

# RODENT SAMPLING METHODS

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*This document was prepared as part of the One Health Workforce – Next Generation Project\* to support activities conducted under One Health Workforce Academies and is intended for an audience of qualified professionals trained in standard best practices. This guide is not intended for use by untrained individuals.*

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*The authors assert that animal surveillance should always occur in compliance with all applicable laws and regulations and should only be undertaken by trained wildlife professionals after securing all necessary permits and approvals, including approval by an ethics review committee.*

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## 1. Brief overview of PPE

Due to the COVID-19 pandemic, One Health workers who are actively working with rodents and other wildlife must recognize the potential for exposure to both known and novel zoonotic pathogens during animal capture, handling, and sampling activities. A similar risk of “spill-back” of SARS-CoV-2 to rodents from infected humans also has the potential to dramatically impact the health of these important wildlife species. These risks can be mitigated through strict adherence to biosafety and biosecurity practices detailed below and in additional reference materials listed in this protocol.

These documents are in no way a substitute for active hands-on training from experts in the field. We highly encourage persons seeking to do this type of research work with wildlife to seek out in-person training opportunities and guidance before initiating any hands-on work with wildlife.

In addition to vaccination guidelines during times of COVID-19 transmission, field sampling teams should follow all relevant national recommendations to maintain best practices to reduce virus transmission among local communities including physical distancing, face masks, and handwashing (or sanitizer) when engaging with community members and stakeholders at locations of animal capture and sampling.

### Minimum PPE required for handling traps

The minimum PPE for handling of traps (when setting or picking up) includes:

1. Designated long sleeved clothing
2. Nitrile gloves
3. N95 facemask without exhalation valve
4. Washable shoes

### Minimum PPE Required for handling rodents

The minimum PPE for handling of rodents during capture and sampling includes:

1. Tyvek suit
2. Double nitrile gloves, and preferably different colors to facilitate notice of punctures or breaks.  
If possible, use extended cuff gloves
3. Eye protection (face shield or goggles)
4. N95 facemask without exhalation valve
5. Washable shoes

The PPE requirements should be specific to the risk for handling animals during capture or during processing. See the **Biosafety SOP** for detailed instructions regarding PPE Use

## 2. Data Collection

Data should be collected according to data collection templates developed prior to field sampling activities. Relevant data may include information on the sampling site, sampling event, animal, and specimen.

### 3. Rodent Capture, Handling, and Sampling

Capture techniques will vary based on the species being targeted and the location where the samples are being collected. Details of the main techniques, including information on Sherman traps, are available in other documents such as the [CDC guide \*Methods for Trapping and Sampling Small Mammals for Virologic Testing\*](#) (pp 15-18)

*All animal capture, handling, and sampling must also be done in accordance with your current IACUC approval and following local laws and regulations regarding veterinary anesthetic drug use.*

#### 3.a Rodent Capture and Handling

1. Free-ranging rodents should be captured using metal box traps (Sherman/Tomahawk traps). Traps are set open at sunset and checked at sunrise. If adverse weather is expected, such as abrupt changes in precipitation intensity that could cause flash flooding events/animals getting wet, the traps must be closed. Also, if researchers are working in areas where predation is common, traps must be checked more frequently. Traps should be located in areas protected from direct sunshine (e.g. ground cover vegetation or artificial reflective foil insulation) to prevent consequent heat stress. Otherwise, traps should be closed between sunrise and sunset.
2. To avoid hypothermia, allow thermoregulation, and reduce capture stress, **always** provide nesting material (e.g., pulped cotton fiber, such as [Nestlets by Ancare](#)) to each trap. If the animal is in hypothermic shock, wrap the animal in a dry drape, place a hand warmer (e.g., reusable exothermic pad or air-activated disposable pad) in the outer layer of the wrap, place the animal in a dry recovery chamber (i.e. clear plastic box), monitor recovery and then release at the capture location.
3. Remove animals from traps by placing a clear plastic handling bag or large Ziploc-style bag over the mouth of the trap, opening the door into the bag and coaxing the animal out by gently shaking the trap. **Do not blow into the trap.**
4. Weigh the animal in the handling bag using a Pesola scale or a flat digital scale prior to inserting the anesthetic ball (see below). Temporarily record the animal plus bag weight and after the animal is anesthetized and removed, reweigh the empty bag and subtract the bag weight from the total to obtain the animal weight.

5. Scruffing is the best way to handle smaller rodents for sampling. Hold the anesthetized animal by pinching the skin between the thumb and forefinger at the point where the rodent's spine meets the head (Figures 1 and 2). For a more secure hold and/or access to the ventral surface, position the rodent's body firmly across your hand by extending your forefinger and thumb back as far as possible while maintaining a firm grip on the scruff. Place the tail between the fingers of this same hand (Figure 3). Gerbils and some other rodents are prone to tail slip (the skin breaks and leaves tissue and bone exposed), so avoid holding them from the tip of the tail.



