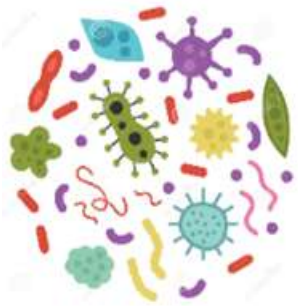

Fever project

Déterminer les causes des maladies fébriles en Guinée

PRACTICAL GUIDE TO LIVE SAMPLING OF LIVESTOCK AND WILDLIFE FOR INFECTIOUS DISEASE SURVEILLANCE

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TABLE OF CONTENTS

1. PREFACE.

Under- or misdiagnosed cases of disease caused by especially dangerous pathogens present public health and proliferation risks. Numerous studies have demonstrated that in low-resource settings, and particularly where malaria is endemic, patients presenting to healthcare facilities with febrile illness may not receive an accurate diagnosis due to, among other reasons, shortcomings in differential protocols and lack of available diagnostic tests (Maze et al. 2018; Halliday et al. 2020). In Guinea, when patients present at the community or prefectural level with febrile illness, weaknesses in clinical differential protocols usually result in the patient being diagnosed with a common infection, notably malaria and occasionally typhoid, with no further testing performed. As such, acute febrile illness presents an unquantified and undifferentiated burden to the Guinean healthcare system. The specific challenge for public health authorities is that pathogens causing febrile episodes in the population, and the zoonotic transmission pathways that may drive them, have not been systematically nor comprehensively identified (Kourouma et al. 2022).

Fever Project is a three-year research project (from September 2021-2024, with the possibility of two additional option years through September 2026) funded by DTRA (grant no. HDTRA12110028). This work has been reviewed and approved by both the CNERS in Guinea (064/CNERS/23) and IRB of Georgetown University in the USA (STUDY00002481). It involves scientists from Guinea, USA, and UK, with the objective to build capacity for threat reduction in Guinea and improve health security through an integrated approach to human and animal health for the identification of high-consequence aetiologies of acute febrile illness in humans. The project's goals are to:

1. Identify the causative agents of acute febrile illness in humans in Guinea.
2. Uncover evidence for transmission of high-consequence zoonotic pathogens associated with acute febrile illness.
3. Investigate risk factors for acute febrile illness in humans, including exposure to domestic and peri-domestic animals.
4. Improve capacities for the detection of high-consequence pathogens associated with acute febrile illness.

Across Guinea, and West Africa as a whole, major anthropogenic interventions are rapidly driving large-scale deforestation, conversion of savannah to pasture and cropland, and re-routing or damming of waterways. These changes have been shown to be associated with altered interactions between the environment, humans, and animals within these communities, as well as increased risks of emergence or transmission of zoonotic pathogens

(Allen et al. 2017; Plowright et al. 2021). Among its overarching aims, Fever Project's objective is to uncover evidence of circulating zoonotic pathogens directly responsible for acute febrile illness in humans. Therefore, we are collecting and analysing samples from human communities, their domestic livestock (i.e., ruminants, poultry, and dogs), and wildlife living in close proximity to these communities (i.e., bats and small terrestrial mammals). Various sites within the prefectures of Dalaba and Guéckédou in Guinea have been selected: these localities represent a gradient of deforestation and human pressure, from highly forested areas with lower human density to lowly forested grassland at higher elevation and more densely inhabited. Sites within both prefectures are sampled twice, both during the dry season (from November to May) and the rainy season (from June to October).

The animal multi-species approach and the procedures detailed in this manual must be evaluated within the contexts of the Fever Project and its sampling localities across Guinea. We have strived to provide accurate and practical workflows that could be applied to any future field-based studies focusing on multi-host pathogen transmission. The domestic and wild animals depicted by photographs taken during field activities within the Fever Project are used with the sole purpose of illustrating sampling procedures. We thank Dr Marc Valitutto, Dr Claire Standley, Dr Ellen Carlin, and Brian Samuelson for these enlightening pictures.

As a team, we have learned every day from local communities and institutions supporting the Fever Project and making its realisation possible. Our list of authors is long, but every member has played a pivotal role in the development of this manual. We also appreciate the expertise, advice, and support provided by various scientific and veterinary subject matter experts, including Professor Alpha Camara, Dr Jean DeMarco, Dr Jim Desmond, and Dr Jonathan Epstein. We are immensely grateful to the DPS and DPE of Dalaba and Guéckédou, and to the communities within the prefectures of Ditinn, Koundou, Mitty, and Termessadou-Djibo for their friendly welcome and engagement. Furthermore, we thank our project partners and collaborators at CNFRSR, EcoHealth Alliance, INSP, ISSMV, and LFHVG. We acknowledge the assistance from the ANSS in supporting epidemic surveillance efforts in Guinea and the CNERS for granting ethical approval to the Fever Project. In addition to these specific entities, our sincere appreciation goes to the governmental MAGEL, MSHP, MEDD, and MESRSI for their overall support of One Health research and capacity strengthening in Guinea, including the specific guidance, advice, and input that their officials have provided to the Fever Project throughout its conception and implementation. Lastly, we thank the whole team at Santé Plus Organisation for their outstanding efforts on all aspects of the Fever Project.

List of abbreviations

- **ANSS:** Agence Nationale de Sécurité Sanitaire.
- **CAT:** Category.
- **CNERS:** Comité National d'Éthique pour la Recherche en Santé.
- **CNFRSR:** Centre National de Formation et de Recherche en Santé Rurale.
- **DPE:** Direction Préfectorale de l'Élevage.
- **DPS:** Direction Préfectorale de la Santé.
- **DTRA:** Defense Threat Reduction Agency, United States Department of Defense.
- **FBSL:** Field Biosafety Level.
- **INSP:** Institut National de Santé Publique.
- **IRB:** Institutional Review Board.
- **ISSMV:** Institut Supérieur des Sciences et de Médecine Vétérinaire.
- **LFHVG:** Laboratoire des Fièvres Hémorragiques Virales de Guinée.
- **MAGEL:** Ministère de l'Agriculture et de l'Élevage.
- **MEDD:** Ministère de l'Environnement et du Développement Durable.
- **MESRSI:** Ministère de l'Enseignement Supérieur, de la Recherche Scientifique et de l'Innovation.
- **MSHP:** Ministère de la Santé et de l'Hygiène Publique.
- **PPE:** Personal Protective Equipment.

2. APPLIED BIOSAFETY AND PPE.

The nature of the activities within the Fever Project, particularly the direct interaction with animals which may carry high-risk infectious disease agents, translated into the implementation of biosafety rules whose main goal is to ensure that sampling procedures are performed in a safe and ethical manner for both the personnel and the animals involved. Therefore, fieldwork staff within the Fever Project received specific training in human subject protection, animal welfare, biosecurity, and biosafety. This essential knowledge was applied to building risk assessments and, as a response, ad-hoc biosafety measures for the conditions that the fieldwork team would be exposed to as part of the Fever Project. The information provided in this section is a brief overview of FBSL, animal sampling associated, and the CAT of PPE required. The guidance offered herein is extracted from *Field Biosafety Manual* by Dr Marc Valitutto (in preparation), which we strongly encourage to consult when designing animal sampling activities as part of a research study.

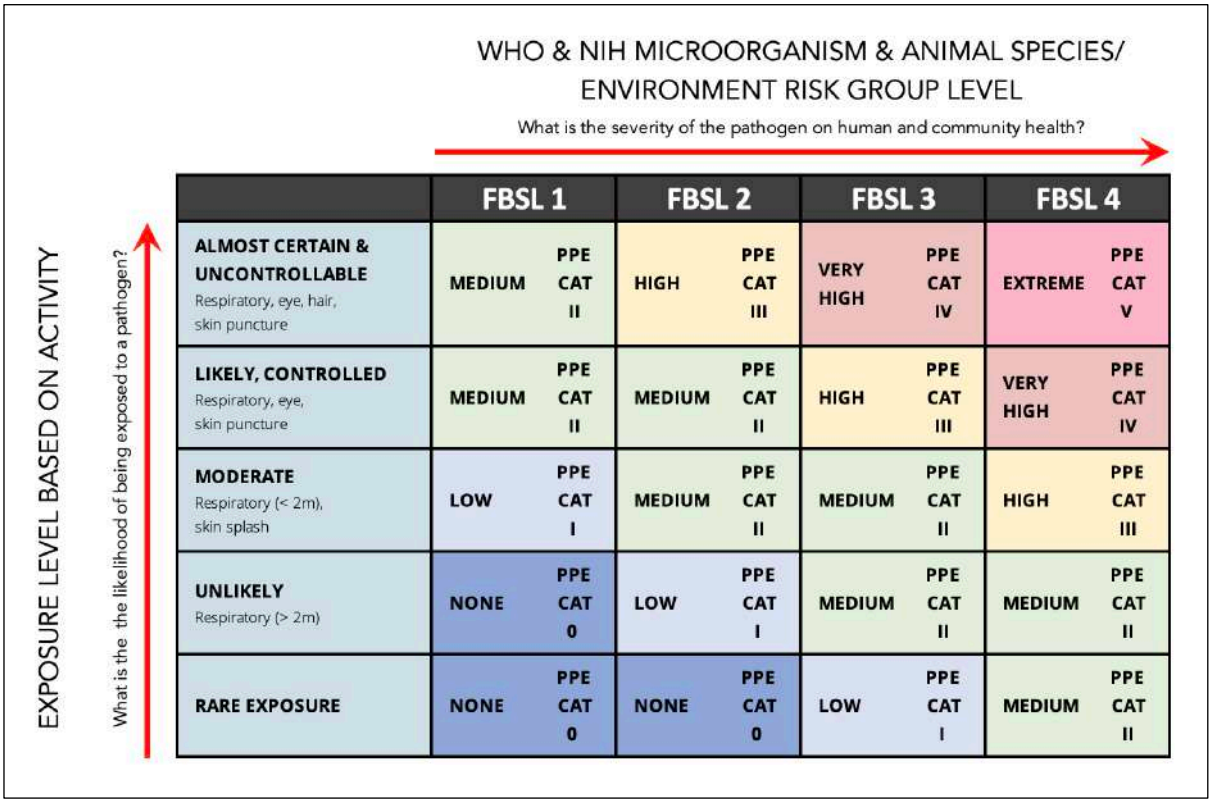

Figure 2.1. How to determine which CAT of FBSL, and therefore PPE, is applicable based on the risk of exposure when conducting activities (y-axis) and the potential implications of the risk of exposure to human and community health (x-axis) (modified from: *Field Biosafety Manual* (Valitutto, in preparation)).

