Talent loading the tubes into the centrifuge and closing the lid.

3.2.2. Talent placing the antibody cocktail solutions at the workstation.

3.3. After centrifugation, discard the supernatant from each tube **[1]**.

3.3.1. Talent carefully pouring off the supernatant from the centrifuged tubes.

3.4. Then, using forceps, remove the lancets from the tubes **[1]** and add 30 microliters of antibody cocktail into each sample **[2]**.

3.4.1. Talent using forceps to extract the lancets from the tubes.

3.4.2. Talent pipetting antibody cocktail into each tube.

3.5. Vortex the samples for 2 to 3 seconds to resuspend the cells **[1]** and incubate the samples at 4 degrees Celsius for 25 to 30 minutes **[2]**.

3.5.1. Talent vortexing the sample tubes briefly.

3.5.2. Talent placing the samples into a refrigerator.

3.6. Next, turn on the flow cytometer and configure the flow plots according to the experimental requirements **[1]**.

3.6.1. Talent operating the flow cytometer.

3.7. Using a transfer pipette, add 150 to 250 microliters of Hank's Balanced Salt Solution or other media to each sample **[1]**. If required, add DAPI at a final concentration of 1 microgram per milliliter by using 100 microliters of DAPI-containing media **[2]**.

3.7.1. Talent pipetting Hank's Balanced Salt Solution into each sample tube. NOTE: Shots 3.7.1 and 3.7.2 are filmed in a single shot

3.7.2. Talent tapping the sample tube to mix content.

3.8. Vortex each sample for 2 to 3 seconds just before acquisition **[1]**.

3.8.1. Talent vortexing sample tubes briefly before placing them in the cytometer.

3.9. Finally, check the reagents and the run settings of the flow cytometer for data acquisition **[1]**. NOTE: The VO is edited as per the shot

3.9.1. ~~Show the flow cytometer interface displaying sample acquisition.~~ clip of me operating flow (exchanging the tubes, changing the run settings, etc.) NOTE: This shot is modified

Results

4. Results

4.1. The flow cytometry of wild-type mice showed no tdTomato *(T-D-tomato)* signal in platelets **[1]**, whereas Pf4cre *(P-F-4-cre)* reporter mice showed distinct tdTomato-positive platelet populations **[2]** and larger tdTomato-positive platelet-bound cells **[3]**.

4.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the top right plot from the "WT mice.*

4.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the cluster labeled "Platelets" in the tdTomato versus FSC plot on the bottom right, corresponding to the "Pf4cre reporter mice"*.

4.1.3. LAB MEDIA: Figure 2. *Video editor: Highlight the area labeled "Platelet bound cells" in the tdTomato versus FSC plot on the bottom right, corresponding to the "Pf4cre reporter mice".*

4.2. Side scatter versus forward scatter plots from both wild-type and Pf4cre mice showed clear separation between cellular debris, lymphocytes, and granulocytes, indicating effective red blood cell lysis and preserved sample structure **[1]**.

4.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the plots on the left-side "SSC vs FSC".*

4.3. CD19-positive IgMa *(I-G-M-A)* positive B cells were detected in MD4 IgHEL *(I-G-H-E-L)* mice **[1]**, whereas wild-type mice lacked the IgMa signal despite CD19 positivity **[2]**.

4.3.1. LAB MEDIA: Figure 3. *Video editor: Highlight the blue circle in the bottom right plot from MD4IgHEL mice*.

4.3.2. LAB MEDIA: Figure 3. *Video editor: Highlight the blue circle from the top right plot from wild-type mice*.

1. **phenotype**
   Pronunciation link: https://dictionary.cambridge.org/dictionary/english/phenotype (Cambridge Dictionary)
   IPA: /ˈfiː.noʊˌtaɪp/ (Cambridge Dictionary)
   Phonetic Spelling: *FEE-noh-type*

---

2. **lancet**
   Pronunciation link: https://www.merriam-webster.com/dictionary/lancet (Wikipedia)
   ("lancet" appears as the name of the journal too, but same word)
   IPA: /ˈlæn.sɪt/ (Wikipedia)
   Phonetic Spelling: *LAN-sit*

---

3. **lysis** (as in "lysis buffer")
   Pronunciation link: https://www.merriam-webster.com/dictionary/lysis (Wikipedia)
   IPA: /ˈlaɪ.sɪs/ (Wikipedia)
   Phonetic Spelling: *LYE-sis*

---

4. **cervical** (region)
   Pronunciation link: https://www.merriam-webster.com/dictionary/cervical (Wikipedia)
   IPA: /ˈsɜr.vɪ.kəl/ (Wikipedia)
   Phonetic Spelling: *SUR-vih-kul*

---

5. **centrifuge**
   Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge (Wikipedia)
   IPA: /ˈsɛn.trə.fjuːdʒ/ (Wikipedia)
   Phonetic Spelling: *SEN-truh-fyooj*

---

6. **supernatant**
   Pronunciation link: https://www.merriam-webster.com/dictionary/supernatant (Wikipedia)
   IPA: /ˌsuː.pərˈneɪ.tənt/ (Wikipedia)
   Phonetic Spelling: *SOO-per-NAY-tent*

---

7. **antibody**
   Pronunciation link: https://www.merriam-webster.com/dictionary/antibody (Wikipedia)
   IPA: /ˈæn.tiˌbɑː.di/ (Wikipedia)
   Phonetic Spelling: *AN-tee-bah-dee*

---

8. **flow cytometer**
   - **flow**: /floʊ/ — *floh*
   - **cytometer**: Pronunciation link: https://dictionary.cambridge.org/dictionary/english/cytometer (Wikipedia)
     IPA: /ˌsaɪˈtɑː.mə.tər/ (Wikipedia)
     Phonetic Spelling: *sigh-TAH-muh-ter*

---

9. **tdTomato**
   Pronunciation link: *No major dictionary entry, but usage in literature strongly suggests:* /tiː diː təˈmeɪ.toʊ/ – "tee-dee toh-MAY-toh" (FPBase)
   IPA: /tiːdi təˈmeɪtoʊ/
   Phonetic Spelling: *TEE-dee tuh-MAY-toh*

---

10. **Pf4-Cre**
    Explanation: a genetically modified mouse line where *Pf4* (platelet factor 4) promoter drives *Cre* recombinase expression. (Jackson Laboratory)
    Pronunciation link: *No single dictionary entry, combine known pronunciations of parts*
    IPA: /piː-ef-four kree/
    Phonetic Spelling: *PEE-EF-four KREE*