Figure 1 (left): scruff restraint technique. Figure 2 (right): positioning the animal in the hand while scruffing, in order to collect samples. Photos: © Leticia Gutiérrez Jiménez.

**Note:** If you grasp too much skin, the airway will become restricted and the rodent will become cyanotic. Monitor the condition of the animal the entire time it is restrained, being careful to observe the breathing rate and color of the ears, nose, and oral cavity. The animal should be released immediately if there are any signs of gasping or change in coloring from pink to blue.

### 3.b Rodent Anesthesia

Anesthetize rodents with isoflurane. **Note:** Pregnant women should avoid or minimize exposure to isoflurane.

1. Keep in mind species-specific isoflurane hypersensitivity (e.g. voles) and that age, sex, and reproductive stage can affect drug potency, which in turn may cause abrupt respiratory depression, especially in older individuals.
2. Apply 0.4 ml of isoflurane to a cotton ball and put the cotton ball into a metal tea ball or plastic tube (nose cone; 0.4 ml is the appropriate dose for a ca. 20g mouse, adjust the dose as needed for larger rodents). If tea ball is used, place the metal ball into the clear plastic holding bag or container with the rodent; if nose cone is used, place the rodent's nose into the tube (Figure 3).

3. Watch the animal closely until anesthetized. Breathing rate will increase then slow as the animal progresses further under anesthesia.
4. When withdrawal reflex is suppressed (use toe pinch to measure the withdrawal reflex), remove rodent from bag for processing.
5. Place an additional cotton ball soaked with 0.2 ml isoflurane into a 50 ml tube and screw on the cap. If the animal begins to wake up during blood collection, unscrew the cap of the nose cone and position over the animal's nose for as brief a time as possible to re-anesthetize the animal (see Figure 3 below). A nose cone for supplementary anesthesia should be used with care as different animals respond differently to isoflurane inhalation.



Figure 3: Nose cone for anesthesia. Photo: PREDICT project

6. Collect samples immediately after the rodent is anesthetized.

**Note:** For larger rodents, chemical restraint and anesthesia (ketamine alone 25-50mg/kg, or ketamine combined with xylazine 80-100 mg/kg ketamine + 10-12.5 mg/kg xylazine) can be applied. Drugs can be administered to manually-restrained or squeeze-caged animals by syringe or by blow dart, depending on size and situation.

### 3.c Basic Sample Set for Rodent Sampling

A suite of samples can be collected, and the specific samples and preservation methods will vary according to the goals of each specific study. Here we provide guidance on collection of samples aimed at virus surveillance and discovery.

The following basic set of samples should be collected from each animal when possible, in the quantities and media outlined below: oral swab, feces/rectal swab, blood, urine/urogenital swab. For any longitudinal sampling, the same individual may be sampled no more than once every 3 months.

The table below provides details on the suggested suite of samples to be collected from each animal handled and their preservation method. MagMAX lysis/binding buffer concentrate, also referred to as

MagMAX lysis buffer, and viral transport medium (VTM) are the two primary sample preservation media, though serum is stored without media. *The lysis/binding solution mentioned in this protocol is part of the MagMax Pathogen nucleic acid extraction kit. If you will be using a different kit/solution please refer to the manual recommendations.*

Ideally, all samples will be frozen in liquid nitrogen immediately in the field and transferred to a -80°C lab freezer when field teams return.

**NOTE: In all cases, if only one sample can be collected, then place it into VTM.**

Sample type	Total samples	Preservation method						Comments
		500 µL VTM		500 µL MagMAX lysis buffer		No Media		
		#	Sample volume	#	Sample volume	#	Sample volume	
Oral swabs	2	1	swab	1	swab			
Feces/ Rectal swabs	2	1	0.5cc feces or swab	1	0.5cc feces or swab			Feces OR swab
Urine/ Urogenital swabs	2	1	100 µL urine or swab	1	100 µL urine or swab			Urine OR swab
Blood*	2-4	1-2	100 µL	1-2	100 µL			For larger species only, collect two 100 ul aliquots per media type (4 samples total)
Serum	2					2	250 µL	minimum 50 µL per aliquot recommended

\* Note: If animals are too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in VTM after serum separation.

### 3.d Collection details

1. Collect **two oral swab** samples using sterile, polyester-tipped swabs with plastic or aluminum shafts. A sterilized handle of a pair of forceps can be used to safely open the animal’s mouth. Rub the polyester tip of the swab gently, but thoroughly, against the back of the animal’s throat. Place one swab into a cryovial containing 500 µL of VTM and one in a cryovial with 500 µL of Lysis/binding solution. Cut the shaft of the swab with alcohol-wiped (or ethanol-wiped), flame-sterilized scissors. To cut the swab shaft, lift the swab a little above the bottom of the vial and then cut it. If the shaft is cut when the swab tip is resting on the bottom, the swab will be too long and the cryovial will not close.