2.1. FBSL AND PPE.

All fieldwork activities involving animal sampling within the Fever Project adhere to FBSL guidelines for applicable PPE and setting up the processing station. The selection of the

appropriate FBSL for the different procedures was partially based on the risk classification system for infectious diseases detailed by World Health Organization and National Institutes of Health. This classification combines various factors such as pathogen virulence, transmission mode, occupational health, hygiene measures, and availability of preventive care and effective treatment (**Figure 2.1**). This risk classification system was integrated by information contained in *Field Biosafety Manual* (Valitutto, in preparation) on inherent risks of pathogen circulation in specific animal populations and environments.

The careful assessment of substantial and potential risks within the Fever Project determined the implementation of FBSL 2 with PPE CAT 0 to CAT III for all activities involving domestic animals (i.e., ruminants, poultry, and dogs). However, FBSL 2 may be raised to FBSL 3, or even FBSL 4, if sampling procedures on domestic animals become part of zoonotic disease outbreak investigations locally. In contrast, it has been deemed necessary to strictly apply FBSL 3 with PPE CAT I to CAT IV for all activities involving wildlife (i.e., bats, rodents, and shrews). As stated above, the recommended level of protection depends on the specific role within the team and whether field activities are carried in controlled settings (**Table 2.1**).

Table 2.1. CAT of FBSL and PPE applied to personnel during animal sampling activities within the Fever Project.

FBSL	Scenario	PPE	Example
2	Biological sampling of medium-risk livestock	0	Personnel and observers at ≥2m distance
		I	Personnel partially involved in fieldwork activities at ≥2m distance
		II	Personnel handling/sampling animals or at <2m distance
		III	Personnel conducting post-mortem examination
3	Biological sampling of high-risk wildlife	I	Personnel and observers at ≥2m distance
		II	Personnel partially involved in fieldwork activities at ≥2m distance or transporting small mammals inside cloth bags or traps
		III	Personnel handling/sampling animals, conducting post-mortem examination, or at <2m distance
		IV	Personnel conducting fieldwork activities under bat roosting/foraging site
4*	Biological sampling during outbreak or mortality event(s) caused by high-risk or unidentified pathogens	II	Personnel and observers at ≥2m distance
		III	Personnel partially involved in fieldwork activities at ≥2m distance or transporting small mammals inside cloth bags or traps
		IV	Personnel handling/sampling animals, conducting post-mortem examination of small animals, or at <2m distance
		V	Personnel conducting fieldwork activities under bat roosting/foraging site or conducting post-mortem examination of large animals

* FBSL 4 is only applicable during outbreak investigations known or suspected to be caused by a high-risk zoonotic pathogen. To date, Fever Project's personnel has never undertaken animal sampling activities as a response to an outbreak.

After determining the applicable FBSL to the different fieldwork activities within the Fever Project, we assessed objective and perceived risks of exposure to zoonotic pathogens to select the appropriate PPE CAT (**Figure 2.2**):

CAT	MANDATORY AND FACULTATIVE PPE
PPE CAT 0	   Personal clothing and shoes (short sleeves and short trousers allowed).
PPE CAT I	  +/- Mandatory: long sleeves and long trousers (dedicated clothing advised). Facultative: impermeable boots or shoe covers.
PPE CAT II	  +/-  +/-  +/-  +/- Mandatory: dedicated clothing (i.e., long sleeves, long trousers, and shoes) and one layer of nitrile gloves. Facultative: impermeable boots or shoe covers, two layers of nitrile gloves, respirator, and safety glasses.
PPE CAT III	       Mandatory: dedicated clothing (i.e., long sleeves and long trousers), impermeable boots or shoe covers, apron, sleeves, two layers of nitrile gloves, N95 or equivalent respirator, and safety glasses.
PPE CAT IV	      Mandatory: dedicated clothing (i.e., long sleeves and long trousers), hooded coveralls, impermeable boots, two layers of nitrile gloves, N95 or equivalent respirator, and indirectly vented or ventless goggles.
PPE CAT V	     Mandatory: dedicated clothing (i.e., long sleeves and long trousers, hooded coveralls, impermeable boots, two layers of nitrile gloves, and powered air-purifying respirator.

Figure 2.2. PPE CAT applicable to the various FBSL during animal sampling activities (modified from: *Field Biosafety Manual* (Valitutto, in preparation)).

- **Rare exposure: PPE CAT 0 for FBSL 2 conditions or CAT I for FBSL 3.** Personnel and members of the public minimally involved in fieldwork activities (e.g., chauffeurs, porters of clean supplies, and observers standing at ≥2m distance).
- **Unlikely exposure: PPE CAT I for FBSL 2 conditions or CAT II for FBSL 3.** Personnel partially involved in fieldwork activities and likely to interact with team members practicing a higher PPE CAT (e.g., field assistants standing at ≥2m distance).
- **Moderate exposure: PPE CAT II for either FBSL 2 or FBSL 3 conditions.** Personnel moderately involved near the processing station and manipulating animals in controlled settings (e.g., transporting wild small mammals contained inside cloth bags or traps, field assistants, data collectors, and observers standing at <2m distance).
- **Likely, controlled exposure: PPE CAT II for FBSL 2 conditions or CAT III for FBSL 3.** Personnel only, directly involved in restraining, handling, and/or collecting biological specimens of animals (e.g., controlled manipulation/handling, anaesthesia, and post-mortem examination of small domestic and wild animals).
- **Almost certain, uncontrollable exposure: PPE CAT III for FBSL 2 conditions or CAT IV for FBSL 3.** Personnel only, directly involved in activities which make the contact with animals, and/or their biological fluids, suspected to harbour high-risk pathogens certain and unavoidable (e.g., work under a roosting or foraging site amid a large colony of bats, work indoors amid a large colony of rodents, uncontrolled handling of large or fractious animals, and post-mortem examination of large domestic and wild animals).

2.2. PREPARATION OF THE PROCESSING AND DONNING/DOFFING STATIONS.

☐ Large basin with water and soap
☐ Large basin with water and bleach
☐ Bucket with water for handwashing station
☐ Bucket for biohazardous waste bags
☐ Biohazard bag (yellow) for dedicated clothing
☐ Small basin for safety glasses or goggles
☐ Biohazard bags (red) for waste
☐ Tarp
☐ Spray bottle with bleach
☐ Spray bottle with 70% ethanol
☐ Scrubs for impermeable boots
☐ Folding chairs
☐ Folding table

The processing station simply consists of folding table and chairs which, after the end of animal sampling activities, are easy to decontaminate as detailed in **Section 8.1**. When FBSL 3 is applied (i.e., wildlife sampling within the Fever Project), the table is covered with one layer of plastic (i.e., yellow biohazard bags) fixed by masking tape. The donning/doffing

station should be situated in proximity of the processing station for ease of access (**Figure 2.3**).

Figure 2.3. Processing station (A) and donning/doffing station (B) under FBSL 3.

The lists of procedures and supplies included herein are basic components for setting up the donning/doffing station before the start of animal sampling activities within the Fever Project. Items and their placement can be adapted based on the personnel's workflow, landscape features, weather conditions, and the applied FBSL. The steps for setting up the donning/doffing station are the following (**Figure 2.4**):

1. Lay out the tarp on a level surface within proximity of the processing station (the tarp is meant to remain clean of debris so that shoeless feet may be protected from the ground).

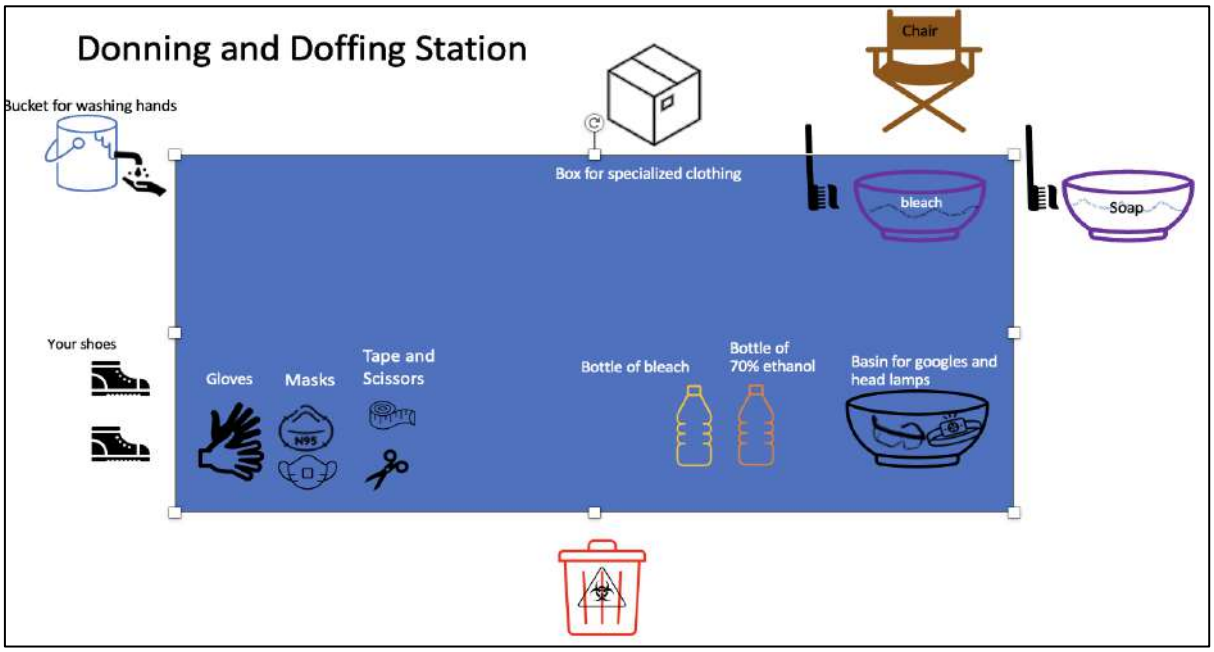

Figure 2.4. Schematic representation of a donning/doffing station (modified from: *Field Biosafety Manual* (Valitutto, in preparation)).