2. Collect **two rectal swabs** using appropriately-sized, sterile polyester swabs. For smaller animals, use urethral or pediatric swabs. Rectal swabs can be moistened with sterile saline prior to animal sampling.

- **Do not use VTM to moisten swabs before sampling.**
- **Do not use** Lysis/binding solution **as a lubricant** – it is highly irritating to tissue.
- **Do not force tip of swab into rectum**, if it will not enter easily, do not collect this sample.

Gently insert one sterile swab tip at a time into the animal's rectum. Place 1 swab in a cryovial filled with 500 µL of VTM, and using alcohol-wiped (or ethanol-wiped), flame-sterilized scissors, cut the shaft of the swab. Place the other swab into a cryovial with 500 µL of Lysis/binding solution. Store in a dewar or dry shipper with liquid nitrogen and transfer to -80°C freezer when possible.

**Alternatively, collect fresh feces:** Add 300 µL (pea-sized) pieces of feces directly into two vials, one containing 500 µL VTM (maximum final ratio of 1:1) and one containing 1 mL Lysis/binding solution (maximum final ratio of 1:2) and mix each tube well. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

3. Collect **two urogenital swabs** and place one in 500 µL of VTM and one in 500 µL of Lysis/binding solution. Swabs can be moistened with sterile saline prior to animal sampling. If the animal urinates, collect up to 500 µL of **urine** using pipettor and place into one cryotube with 500 µL of VTM and mix well. Pipette up to another 500 µL of urine into a cryotube with 500 µL of Lysis/binding solution and mix well.

### 3.e Blood Sample Collection

**Make sure the total blood collected from the animal does not exceed 1% of weight of animal (i.e., a maximum of 500 µL should be collected from a 50g rodent).**

If animal size allows, collect blood and place in a red-top vacutainer tube with clotting factor and in a lavender-top tube (e.g., EDTA). Allow blood in red-top tube to clot, then centrifuge and pipette two 500 µL serum samples and freeze without media. From lavender top tubes, add up to 500 µL of whole blood to a cryotube with 500 µL of VTM and 100 µL of blood to a second cryotube with 500 µL of Lysis/binding solution (if blood volume is <500 µL then only collect into VTM).

If animals are too small to collect vacutainer blood tubes, blood collection can be done with [Sarstedt Inc Microvette Capillary](#) Blood Collection Tubes (100ul to 600ul capacity), Becton Dickenson [BD Microtainer™ Capillary](#) Blood Collector and [BD Microgard™ Closure tubes](#) (250ul-500ul capacity), [Terumo™ Capiject™ capillary blood collection tubes](#) (500ul capacity), or heparinized 75 µL glass hematocrit tubes.



For blood collected into hematocrit tubes, use a **bulb** to expel the whole blood in a cryotube with 500 µL of VTM. Hematocrit tubes can be centrifuged using portable hematocrit or standard vacutainer centrifuges to separate plasma. Score glass tube using a razor blade or X-acto knife where the serum meets the red cell fraction and snap the tube. Use a bulb to expel plasma into a micro-cryovial and freeze. If two or more capillary tubes are filled, collect two aliquots of plasma. Preserve the remaining red cell fractions in a separate cryovial with VTM and freeze.

### 3.e.1 Guidelines for Selecting Bleeding Method

Personnel should be acquainted with the local fauna and their anatomy and apply this knowledge to determine the most appropriate sampling methods to use in the field. Do not attempt venipuncture without at least one field person present with prior experience, especially with the facial vein and jugular vein bleeding approaches.

**Table 1: Recommended Bleeding Technique(s) by Rodent Taxa**

Rodent Taxa		Mice, field mice, jumping mice	Voles, gerbils, and hamsters	Squirrels and chipmunks	Rats and rat-like species
Bleeding Method		1) Facial vein 2) Saphenous vein	1) Facial vein 2) Saphenous vein	1) Saphenous vein	1) Saphenous vein 2) Lateral tail vein  <b>Larger animals:</b> Ventral tail vein, jugular vein

### 3.e.2 General Techniques for Bleeding Rodents

**Note: Bleeding by any method should only be performed on anesthetized animals.**

#### Submandibular or facial vein blood collection

Adapted from the MEDipoint, International Inc. Goldenrod lancet ‘For use on mice’ website.