2. Place the handwashing station (i.e., a bucket with tap filled with water and a soap bar) near the tarp.
3. Place two large basins with water side-by-side and next to the tarp:
   a. Add soap to one basin for the removal of debris from the impermeable boots.
   b. Add sodium hypochlorite at 3% concentration (i.e., bleach) to the other basin for the decontamination of the impermeable boots and any cloth drawstring bags used during sampling activities (place this basin closest to the tarp for stepping on it immediately after doffing the impermeable boots).
4. Place scrub brushes with long handles in each of the two large basins.
5. Place a folding chair next to the large basin with water and bleach to assist with doffing the impermeable boots.
6. Place two spray bottles, one with bleach and the other with 70% ethanol, next to the tarp for their use during decontamination and doffing of PPE.
7. Place a bin with a biohazard bag (red) lining next to the tarp for the disposal of contaminated PPE and biohazardous waste.
8. Place a small basin on the tarp for the decontamination of safety glasses or goggles and headlamps.
9. Place a bin with a biohazard bag (yellow) lining next to the tarp for the collection of contaminated dedicated clothing after their removal.
10. Lay out aprons, sleeves, and all PPE based on the applied CAT and FBSL (including gloves, scissors, and masking tape) near each other on the tarp.
11. Wear PPE in the exact order illustrated in **Figure 2.5**, **Figure 2.6**, and **Figure 2.7** for each PPE CAT (when wearing two layers of nitrile gloves, use masking tape to join the edges of sleeves and internal gloves).

Figure 2.5. Step-by-step guide for donning PPE CAT II (modified from: *Field Biosafety Manual* (Valitutto, in preparation)).

Figure 2.6. Step-by-step guide for donning PPE CAT III (modified from: *Field Biosafety Manual* (Valitutto, in preparation)).

Figure 2.7. Step-by-step guide for donning PPE CAT IV (modified from: *Field Biosafety Manual* (Valitutto, in preparation)).

3. BAT SAMPLING METHODS.

Before setting traps around a new site, carefully communicate Fever Project's objectives with the local community, including the rationale behind sampling bats. The team can start exploring the environment and planning where to capture only after receiving clear consent from the community, including reassurance that the public will not stand too close to the capture site and the processing station, permission to conduct trapping activities over multiple consecutive nights (if required), and acceptance to release each captured bat at its point of capture. In case of a negative answer by the community, bat trapping activities cannot proceed for that specific site.

3.1. PROTOCOL FOR SETTING MIST NETS.

☐ FBSL 3

☐ Triple high mist net pole system

☐ Two mist nets 9m

☐ Pickaxe

☐ GPS

☐ Clipboard/Folder

☐ Data collection sheet

☐ Pencil

☐ Mobile phones

Personnel directly executing the protocol apply PPE CAT II. The only trapping technique applied within the Fever Project utilises the triple high mist net system with two 9m mist nets (**Figure 3.1**) opportunistically placed nearby known sites where bats forage or roost.

Figure 3.1. Setting up the triple high mist net pole system.

1. Set the triple high mist net pole system and mist nets before dusk (place one net on the first pole to determine where to dig a hole in the ground for the second pole).
2. After completing the system's setup, always check its stability and proper functioning.
3. Record the trap-night date and time, administrative location, landscape category (e.g., village, farm, forest), and generic GPS coordinates of the study site on the appropriate data collection sheet.
4. Repeat trapping at the same site for a second consecutive night if the first night yielded <50 captures.

Advice box
• Check the weather forecast before fieldwork and avoid capture activities when heavy rainfall and wind gusts are predicted during the rainy season (from June to October), or extreme heat during the dry season (from November to May). Adverse weather conditions may impact animals' survival during trapping. • On full moon nights, bats may be less active since their foraging activity may be negatively correlated with moonlight intensity (Saldaña-Vázquez & Munguía-Rosas 2013). Therefore, avoid trapping activities during full moon nights.

3.2. PROTOCOL FOR INSPECTING MIST NETS.

☐ FBSL 3	☐ Clipboard/Folder	☐ Marker pen
☐ Cloth drawstring bags	☐ Data collection sheet	☐ Pencil
☐ Leather gloves	☐ Coloured masking tape	☐ Crochet hooks
☐ Headlamp	☐ Mobile phones	

Personnel directly executing the protocol apply PPE CAT III. The mist nets are checked as soon as possible after dusk and equipped with adequate numbers of clean and dry cloth bags, leather gloves, and fine scissors. Each member of staff focuses on delicately releasing each captured bat into a separate cloth bag. In case of difficulty when freeing the animal, support from a second team member and use of a crochet hook to loosen the entangled net should be sought.

1. Each member of staff focuses on delicately releasing each captured bat into a separate cloth bag, holding the head and/or body with the gloved hand while delicately freeing the wings with the other.
2. Immediately label the cloth bag containing captures by sticking masking tape to the bag and noting the animal identification number on it, then carefully hang all the cloth bags containing captures from a line in a secluded area by ordering them based on the timing of capture (to ensure time lapse <4h between capture and release).
3. If a sufficient number of captures is reached (i.e., between 20 and 40 depending on the fieldwork team's experience), lower the mist nets to avoid any excess captures, then start the sampling procedures as detailed in **Section 3.3**. If the number of captures is low, keep the mist nets active and check them every 15min for a maximum of 4h while getting started with the sampling procedures.
4. Record the time the trapping activity ends on the data collection sheet.

Advice box

- Carefully check the direction of entry when freeing each bat from the mist net since it will greatly facilitate release.
- When capturing mothers with their pups, be cognisant not to displace the pup during manipulation and do not sample the pup.
- The application of PPE CAT IV, as detailed in **Section 2**, becomes necessary when exposure to a high number of bats within certain environments, such as bat roosts in a cave or a forested area, and, therefore, contact with their excreta is unavoidable (**Figure 3.2**).

Figure 3.2. Applying PPE CAT IV in a cave where bats roost.

3.3. PROTOCOL FOR HANDLING, SAMPLING, AND DATA COLLECTION.

☐ FBSL 3	☐ Clipboard/Folder	☐ Swabs large
☐ Cloth drawstring bags	☐ Data collection sheet	☐ Swabs small
☐ Leather gloves	☐ Pen/Pencil	☐ Needles 25-27g
☐ Headlamp	☐ Marker pen for tubes	☐ Syringes 1mL
☐ Callipers	☐ Forceps for tick removal	☐ Syringe 3mL
☐ Spring balances 100-500g	☐ Coloured tube stickers	☐ Pipette 100-1,000µL
☐ Scissors	☐ Disinfection wipes	☐ Pipette 20-200µL
☐ DNA/RNA Shield™	☐ Sterile saline solution	☐ Pipette tips
☐ Whatman™ 903 cards	☐ Fruit juice	☐ Non-toxic nail polish
☐ Tubes 1.5mL	☐ Smartphone or camera	

Personnel directly executing the protocol apply PPE CAT III. The handling and sampling activities are performed on one individual capture at a time.

Figure 3.3. Manipulation of a bat from inside the cloth bag.

1. Place the different tube stickers (i.e., blue, brown, red, and green) on the 1.5mL tubes and pipette 500µL DNA/RNA Shield™ into each (see **Appendix C**).
2. Prior to retrieving every single bat, check that all bats are alive by gently agitating each bag for an activity response. Bats with low activity should be checked visually for health. Prioritize sampling of the lower activity bats first if they are deemed able to withstand handling.
3. Carefully weigh the bat into its cloth bag using the appropriate 100g or 500g spring balance.
4. While the animal is in the bag, record the following information on the data collection sheet: initials of the transcriber, date, species (see **Section 3.4**), animal identification number, and weight including cloth bag.

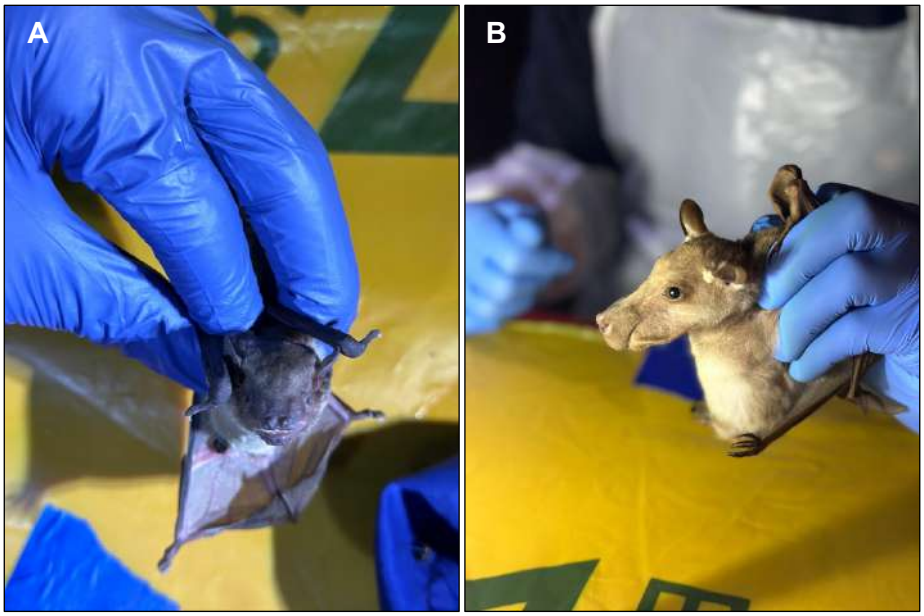

Figure 3.4. A bat restrained via wing hold for sampling purposes.