For videos of the technique, please visit [http://www.medipoint.com/html/pc\\_video\\_instructions.html](http://www.medipoint.com/html/pc_video_instructions.html) (for PC) [http://www.medipoint.com/html/mac\\_video\\_instructions.html](http://www.medipoint.com/html/mac_video_instructions.html) (for Mac)

For this technique you will need to purchase lancets to prick the skin on the rodent’s cheeks. Lancets provide the greatest control of puncture depth and are the recommended device for facial bleeding; however, sterile 20G-22G hypodermic needles may also be used **after** the facial bleeding technique has been completely mastered.

You can use any lancet, but as guidance, **Goldenrod lancets** are recommended. They come in four point lengths: 3, 4, 5 and 5.5mm point. You should use the best option depending on the age/size of the rodents you will sample. If you only need one or two drops, use 3mm point (keep in mind that a

standard blood drop is equal to ~50ul). Always be careful that the volume of blood harvested does not exceed the total volume recommended for that size animal's safety and health ( $\leq 1\%$  body weight).

**Step-by-step procedure:**

1. Choose the proper lancet point length (see above), corresponding to the size of rodent.
2. Open the lancet/needle wrapper and prepare a collection vial.
3. Hold the anesthetized animal by pinching the skin at the back of the head (scruff restraint technique – see Figures 1 and 2) and taking the tail between the fingers of the same hand.
4. Locate the back of the jaw bone, the submandibular area. There is a vascular bundle located at the rear of the jaw bone providing a convenient and consistent source of blood (Figures 4, 5, and 6).

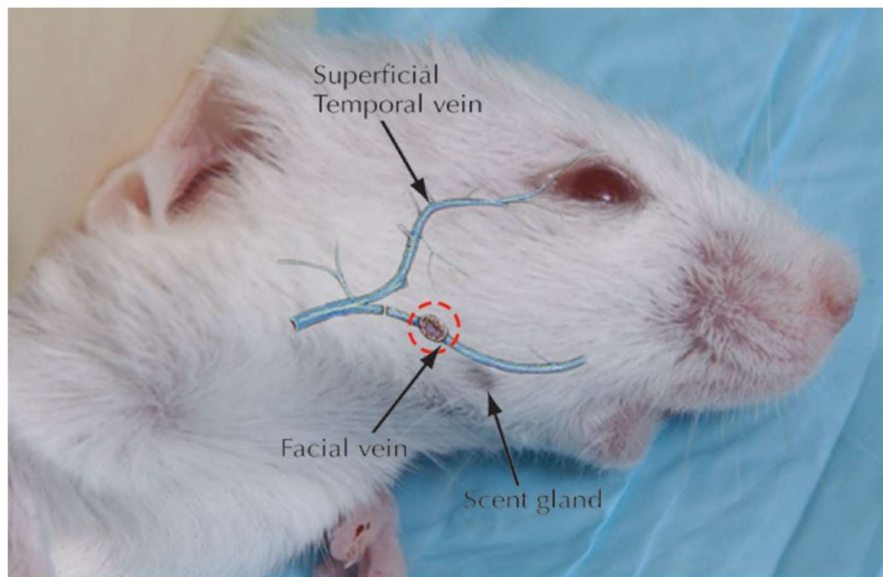


Figure 4: The vascular bundle targeted during blood collection from the facial vein. The dashed circle indicates the puncture site. Figure: Golde, Gollobin and Rodriguez, 2005.

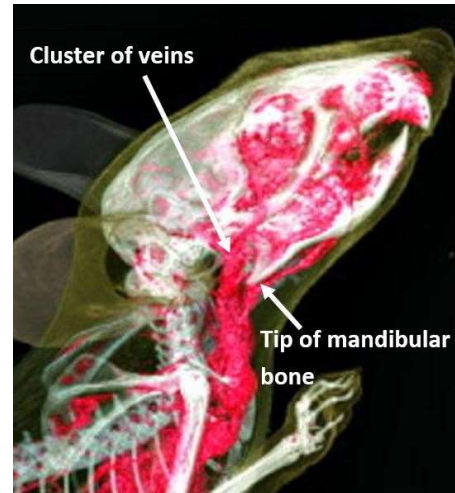
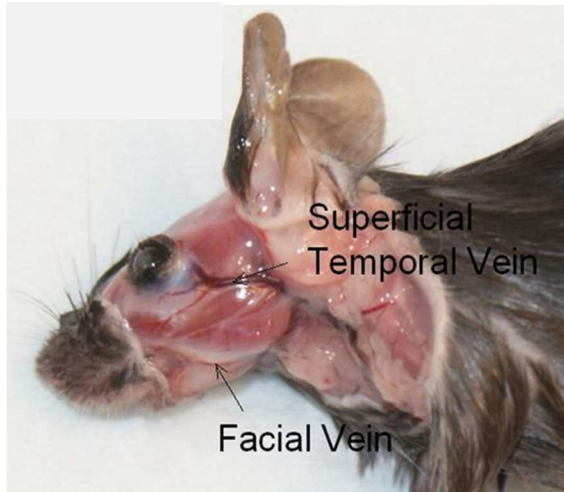


Figure 5 (left): Superficial temporal vein and facial vein in the mouse. Figure 6 (right): The targeted cluster of veins is just behind and above the tip of the mandibular bone. Photos: MEDpoint International, Inc.