5. Bat handling can be performed by either:
   a. Keeping the bat within the bag and gently restraining it while only exposing each anatomical feature as needed for data and sample collection (**Figure 3. 3**).

b. Carefully removing the bat from the bag and restraining its wings by holding both in one hand, closest to the animal's shoulders. The index finger separates the wings (**Figure 3.4**).

Figure 3.5. A tick (highlighted by the red arrow) attached to the head of a bat during sampling procedures.

6. While restraining the animal, collect the following data:
   a. Identify sex, reproductive status, and age by inspecting its genitalia, abdomen, and wings (see **Table 3.1** for definitions; if uncertain about age or sex, include "?" in the record and try explaining the uncertainty in the **Section "Notes"** of the data collection sheet).
   b. Evaluate the presence and number of ectoparasites (i.e., ticks, fleas, and/or lice) by visually inspecting its body (**Figure 3.5**). If any are present, remove a known number using forceps and place them into a green-top 1.5mL tube. Label the tube both at its top and side.
   c. Measure forearm/radius length using the callipers (**Figure 3.6**).

Table 3.1. Codes relative to age (J for juveniles and A for adults) and reproductive status of the sampled female (F) and male (M) bats.

Age F	Reproductive status F	Age M	Reproductive status M
J	N (not reproducing; the vagina is imperforated and the fusion of the phalangeal symphysis is incomplete)	J	AB (abdominal testes; testes are not fully descended/visible and the fusion of the phalangeal symphysis is incomplete)
A	N (not reproducing; the vagina may be perforated but the fusion of the phalangeal symphysis is complete)	A	AB (testes are neither abdominal nor fully descended/visible but the fusion of the phalangeal symphysis is complete)
	E (pregnant; the abdomen is swollen and nipples are prominent)		SC (scrotal testes; testes are descended and large and the fusion of the phalangeal symphysis is complete)
	L (lactating; the nipples are prominent and a pup may be clinging to the dam)		

7. While restraining the animal, swab the animal to collect the following samples (**Figure 3.7**):
   a. One oral swab using a large sterile polyester-tipped swab. Then, place the swab's tip into a blue-top 1.5mL tube and cut the swab's shaft using scissors wiped with 70% ethanol. Label the tube both at its top and side.

b. One rectal swab using a small sterile polyester-tipped swab moistened with few drops of sterile saline solution. Then, place the swab's tip into a brown-top 1.5mL tube and cut the swab's shaft using scissors wiped with 70% ethanol. Label the tube both at its top and side.

8. Release the animal into its cloth bag, then gently restrain its body while only exposing one wing (**Figure 3.8**) to collect blood samples:

Figure 3.6. Measurement of forearm/radius length.

Figure 3.7. Swabbing the oral (A) and rectal (B) cavities.

a. Locate the brachial vein dorsally to the elbow joint and clean the surface with a disinfection wipe; quickly poke the vein with a sterile 25g needle (use a 27g needle for animals ≤30g); a blood drop will form instantly if the brachial vein is penetrated.

b. Immediately collect blood drops using the 20-200µL pipette fitted with a sterile tip and set at 50ul. Fill a circle of the Whatman™ 903 card first, then expel ≤100µL into a red-top 1.5mL tube. Label the tube both at its top and side. Label the blood spot.

c. Ensure haemostasis by applying pressure to the site for 30sec to 1min using a cotton ball (if the artery is hit instead of the brachial vein, the blood flow is rapid and, in this case, haemostasis may take longer; in such cases the operator must patiently wait for >1min without constantly checking for stoppage of blood flow).

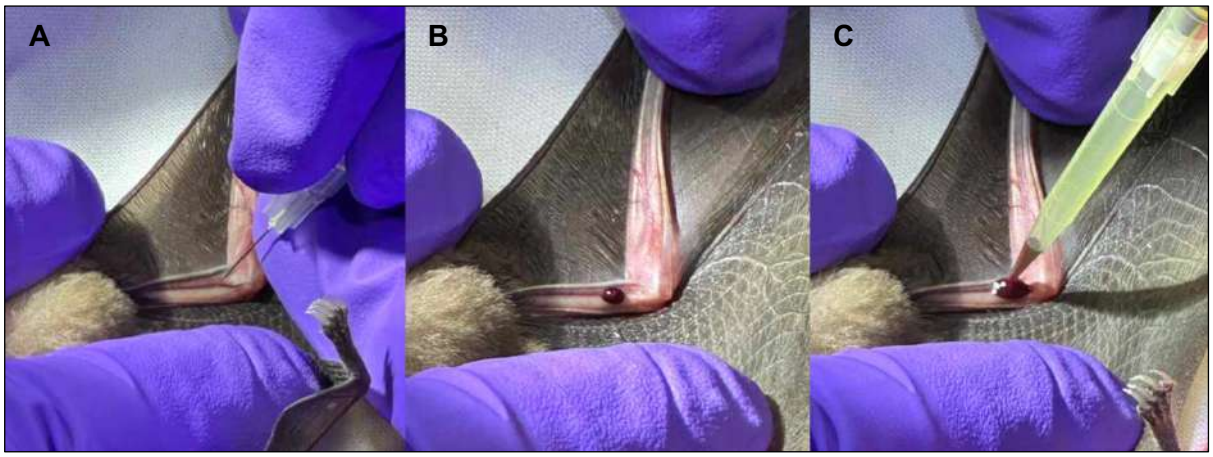

Figure 3.8. Blood collection by puncture of the brachial vein.

⚠ If unsuccessful, or more blood is necessary, blood collection can be tried twice on each wing, but it will stop after the fourth trial or before then if the animal appears fatigued.

9. Release the animal into its cloth bag, then gently restrain its body while only exposing the head and offer 20µL/1g fluids (e.g., 1mL of fluids for 50g individual) using a syringe without needle (**Figure 3.9**):
   a. Fruit juice for frugivorous bats.
   b. Saline solution for insectivorous bats.
10. Use a non-toxic nail polish or permanent marker to mark the animal's wing with a dash in order to avoid sampling re-captures during the following night(s) of fieldwork.
11. Check with the transcriber that the data collection is completed.
12. Ensure that the animal appears active and alert into its cloth bag, then carefully carry it at the point of capture (or within 100m from it), open the bag, and let the animal exit and fly away.
    a. If it does not, administer sterile saline solution subcutaneously as described in **Section 9.1**.
13. Weigh the empty cloth bag using the 100g spring balance. Communicate the information to the transcriber and check with them that the data collection is completed.
14. Between one animal and the other, ensure to:
    a. Clean the processing station by using 70% ethanol on areas that have been in contact with the animal's body and its fluids.
    b. Dispose of labelling tape, debris, and faeces from the dirty cloth bag into the biohazard bag.

Figure 3.9. Administering oral hydration.

c. Place the dirty cloth bag into the basin with water and bleach prepared for boot decontamination.
d. Use 70% ethanol to wipe the scissors used for cutting the swabs' shaft.

15. At the end of the sampling process, ensure to:
    a. Decontaminate the processing station and dispose of PPE as detailed in **Section 8.1** and **Section 8.2**.
    b. Take a photo of the completed data collection sheet as a digital record.
    c. Transport and store the collected specimens as described in **Section 10.2**.

Advice box
• If personnel find that larger frugivorous bats, such as *Hypsignatus monstrosus* (**Section 3.4**), are intimidating to directly handle, it is recommended to anaesthetise these animals as detailed for small mammals in **Section 4.3**. The anaesthesia procedure applies the same biosafety and sampling protocol detailed above. • General guidance for ageing bats is to observe their open wing against artificial light to verify whether cartilage (in juveniles) or fused bones (in adults) form the phalangeal symphysis. • The oral swab should be gently inserted without forcing it and allowing the animal to chew on it for 15sec. • The rectal swab should be gently inserted but, if difficult to insert, it must be avoided due to the risk of rectal prolapse. Once inside the rectum, apply a rotational movement to the swab for 15sec. • The total amount of blood collected from each animal must not exceed 0.75% of its weight (e.g., ≤200µL blood for 25g individual; one blood drop is approximately 50µL). • Styptic powder or gel may be applied to ensure haemostasis after blood collection, especially when puncture of an artery may have occurred. • When administering fluids, allow the bat to drink without forcing them and stop when intake is refused.

3.4. BAT IDENTIFICATION KEYS.

The chiropterans listed herein (**Figure 3.10**) were selected based on pilot data collected as part of the Fever Project, in addition to occurrence data obtained from the Global Biodiversity Information Facility database (www.gbif.org). This limited list is meant to provide guidance to fieldwork personnel for a presumptive morphological identification of the captured individuals rather than being a comprehensive taxonomic guide of West African bats (consult *Flying Mammals: Quick ID Guide to the Bats of Africa* (Stuart & Stuart 2021) for more information).

3.4.1. Fruit bats.

1. Collared fruit bat (genus *Myonycteris*). Weight: 35-80g. Forearm length: 55-70mm. Brown bats with pointed ears and, in males, broad shoulder ruffs resembling a collar. *Myonycteris* bats roost singly or in small groups in dense, low vegetation. *Myonycteris leptodon* and *Myonycteris torquata* are considered distinct species and may both occur across Guinea.
2. Egyptian fruit bat (*Rousettus aegyptiacus*). Weight: 83-170g. Forearm length: 82-106mm. Large frugivore bat with black wings and brown-to-grey fur, *R. aegyptiacus* roosts in caves and flies long distances for foraging in a varied range of habitats. This species is widespread and common across sub-Saharan Africa.
3. Epauletted fruit bat (*Epomophorus gambianus*). Weight: 40-120g. Forearm length: 54-100mm. Frugivore bat with brown fur and white tufts at the base of the ears, *E. gambianus* is commonly found in savannahs, woodlands, and forest mosaics across sub-Saharan Africa.
4. Hammer-headed fruit bat (*Hypsignatus monstrosus*). Weight: 220-450g. Forearm length: 118-137mm. The largest frugivore bat across continental Africa, *H. monstrosus* is characterised by brown fur, white tufts at the base of the ears, and tubular nostrils. This species roosts at low altitudes, usually in forested areas, and it can be common on a local scale.