5. Once the site is located, properly align the tip of the lancet: The facial muscles of a mouse or rat run fairly parallel to the bottom of the jaw line, that is, along the face from the nose/whiskers towards the ear. Align the lancet with the striations of the muscles in the animal's face so that when the tip enters the facial muscles, it will go between the striations instead of across them. This will cause less damage to the muscle tissue and therefore cause less scarring, and there will be less chance of hematoma formation.



Figure 7 (left): Facial vein puncture site in *Peromyscus maniculatus*. Photo: © Leticia Gutiérrez Jiménez. Figure 8 (right): After locating the puncture site, apply enough pressure to insert only the tip of the lancet into the cheek. Photo: MEDpoint International, Inc.

6. Quickly poke the cheek of the animal, applying enough pressure to insert only the tip the lancet, and withdraw immediately. A blood drop will form instantly. Approach the tube to collect the blood as it flows freely (Figures 7 and 8).

**Note:** To avoid sample contamination, **do not** rub or press the collection tube against the puncture site.



Figure 9: Collect blood into the vial as it flows freely, avoiding rubbing or pressing the tube against the puncture site. Photo: MEDIpoin International, Inc.

If you are not getting enough blood, try the following technique: When scruffing the animal, hold the skin on the back of its neck between your thumb and middle finger. When more blood is desired, put your index finger on the top of the rodent's head and gently move its head up and down. This will keep the wound open a little longer and pump more blood into the submandibular area and out the puncture site.

Blood may also be drawn from both cheeks at one sampling event if the amount of blood to be collected is large.

7. Stop the blood flow at any time by releasing the head/neck scruffing pressure point and applying a compress (gauze, cotton) to the puncture site for a few seconds (or 1-3 minutes to prevent hematoma formation), as needed.

**Note:** Always ensure complete hemostasis before returning the animal to its individual trap.

8. To avoid hypovolemic shock in small animals ( $\leq 200\text{g}$ ), fluid volume replacement should be administered subcutaneously after collecting all biological samples and immediately before placing the individual into its trap/recovery chamber. (See Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines)

**Note:** Recumbent or dehydrated individuals must be treated immediately and should **NOT be sampled**. Those animals should be released at the trap capture location as soon as they recover. To avoid dehydration and hypoglycemia, always provide a small apple or other fruit slice (1 apple = ~16 slices) as part of the bait mix **and** to each trapped individual while it is waiting to be processed.

### Lateral saphenous vein blood collection

Adapted from *Guide to the Care and Use of Experimental Animals*. Volume 1, Second Edition. University of British Columbia. For videos of this technique, please visit Blood sampling of the rat from the saphenous vein: <https://norecopa.no/films-and-slide-shows/mouse>

A minimum of two people are required for this type of blood collection in rodents: one for anesthetizing, handling and bleeding, and one for collecting the blood sample and data recording.

A volume of 100 $\mu$  -300  $\mu$ l can readily be collected using the lateral saphenous vein bleeding technique; however, the number of attempts to take a blood sample should be minimized. Staff should make **no more than three needle sticks in any one attempt**. Warming the animal immediately prior to blood collection will increase blood flow considerably.

### Step-by-step procedure

1. Choose the appropriate anesthesia protocol and secondary restraining technique for the species. Mechanic and manual restraint causes stress, and therefore the duration of restraint should be minimized. Where a restraint tube is used, it should be appropriate to the size of the rodent. All forms of restraining equipment should be frequently washed to prevent pheromonally-induced stress or cross-infection.
2. The animal is held head-first in a restrainer so that only the rear legs and tail are free. The rear leg can be stretched out to its natural position. The head, nose, and ear color should be monitored closely. Apply the isoflurane nose cone for a couple of seconds if needed. For larger – and feisty – rodents, it is recommendable to wrap the animal in a drape or towel in case of premature awakening.



Figure 10 (left): Mouse or vole restraint. Figure 11 (right): Rat or squirrel restraint. Photos: Guide to the Care and Use of Experimental Animals.