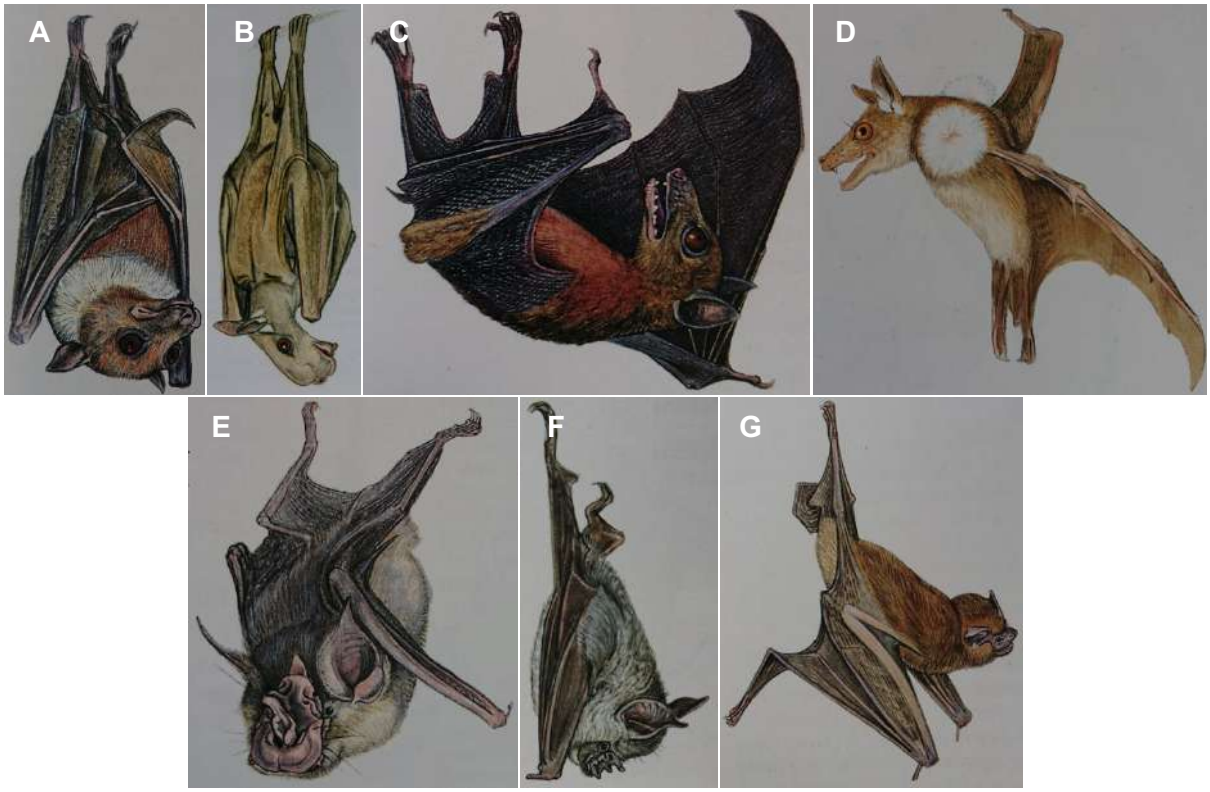

Figure 3.10. Illustrations of *Myonycteris torquata* (A), *Hypsignathus monstrosus* (B), *Rousettus aegyptiacus* (C), *Epomophorus labiatus* (D), *Rhinolophus hildebrandti* (E), *Hipposideros cyclops* (F), and *Miniopterus schriebersi* (G) (modified from: *The Kingdon Field Guide to African Mammals* (Kingdon 2015)).

3.4.2. Insectivorous bats.

1. Horseshoe bat (genus *Rhinolophus*). Weight: 5-33g. Forearm length: 35-69mm. The name of this genus derives from a flat, leaf-shaped lappet that surrounds the nostrils. *Rhinolophus* bats prefer to roost in caves, tree hollows, or buildings, and to hunt in sheltered areas.
2. Leaf-nosed bat (genus *Hipposideros*). Weight: 6-138g. Forearm length: 34-124mm. A very diverse genus of insect-eating bats with leaf-shaped ears and a series of lappets that surrounds the nostrils. *Hipposideros* spp. inhabit woodlands and savannas at low-to-medium altitudes and roost in tree hollows, caves, or house lofts.
3. Long-fingered bat (genus *Miniopterus*). Weight: 3-19g. Forearm length: 37-52mm. The most distinctive anatomical feature of *Miniopterus* bats is exceptionally long, double-folding digits, in addition to a large cranium with small, pointed muzzle. These bats roost in large numbers deep in caves and can be found in a wide variety of habitats.

4. SMALL MAMMAL SAMPLING METHODS.

Before setting traps around a new site, carefully communicate Fever Project's objectives with the local community, including the rationale behind sampling terrestrial small mammals. The team can start exploring the environment and planning where and how many traps could be set only after receiving clear consent from the community, including reassurance that traps will not be removed or displaced, permission to enter houses and bedrooms for trap setting/checking (ensure that the doors will be found unlocked), and acceptance to release each captured small mammal at its point of capture (or as close as possible in order to avoid human-wildlife conflicts). Often, locals become keen informants of rodent presence, although their attention may be towards bigger rats rather than small-sized mice. In case of a negative answer by the community, small mammal trapping activities cannot proceed for that specific site.

4.1. PROTOCOL FOR SETTING SMALL MAMMAL TRAPS.

☐ FBSL 3	☐ Clipboard/Folder	☐ Desiccated fish
☐ Sherman traps	☐ Data collection sheet	☐ Peanuts
☐ Tomahawk traps	☐ Pencil	☐ Fruit/Vegetable
☐ Marker pen	☐ Mobile phones	☐ Headlamps
☐ Coloured masking tape	☐ GPS	

Figure 4.1. Trapping activities using Sherman (A) and Tomahawk (B) traps.

Personnel directly executing the protocol apply PPE CAT I. The only trapping technique applied within the Fever Project utilises live collapsible Sherman traps (9x3x3.5in) and

Tomahawk traps with one trap door (19x6x6in) opportunistically placed indoors and along lines outdoors (**Figure 4.1**). Before setting, bait Sherman traps with a standard amount (i.e., a tablespoon) of a mixture of peanuts, desiccated fish, and a fruit/vegetable locally available (e.g., corn, tomatoes, or bananas). Bait Tomahawk traps with peanut butter on metal/plastic bottle lids.

Figure 4.2. Flagging tape attached to the vegetation for delimiting trap lines.

1. Set the traps before dusk indoors (two Sherman traps per room) and outdoors (several lines of 10 Sherman traps at 5-to-10m distance, and of 3 Tomahawk traps opportunistically placed around burrows of giant pouched rats (*Cricetomys* spp.)). In total, place a minimum of 60 Sherman traps and 9 Tomahawk traps per site each evening.
2. Mark each surveyed household with flagging tape and the number of traps which were set. Mark each trap line at its beginning, halfway, and end with flagging tape attached at chest height to the vegetation closely situated (**Figure 4.2**). In addition, each trap may be numbered before setting them in ascending order (**Figure 4.3**).

Figure 4.3. Numbering Sherman traps in ascending order before trapping activities can start.

3. Always check the proper functioning of each trap's trigger in situ to ensure bait is not in the way of the mechanism.
4. Record the trap-night date and time, administrative location, landscape category (e.g., indoors, village, farm, cultivated field, forest), and generic GPS coordinates of the study site on the appropriate data collection sheet.
5. Repeat the trapping strategy at the same site for ≤4 consecutive nights (a fourth consecutive night may be necessary only when capture rates are low (i.e., ≤10 rodents per night) but re-captures remain infrequent).

Advice box
• Check the weather forecast before fieldwork and avoid capture activities when heavy rainfall and wind gusts are predicted during the rainy season (from June to October),

or extreme heat during the dry season (from November to May). Adverse weather conditions may impact animals' survival during trapping.

- On full moon nights, rodents may be less active since their foraging activity may be negatively correlated with moonlight intensity (Bovendorp et al. 2017). Therefore, avoid trapping activities during full moon nights.
- For optimal indoor trapping success, place the traps underneath beds, alongside, walls, near kitchen corners, and alongside holes in the wall/floor that may act as burrows, always with the trap's door facing potential runways. For optimal outdoor trapping success, place the traps on potential runways (with the trap's door facing the entrance), alongside edges and fences with underbrush cover, and alongside logs or landmarks that may act as burrows.

4.2. PROTOCOL FOR INSPECTING SMALL MAMMAL TRAPS.

☐ FBSL 3	☐ Clipboard/Folder	☐ Marker pen
☐ Nitrile or latex gloves	☐ Data collection sheet	☐ Coloured masking tape
☐ N95 respirator	☐ Pencil	☐ Mobile phones
☐ Headlamp	☐ Carrier bags	

Personnel directly executing the protocol apply PPE CAT II (N95 respirator must be worn if captures occur into Tomahawk traps). The traps are checked as soon as possible after dawn by systematically inspecting the trap lines (remember to bring new traps in a carrier bag to replace those with captures in case the traps will be kept on site at point of capture during the day). Record the type/number of traps that are missing and misfired (i.e., any traps that was found triggered or with by-catch (i.e., the capture of non-target animals such as birds and amphibians)) on the data collection sheet. By-catch is immediately released at the point of capture.

1. Record the time the trapping activity starts on the data collection sheet.
2. If a Sherman trap is triggered, open carefully to verify if there is presence of captures or it is a misfire.
3. Immediately label small mammal captures by sticking masking tape to the trap and noting the animal identification number and landscape category on it (**Figure 4.4**). Also note the animal

Figure 4.4. Flagging tape attached to the trap for capture identification.

identification number on the flagging tape marking the trap's location (place it if not present).

4. Carefully place the traps containing captures in a separate carrier bag and avoid stacking them on top of each other. This is particularly important for rodents in Tomahawk traps since it reduces visual stimuli and stress.
5. The traps containing captures are removed from the carrier bag and placed in a shaded, secluded area with protective visual covering and at least 1m apart to minimise stress prior to sampling procedures.
6. If there is chance of tampering/theft during the day, remove all traps by also collecting untriggered traps and misfires in a separate carrier bag. If the chance is negligible, replace the traps with captures, ensure to deactivate all traps to avoid undesired captures during the day, and keep traps on site at point of capture.
7. On the last morning of trap inspection, ensure that flagging tape on houses and vegetation is removed and disposed of in the biohazard bag.