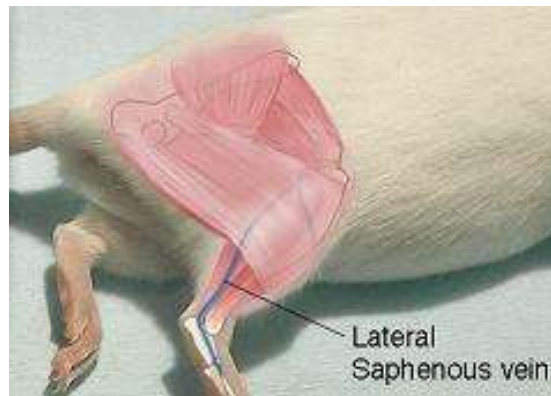
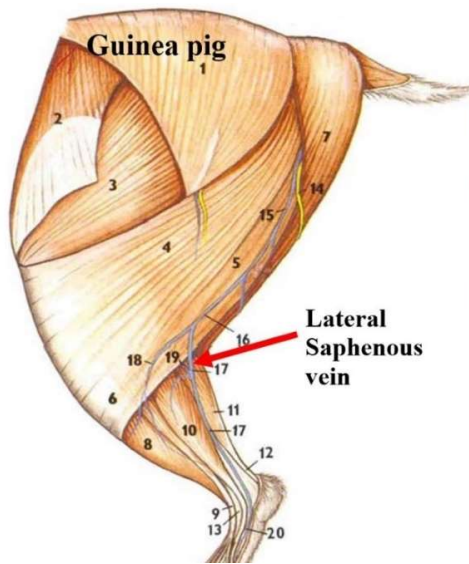
3. To secure the animal and elevate the vein, the skin on the upper thigh is gently but firmly squeezed, using the same hand holding the restrainer.



- The vein is easier to see when the fur is shaved either by clippers or by using scissors. A thin film of bland ointment, such as petroleum jelly or Glycerin, can be applied to prevent blood from seeping and allow for blood drop formation. If you are not shaving the area, find the vein by “combing” or clearing away the fur using a cotton-tipped swab that has been dipped in jelly.



Figures 12-14 (left to right): Clearing the fur on the mouse, the rat or squirrel and the vole. Figures 12 and 13: Guide to the Care and Use of Experimental Animals. Figure 14 Photo: © Leticia Gutiérrez Jiménez



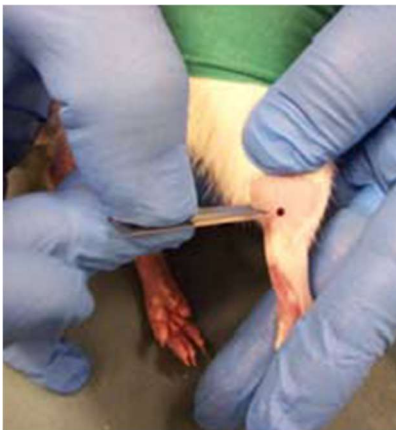
- Locate the vein.

Figure 15 (left): The lateral saphenous vein in the guinea pig. Credit: Guide to the Care and Use of Experimental Animals. Figure 16 (right): The lateral saphenous vein in the mouse. Credit: Theodora.com



Figure 17: Lateral saphenous vein in the Least chipmunk (*Tamias minimus*). Photo: © Leticia Gutiérrez Jiménez

- Using a **23-27 gauge needle or an appropriate depth size animal lancet**, puncture the vessel at a 90° angle at the most proximal visible aspect. Collect blood as it drips from the vein.



Figures 18-20 (left to right): Puncturing the lateral saphenous vein of the mouse, rat or squirrel, and vole. Figures 18 and 19: Guide to the Care and Use of Experimental Animals. Figure 20: © Leticia Gutiérrez Jiménez

Rodent taxa	Mouse (25g)	Hamster/Vole	Rat (250g)
Max. sample volume	200ul	0.5% bodyweight	1600ul
Needle size	27G-25G needle or lancet	25G needle or lancet	23G needle or lancet



7. Collect your sample.



Figures 21(left) and 22 (right): Collecting blood from the rat/squirrel and vole via the lateral saphenous vein. Figure 21: Guide to the Care and Use of Experimental Animals. Figure 22: © Leticia Gutiérrez Jiménez

8. Use a dry gauze pad and/or povidone-iodine prep pad to apply pressure to the puncture site as the pressure on the upper thigh is released. If bleeding does not stop, use a silver nitrate styptic pencil (moisten the pen slightly with sterile saline solution and then carefully place onto the puncture site). Silver nitrate will stop vigorous hemorrhage, but it stings sharply on the application site and should not be used habitually to stop minor bleeding.

9. In small animals ( $\leq 200\text{g}$ ), fluid volume replacement should be administered subcutaneously after collecting all biological samples, as described in Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines.

**Note:** Recumbent or dehydrated individuals must be treated immediately and should **NOT be sampled**. Those animals should be released at the trap capture location as soon as they recover. To avoid dehydration and hypoglycemia, always provide a small apple or other fruit slice (1 apple = ~16 slices) as part of the bait mix **and** to each trapped individual while it is waiting to be processed.