Advice box
• Do not wait >15h between deployment in the evening and inspection in the morning; the risk of dehydration or starvation is higher for the trapped animals if they are contained for too long. • When capturing mothers with their pups, be cognisant not to displace the pup during manipulation. • The application of N95 respirators becomes necessary only when personnel are exposed to captures in Tomahawk traps and, therefore, close contact with the animal is unavoidable (**Figure 4.1**).

4.3. SMALL MAMMAL ANAESTHESIA.

☐ FBSL 3	☐ Pelican™ case	☐ Spring balance 100g
☐ Oxygen concentrator	☐ Anti-spill funnel filler	☐ Connecting tubes
☐ Vaporiser	☐ Induction chamber	☐ Clean biohazard bag
☐ Ophthalmic lubricant	☐ Input port adapter	☐ Isoflurane
☐ Masking tape	☐ Anaesthetic facemask	

Personnel directly executing the protocol apply PPE CAT III. Direct handling of giant pouched rats (*Cricetomys* spp.) and shrews (*Crocidura* spp.) trapped within the study is only done on anaesthetised animals. A step-by-step guide to small mammal anaesthesia and blood collection is described below.

1. Prepare all the supplies and equipment for anaesthesia:
   a. Open the Pelican™ case and place the isoflurane vaporiser on the processing table ensuring its stability and straight position.
   b. Place the oxygen concentrator in a safe location on the ground near the processing station.
   c. Connect the tubes as needed based on the size of the animal to be anaesthetised:
      i. Connect a first tube from the oxygen concentrator to the input valve of the vaporiser.
      ii. Connect a second tube to the output valve of the vaporiser while the other end remains unplugged.
2. Open the bottle of isoflurane and fit it with the anti-spill funnel filler. Unscrew the lid at the top of the reservoir and pour the isoflurane until the liquid filled the reservoir approximately halfway (**Figure 4.5**).
   a. For *Cricetomys* rats, carefully place the Tomahawk trap into an unpierced biohazard bag, insert the unplugged end of the second tube into the bag's mouth and close the bag with your hands.
   b. For *Crocidura* shrews, carefully place the trapped individual into a cloth bag, then weigh it using the 100g spring balance and record the weight (including cloth bag) on the data collection sheet.
      i. Plug the adapter into the input port of the induction chamber, then insert the unplugged end of the second tube into the input port.
      ii. Cover the output port of the induction chamber with masking tape to prevent isoflurane leakage.
      iii. Release the animal into the induction chamber (use its lid to prevent the animal from escaping, then hold the lid down since it does not lock) (**Figure 4.6**).

Figure 4.5. Anti-spill funnel filler fitted to the bottle of isoflurane for pouring it into the reservoir.

Figure 4.6. *Mastomys* mouse after release in the induction chamber.

3. Switch the oxygen concentrator on (hold the on/off button for few seconds) and set it to 3L/min, set the vaporiser to 5%, then wait approximately 5min to induce the animal into anaesthesia (e.g., tilt the Tomahawk trap or induction chamber to verify whether the animal freely falls without any response).
4. Quickly switch the vaporiser to 0% and disconnect the tube from the Tomahawk trap or the induction chamber (**Figure 4.7**).
    a. For *Cricetomys* rats, fit the tube with the facemask then remove the rat from the trap and fit the facemask on its snout.
    b. For *Crocidura* shrews, the facemask is not necessary; remove the shrew from the induction chamber and fit the unplugged end of the tube on its snout.
5. Switch the vaporiser to 2% and the oxygen concentrator to 2L/min for *Cricetomys* rats (1L/min for *Crocidura* shrews) to maintain the anaesthetic level (at this stage, one staff member becomes the anaesthetist, solely dedicated to monitoring the animal's level of anaesthesia (i.e., monitoring the heart rate by keeping the index and middle fingers under the animal's elbow, and the respiratory rate by observing its breathing pattern)).

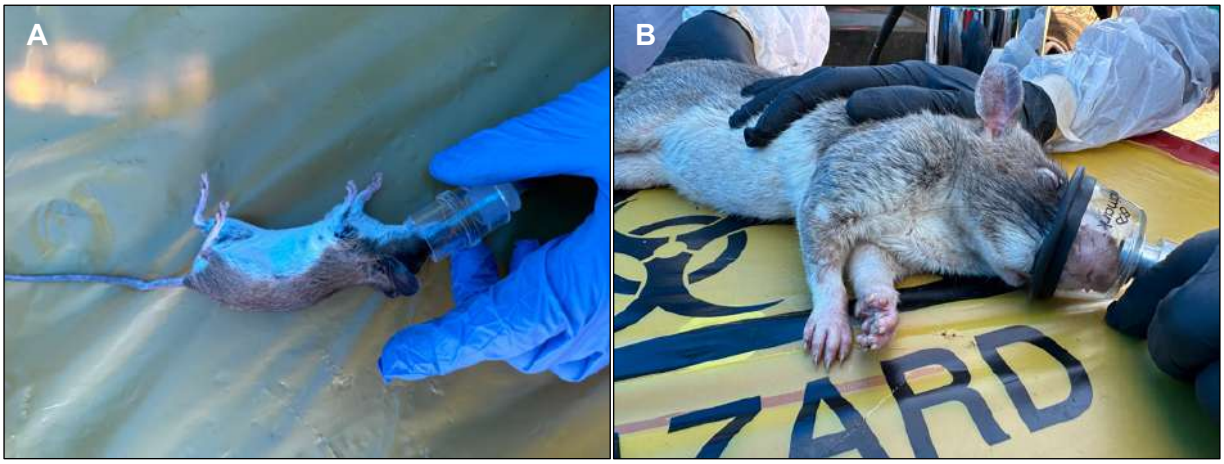

Figure 4.7. Maintaining the anaesthetic level in *Mastomys* mouse (A) and *Cricetomys* rat (B).

6. Apply ophthalmic lubricant to both eyes.
7. After clear affirmation from the anaesthetist, the sampling and data collection activities detailed in **Section 4.4** can start while the animal remains anaesthetised.
8. At the end of the sampling activities, weigh the empty cloth bag using the 100g spring balance (for shrews). Communicate the information to the transcriber and check with them that the data collection is completed.
9. Quickly switch the oxygen concentrator off and the vaporiser to 0%, then gently place the animal into its trap.
10. With the trap back to a shaded and secured area, ensure the safe awakening of the animal and, when it appears active and alert, place a slice of fresh fruit into its trap.

11. Between one anaesthesia and the other, ensure to:
    a. Clean the processing station by using 70% ethanol on areas that have been in contact with the animal's body and its fluids (including, if used, the induction chamber, the unplugged end of the tube, or the facemask).
    b. Use 70% ethanol to wipe the scissors used for cutting the swabs' shaft.
12. At the end of the sampling process, ensure to:
    a. Release animals at their precise point of capture (or as close as possible in order to avoid human-wildlife conflicts).
    b. Ensure that labelling tape on the trap is removed and disposed of in the biohazard bag.
    c. Decontaminate all the supplies and equipment for anaesthesia, the processing station, and dispose of PPE as detailed in **Section 8.1** and **Section 8.2**.
    d. Take a photo of the completed data collection sheet as a digital record.
    e. Transport and store the collected specimens as described in **Section 10.2**.

Advice box

- Fully re-charge the oxygen concentrator after each use and before its potential use the next day.
- Conduct activities in sheltered areas that are free from weather extremes (e.g., direct sunlight, rain, and/or wind gusts). If no sheltered area is found, create it by using tarps and ropes.
- During the anaesthesia procedures, act quickly after the induction and apply principles of restraint since the animal may suddenly awaken (**Figure 4.8**).

Figure 4.8. Restraint of *Cricetomys* rat after induction.

- When not directly inhaled by the animal due to the sampling procedures, remember to switch the level of isoflurane to 0% in order not to waste it.
- Troubleshooting guide in case the animal is not being induced or staying under anaesthesia: i) properly connected tubes with no leakage; ii) ensure that the isoflurane is flowing by smelling the unplugged end of the second tube; iii) oxygen concentrator with fully charged battery; iv) reservoir filled with appropriate amount of isoflurane; v) incorrect percentage of vaporiser.

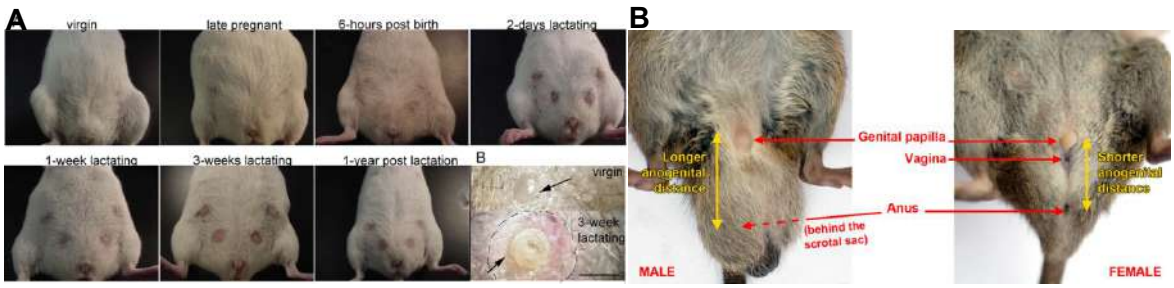

Figure 4.9. Nipple and areola growth during pregnancy and lactation (A) (modified from: Wu et al. (2015)). External comparison of male and female reproductive organs (B) (modified from: Herbreteau et al. (2011)).

4.4. PROTOCOL FOR HANDLING, SAMPLING, AND DATA COLLECTION.

☐ FBSL 3	☐ Clipboard/Folder	☐ Swabs large
☐ Cloth drawstring bags	☐ Data collection sheet	☐ Swabs small
☐ Callipers	☐ Pen/Pencil	☐ Needles 25-27g
☐ Spring balances 100-500g	☐ Marker pen for tubes	☐ Syringes 1mL
☐ Scissors	☐ Forceps for tick removal	☐ Syringe 3mL
☐ DNA/RNA Shield™	☐ Coloured tube stickers	☐ Pipette 100-1,000µL
☐ Whatman™ 903 cards	☐ Disinfection wipes	☐ Pipette tips
☐ Tubes 1.5mL	☐ Sterile saline solution	☐ Non-toxic nail polish
☐ Fresh fruit slices	☐ Smartphone or camera	

Personnel directly executing the protocol apply PPE CAT III. The traps containing captures are removed from the carrier bag and placed in a shaded, secluded area. The handling and sampling activities are performed on one individual capture at a time.

1. Place the different tube stickers (i.e., blue, brown, red, and green) on the 1.5mL tubes and pipette 500µL DNA/RNA Shield™ into each (see **Appendix C**).
2. Carefully place the rodent (see **Section 4.3** for giant pouched rats and shrews) into a cloth bag.
3. Weigh the rodent into its cloth bag using the appropriate 100g or 500g spring balance.
4. While the animal is in the bag, record the following information on the data collection sheet: initials of the transcriber, date, species (see **Section 4.5**),

Figure 4.10. *Hemimerus* earwigs infesting a *Cricetomys* rat.

animal identification number, landscape category, and weight including cloth bag.

5. Scruff the animal from inside the cloth bag, then collect the following data:
   a. Identify sex, reproductive status, and age by inspecting its genitalia and abdomen (see **Figure 4.9** and **Table 4.1** for definitions; if uncertain about age or sex, include "?" in the record and try explaining the uncertainty in the **Section "Notes"** of the data collection sheet).
   b. Evaluate the presence and number of ectoparasites (i.e., ticks, fleas, lice, and/or earwigs for *Crycetomys* spp. (**Figure 4.10**)) by visually inspecting its body. If any are present, remove a known number using forceps and place them into a green-top 1.5mL tube. Label the tube both at its top and side.
   c. Measure body and tail lengths using the callipers, then release the animal into its cloth bag to give it a break from manipulation.

Table 4.1. Codes relative to age (J for juveniles and A for adults) and reproductive status of the sampled female (F) and male (M) rodents and shrews*.

Age F	Reproductive status F	Age M	Reproductive status M
J	N (not reproducing; the vagina is imperforated and the weight is ≤ limit indicated in **Section 4.5**)	J	AB (abdominal testes; testes are not fully descended/visible and the weight is ≤ limit indicated in **Section 4.5**)
A	N (not reproducing; the vagina may be perforated but the weight is > limit indicated in **Section 4.5**)	A	AB (testes are neither abdominal nor fully descended/visible but the weight is > limit indicated in **Section 4.5**)
	E (pregnant; the abdomen is swollen and nipples are prominent)		SC (scrotal testes; testes are descended and large and the weight is > limit indicated in **Section 4.5**)
	L (lactating; the nipples are prominent and surrounded by an areola)		

* Age and sex using external traits for *Crocidura* shrews are difficult to determine. Adult males do not possess a scrotum and their testes remain within the body during their whole lives, whereas adult females may be identified by the presence of prominent nipples in their groin area.

6. Scruff the animal from inside the cloth bag a second time, then collect the following samples:
   a. One oral swab using a large sterile polyester-tipped swab. Then, place the swab's tip into a blue-top 1.5mL tube and cut the swab's shaft using scissors wiped with 70% ethanol. Label the tube both at its top and side.
   b. One rectal swab using a small sterile polyester-tipped swab moistened with few drops of sterile saline solution (use large swabs for rodents >150g (**Figure 4.11**)). Then, place the swab's tip into a brown-top 1.5mL tube and cut the swab's shaft using scissors wiped with 70% ethanol. Label the tube both at its top and side.

7. While manipulating the animal, collect blood samples (**Figure 4.12**):
    a. Only for mice (e.g., *Mastomys* spp.), while scruffing the animal also secure its tail between the ring and little fingers of the same hand (the scruffing action creates a tourniquet that makes the submandibular (facial) vein accessible). Locate the back end of its mandible (**Figure 4.13**) and quickly poke its cheek with a sterile 27g needle; a blood drop will form instantly if the submandibular (facial) vein is penetrated.
        i. Immediately approach the Whatman™ 903 card, then a red-top 1.5mL tube, to collect one or two drops of blood each. Label the tube both at its top and side. Label the blood spot.
        ii. Ensure haemostasis by releasing the mouse into its cloth bag (bleeding will stop due to the compression applied by the local muscles).

Figure 4.11. Swabbing the rectal cavity of *Cricetomys* rat.

Figure 4.12. Puncture of the submandibular (facial) vein in *Mastomys* mouse.

    b. Only for anaesthetised *Crocidura* shrews or mice ≤15g, apply pressure to the vessel close to the hip over the femur. Clean the lateral side of the hindlimb with a disinfection wipe. Locate the lateral saphenous vein and quickly poke it with a sterile 27g needle; a blood drop will form instantly if the vessel is penetrated.
        i. Immediately approach the Whatman™ 903 card, then a red-top 1.5mL tube, to collect one or two drops of blood each. Label the tube both at its top and side. Label the blood spot.
        ii. Ensure haemostasis by applying pressure to the site for 30sec to 1min using a cotton ball.
    c. Only for rats (e.g., *Cricetomys* and *Rattus* spp.), while scruffing a smaller rat, or on an anaesthetised large *Cricetomys* rat, also secure the base of its tail between the thumb and index finger of the other hand (press both fingers against the two sides; the

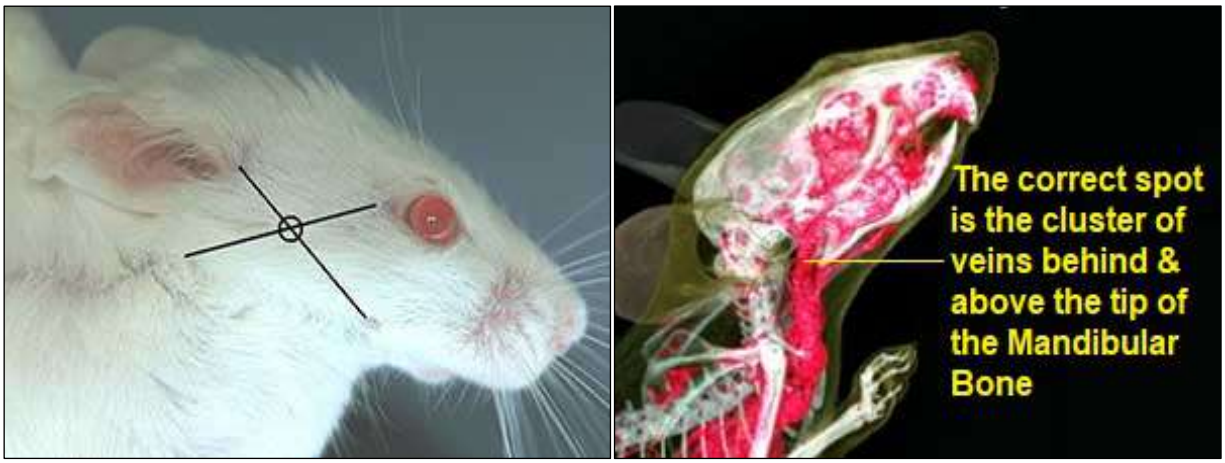

Figure 4.13. Locating the submandibular (facial) vein during scruffing of a laboratory mouse (modified from: https://medipoint.com/for-use-on-mice/).

tourniquet may make the lateral tail vein more visible since it is a superficial vein). Clean the proximal half of the tail with a disinfection wipe. Insert a sterile 25g needle fitted to a 1mL syringe at a shallow angle approximately one third down the tail's length and gently aspirate (**Figure 4.14**).

i. Immediately expel blood drops to fill a circle of the Whatman™ 903 card, then expel ≤100µL into a red-top 1.5mL tube. Label the tube both at its top and side. Label the blood spot.

ii. Ensure haemostasis by applying pressure to the site for 30sec to 1min using a cotton ball.

⚠ If unsuccessful, or more blood is necessary, blood collection can be tried twice on each side, but it will stop after the fourth trial or before then if the animal appears fatigued.

8. Use a non-toxic nail polish or permanent marker to mark the animal's tail with a dash to avoid sampling re-captures during the following morning(s) of fieldwork.
9. Release the animal into its cloth bag, ensure it appears active and alert, then place a slice of fresh fruit into its trap and carefully allow the individual to re-enter its trap.

Figure 4.14. Aspirate from the lateral tail vein in *Cricetomys* rat.

a. If it does not, administer sterile saline solution subcutaneously as described in **Section 9.1**.

10. Weigh the empty cloth bag using the 100g spring balance. Communicate the information to the transcriber and check with them that the data collection is completed.
11. Between one animal and the other, ensure to:
    a. Clean the processing station by using 70% ethanol on areas that have been in contact with the animal's body and its fluids.
    b. Dispose of debris and faeces from the dirty cloth bag into the biohazard bag.
    c. Place the dirty cloth bag into the basin with water and bleach prepared for boot decontamination.
    d. Use 70% ethanol to wipe the scissors used for cutting the swabs' shaft.
12. At the end of the sampling process, ensure to:
    a. Release animals at their point of capture (or as close as possible to avoid human-wildlife conflicts).
    b. Ensure that labelling tape on the trap is removed and disposed of in the biohazard bag.
    c. Decontaminate the processing station and dispose of PPE as detailed in **Section 8.1** and **Section 8.2**.
    d. Take a photo of the completed data collection sheet as a digital record.
    e. Transport and store the collected specimens as described in **Section 10.2**.

Advice box
• Scruffing requires gripping the skin fold where the animal's spine meets its head by using the thumb and index finger. An alternative technique that reduces airway obstruction uses the thumb and middle finger, then substituting the middle with the index finger to create a transverse fold (see also https://norecopa.no/education-training/films-and-slide-shows/refined-technique-for-scruffing-animals). Intentionally, we split our handling procedures in two sections to limit restriction of the upper respiratory tract and stress due to handling. • Juvenile males with not fully descended testes may be confused with females. However, differentiation can be achieved by observing the anogenital distance (i.e., the distance between the genital papilla and the anus) which is always greater in males. • The oral swab should be gently inserted without forcing it and allowing the animal to chew on it for 15sec.