10. Remove the rodent from the restrainer and place it in its individual trap.

11. Monitor the animal for 5-10 min to ensure hemostasis (bleeding has stopped).

### 3.e.3 Techniques for Bleeding Rats and Larger Rodents

#### Lateral tail vein blood collection

1. Use a tourniquet. The veins are located on either side of the tail and are quite superficial.
2. Use a needle with the appropriate gauge (usually 27G or 25G), entering the skin at a shallow angle about one third down the length of the tail. **Be careful not to collapse the vein.**
3. Consider using a small syringe or hematocrit tubes to collect the blood.

4. In small animals ( $\leq 200\text{g}$ ) administer fluids after sampling as described above and in *Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines*.
5. Remove the rodent from the restrainer and place it in its individual trap.
6. Monitor the animal for 5-10 min to ensure hemostasis (bleeding has stopped).

### Ventral tail vein blood collection

1. Use a tourniquet, as above (lateral tail vein bleeding).
2. The vein is located both centrally and ventrally in the tail. The ventral tail vein is deeper than the lateral tail vein used in mice and it is not readily visualized. Take care not to bleed from the ventral caudal artery, which is sheathed by a thick fascia.
3. As with lateral tail vein bleeding, start one third of the way down the tail.
4. After bleeding, ensure hemostasis by applying pressure to site of bleeding using a cotton ball or gauze until bleeding ceases (approximately 1 minute).
5. Administer fluids as described above and in *Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines*.
6. Monitor the animal for recovery.

### Jugular vein blood collection

Jugular vein bleeding is recommended only for larger rodents (e.g., guinea pigs, chinchillas, etc.), and even then should be considered only if appropriate alternatives are impossible or have been unsuccessful. The technique requires a potentially challenging degree of animal restraint and a strenuous physical positioning of the animal to be sampled, which can be difficult with alert and resistant animals. A high level of competence is required in the animal sampling staff to avoid permanent harm to the animal.

1. On the anesthetized animal, clean and apply pressure to the jugular vein on one side.
2. Direct the needle into the vein and collect the sample.
3. After bleeding, ensure hemostasis by applying pressure to site of bleeding using a cotton ball or gauze until bleeding ceases (approximately 1 minute).
4. In the case of small animals or visibly dehydrated animals, administer fluids as described above and in *Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines*.
5. Monitor the animal for recovery.

### 3.f Necropsy Sample Collections

In case of accidental death before or during animal sampling, or where dead animals are available opportunistically for sampling, perform a necropsy examination and collect the samples described above. If euthanasia is required, follow your IACUC approved methods. We recommend following the [AVMA guidelines](#).

In all cases, samples going into VTM and Lysis/binding solution should be collected sterilely, limiting cross-contamination between tissues (see below). Collect as much blood as possible via cardiac puncture. In addition, collect **three** adjacent, approximately 200 mg (pea-sized) samples of the following tissues:

<b>priority</b>	<b>additional</b>
• Liver	• GI (duodenum, colon)
• Lung	• adrenal
• Spleen	• Ovary
• Kidney	• Testes
• Lymph node	• Pancreas
	• Other, if required

One specimen should be frozen in 500 µL VTM in a cryovial, one should be frozen in 500 µL Lysis/binding solution in a cryovial, and one should be stored at room temperature in a vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

To avoid cross contamination among individual specimens, follow sterile tissue collection technique.

1. Use clean scissors and tweezers (Clean instrument = wiped with 70% ethanol and flamed). Use scissors to cut skin and to open body cavities.
2. Open the abdominal and thoracic cavities carefully to prevent contamination between cavities and among organs.
3. Observe organs, noting abnormalities.
4. Dip scissor and tweezers in ethanol
5. Flame them
6. Collect the first tissue. Place a 5mm cube of tissue in Lysis/binding solution and a 1cm piece in VTM. Freeze in nitrogen dewar or -80C freezer. Repeat steps 4-6 for every tissue sample.
7. Collect a third sample and place in 10% formalin fixative. Store at room temperature.

## 4. References

[CDC guide \*Methods for Trapping and Sampling Small Mammals for Virologic Testing\* \(pp 15-18\)](http://www.cdc.gov/hantavirus/pdf/rodent_manual.pdf)  
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## Appendix I. Supply and Equipment List

Note: Supply details, availability, and vendor sources may vary.

### PPE

- Designated clothing (e.g. overalls or other clothes which can be put on before sampling and removed following sampling)
- Flexible face shield or other eye protection
- N95 or P100 respirator without valve
- Nitrile examination gloves
- Washable shoes
- Tyvek suit

### First Aid

- Betadine or (or benzalkonium chloride)
- First aid kit

### Data Collection

- Datasheets (Rodent sampling, Trap form)
- Pencils
- GPS

### Capture and Handling

- Sherman traps
- Flagging tape
- Leather gloves
- Manual restrainer
- Spring/electronic balance
- Dial/digital caliper/ruler
- Nestlets or similar bedding material for traps
- Hand warmers or similar hypothermia treatment method
- Large/gallon-sized Ziploc-style (resealable) plastic bags
- Chemical restraint / anesthesia requirements
- Metal tea ball and/or nose cone for anesthesia maintenance, and/or isoflurane vaporizer
- 20ml plastic tubes
- Cotton balls

- Camera
- Identification guides
- Apples or other fruit (food with high water content) to help prevent animal dehydration

### Sampling

- Clippers or scissors for hair / fur
- Glycerin or petroleum jelly
- Cotton-tipped swabs (for “combing” or clearing fur only, **NOT** for sampling)
- Processing trays
- Permanent lab markers for tube labeling
- Cryotubes
- Lancets
- Needles: 23G, 25G, 27G (Note: 20-22G needles may be used for facial bleeding after the technique has been mastered)
- Needles and syringes for blood draws
- Insulin syringe (27G-29G, x ½ inch, 1.0 ml) for fluid replacement
- Lactated Ringer’s or 0.9% NaCl solution for fluid replacement
- Sterile swabs (dacron/polyester-tipped), appropriate sizes for sampling needs
- Sterile saline
- 2inch x 2inch gauze pads
- Povidone-iodine, 70% Isopropyl Alcohol, or Benzalkonium Chloride prep pads
- Silver nitrate styptic pencil
- Cryo resistant tube labels
- Cryovial rack
- Cryoboxes and dividers
- Plastic hematocrit tubes
- Plastic vacutainers (EDTA and dry)
- Small vials or jars for tissue samples (necropsy sampling only)
- Pipettors and disposable tips
- Portable centrifuge for vacutainers
- Portable centrifuge for hematocrit tubes

- Cryo Gloves
- Fine Point Forceps
- Scissors
- Dissection kit
- 70% ethanol for sterilizing necropsy instruments
- Alcohol burner for sterilizing necropsy instruments and/or sterilizing scissors to cut swabs
- Lysis/Binding buffer
- Viral Transport Medium (VTM)
  
- Buffered formalin

- 95% ethanol
- Lighter
- Liquid nitrogen shipper/liquid nitrogen

**Waste Disposal and Decontamination**

- Paper towels
- Sharps containers
- Bleach
- 95% ethanol
- Biohazard bags
- Sprayers

**Supply References**

Goldenrod lancets: [http://www.medipoint.com/html/for\\_use\\_on\\_mice.html](http://www.medipoint.com/html/for_use_on_mice.html)

Microcapillary tubes with caps:

<https://www.fishersci.com/us/en/catalog/search/products?storeId=10652&nav=122540&sortBy=default&keyword=Microvette+Capillary&searchType=PROD&SWKeyList=%5B%5D&typeAheadCat=>

Microtainers with microgard closures: <https://www.fishersci.com/shop/products/bd-microtainer-capillary-blood-collector-bd-microgard-closure-7/p-178764#tab2>

Capillary blood collection tubes: <https://www.fishersci.com/shop/products/terumo-capiject-capillary-blood-collection-tubes-8/p-178577>

Nestlet bedding material: <http://www.ancare.com/our-products/nestlets-ancare>



## Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines

- a) To minimize contamination, choose the smallest fluid vial volume (i.e. 10mL) of Lactated Ringer's (preferred) or 0.9% NaCl solution. Warm the solution up to ~37°C/100°F.
- b) Check the expiration date of fluid vial/bag. Visually inspect the vial integrity and fluid aspect (discoloration, haziness, crystallization, or particulate matters). Discard any contaminated, punctured or cracked vials/bags.
- c) Select an injection site in the loose skin over the animal's neck or flank that has no evident skin nor tissue damage.
- d) Wipe the chosen area with a 70% Isopropyl Alcohol or Benzalkonium Chloride prep pad.
- e) For small rodents, use an insulin syringe (27G-29G, x ½ inch, 1.0 ml), insert the needle 5-10 mm through the skin before making the injection. Lack of resistance to the injection is indicative that you are in the right location.
- f) Depending on individual size, inject subcutaneously 0.5-1ml (respectively for a juvenile and adult mouse) sterile fluid (see Figure below). Small rodents (~25gr) can safely receive up to 2-3ml subcutaneously and larger rodents (~200gr) up to 3-5ml.
- g) Check for leaking from the injection site, especially if a larger volume is injected.
- h) Discard syringe in sharps container.
- i) You can draw multiple fluid doses from the same vial/bag used during the same sampling day. **Discard any opened fluid vial at the end of the sampling day, even if it is not empty.**



Replace fluids after collecting blood from rodents. Photo credit © Leticia Gutiérrez Jiménez