- The rectal swab should be gently inserted but, if difficult to insert, it must be avoided due to the risk of rectal prolapse. Once inside the rectum, apply a rotational movement to the swab for 15sec.
- Blood collection from mice using the submandibular (facial) vein is further explained by the following video https://medipoint.com/for-use-on-mice/.
- Blood collection from rats using the lateral tail vein and the lateral saphenous vein is further explained by the following resources https://www.jove.com/pdf/52766/jove-protocol-52766-sampling-blood-from-the-lateral-tail-vein-of-the-rat and https://www.nc3rs.org.uk/3rs-resources/blood-sampling/blood-sampling-rat#saphenous-vein-(non-surgical).
- The total amount of blood collected from each animal must not exceed 0.75% of its weight (e.g., ≤200µL blood for 25g individual; one blood drop is approximately 50µL).
- Styptic powder or gel may be applied to ensure haemostasis after blood collection, especially when puncture of an artery may have occurred.
- When collecting blood from mice and shrews, avoid pressing the card or tube against the animal's cheek to minimise contamination risks.

4.5. SMALL MAMMAL IDENTIFICATION KEYS.

The small mammals listed herein (**Figure 4.15**) were selected based on pilot data collected as part of the Fever Project, in addition to occurrence data obtained from the Global Biodiversity Information Facility database (www.gbif.org). This limited list is meant to provide guidance to fieldwork personnel for a presumptive morphological identification of the captured rodents and shrews rather than being a comprehensive taxonomic guide of West African small mammals (consult *Les Rongeurs de l'Afrique Sahélo-Soudanienne* (Granjon & Duplantier 2009) for more in-depth information).

4.5.1. Family Muridae.

1. Domestic mouse (*Mus musculus*). Weight: 9-24g (approximate limit between juveniles and adults is 8-10g). Head-body length: 70-105mm (approximately equal to tail length of 65-100mm). *Mus musculus* is the most ubiquitous representative of a very variable taxonomic genus which also includes *Mus minutoides* (an indicative morphological differentiation between *M. musculus* and *M. minutoides* may be possible based on their size, approximately twice as small for the latter). *Mus musculus* has relatively big ears and brown-to-grey fur with unclear delimitation between upperside and underside. *Mus musculus* is an almost exclusively commensal species found indoors.

2. Multimammate mouse (genus *Mastomys*). Weight: 30-95g (approximate limit between juveniles and adults is 30-32g). Head-body length: 90-170mm (higher than tail length of 80-150mm). Nocturnal rodents with short and soft fur with a brown-to-grey upperside and pale beige or grey underside, *Mastomys* spp. are ubiquitous across savannas, woodlands, human settlements, and cultivated fields across sub-Saharan Africa. An indicative morphological differentiation between *Mastomys natalensis* and *Mastomys erythroleucus* remains ambiguous and genetic identification may be necessary.
3. Black rat (*Rattus rattus*). Weight: 55-185g (approximate limit between juveniles and adults is 54g). Head-body length: 140-210mm (shorter than tail length of 160-250mm). Usually nocturnal rodents (however, they can become diurnal at higher population densities) with large and mobile ears, coarse and brown-to-black fur with unclear delimitation between upperside and underside. *Rattus rattus* is a non-native species to the African continent, adapted to being commensal but increasingly found also outside human settlements.

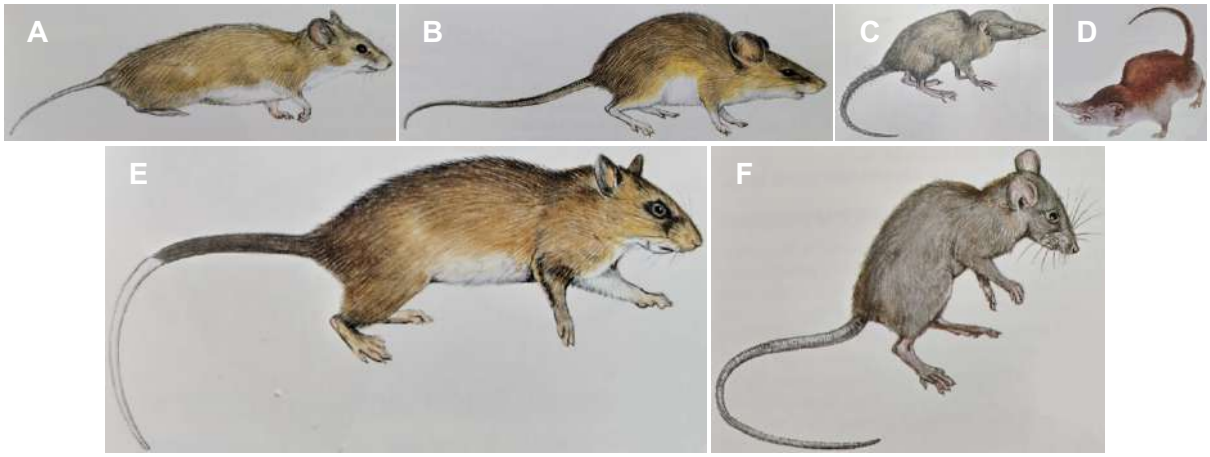

Figure 4.15. Illustrations of *Mus minutoides* (A), *Mastomys natalensis* (B), *Crocidura flavescens* (C), *Crocidura somalica* (D), *Rattus rattus* (E), and *Cricetomys gambianus* (F) (modified from: *The Kingdon Field Guide to African Mammals* (Kingdon 2015)).

4.5.2. Family Nesomyidae.

Giant pouched rat (*Cricetomys gambianus*). Weight: 500-1,500g (approximate limit between juveniles and adults is 520g). Head-body length: 280-400mm (approximately equal to tail length of 280-410mm). Strictly nocturnal large rodents with white fur on the terminal half of the tail, coarse fur with a grey or brown upperside and white or beige underside. *Cricetomys gambianus* are often commensal, found within villages and near houses. An indicative morphological differentiation between *C. gambianus* and *Cricetomys emini/Cricetomys kivuensis* may be possible based on their trapping location (*C. emini/C. kivuensis* may be distributed only across the Nzérékoré Region of Guinea) and coat (*C. emini/C. kivuensis* have a distinct dark brown fur with white-to-grey abdomen).

4.5.3. Family Soricidae.

Shrew (genus *Crocidura*). Weight: 11-40g. Head-body length: 60-130mm (higher than tail length of 50-90mm). Shrews of the genus *Crocidura* are the commonest and most diverse group of shrews across the African continent. *Crocidura* spp. are characterised by a long nose and small eyes, fur variable in its colour with bristles on the tail. They are nocturnal species, most actively at dawn, and occur in a wide range of habitats.

5. AVIAN SAMPLING METHODS.

Herein, we outline the methodologies applied as part of the Fever Project to handle and sample domestic avian species (i.e., chickens and ducks). We deliberately did not include procedures to mark the sampled poultry because each individual may be re-sampled both during the dry and the rainy seasons. Before the sampling activities may start, informed consent from animal owners must be sought (see **Appendix B**). It is important to clarify with community leaders that revision and signature of animal owner consent forms are required before sampling and that the animal owner must be present to provide such consent. In case of a negative answer by the animal owner, sampling activities on poultry cannot proceed for that specific site. Animal owners are also asked to complete an animal health history questionnaire after providing their informed consent to do so.

5.1. PROTOCOL FOR HANDLING, SAMPLING, AND DATA COLLECTION.

☐ FBSL 2	☐ Clipboard/Folder	☐ Pipette tips
☐ Scissors	☐ Data collection sheet	☐ Pipette 100-1,000µL
☐ DNA/RNA Shield™	☐ Pencil	☐ Disinfection wipes
☐ Needles 25g	☐ Marker pen for tubes	☐ Swabs large
☐ Syringes 1mL	☐ Forceps for tick removal	☐ Whatman™ 903 cards
☐ Syringe 3mL	☐ Sterile saline solution	☐ Coloured tube stickers
☐ Tubes 1.5mL	☐ Smartphone or camera	

Personnel directly executing the protocol apply PPE CAT II. The handling and sampling activities are performed on one individual animal at a time.

1. Place the different tube stickers (i.e., blue, brown, red, and green) on the 1.5mL tubes and pipette 500µL DNA/RNA Shield™ into each (see **Appendix C**).
2. To restrain the bird for transport, hold both wings at the proximal end of the humerus in one hand. The index finger separates each humerus to prevent skeletal injuries (**Figure 5.1**).
3. To restrain the bird for sampling purposes, the handler may apply:

Figure 5.1. A domestic duck restrained via wing hold for transport to the processing station.

a. Two-handed grip with hands placed on either side of the bird to hold its wings between the bird's body and the handler's palms. The bird is then lied sideways on the table (i.e., with its left side down and its right side up) with the handler's thumbs on the animal's breast near the sternum. The handler may use the left arm to tuck the bird's body under it while securing the feet with the other hand (**Figure 5.2**).
b. Restraint freely in the air with hold of both wings together behind the body. A finger is secured between the wings so that the bones do not rest on each other. Experienced personnel may find that this type of restraint allows for faster sampling procedures (**Figure 5.3**).

Figure 5.2. A domestic duck restrained sideways on the table to collect blood from the jugular vein.

c. A clean, dry towel to wrap the bird's body is an alternative and effective form of restraint. Additionally, a breathable cloth can be placed over its head to keep it calm. However, this type of restraint limits access to the jugular vein only for blood collection purposes.

Figure 5.3. A domestic duck restrained freely in the air to collect blood from brachial (A) and jugular veins (B).

4. While one member of personnel is restraining the animal, record the following information on the data collection sheet:
   a. Initials of the transcriber, date, species, and animal identification number.
   b. Identify sex, reproductive status, and age (if uncertain about age or sex, include "?" in the record and try explaining the uncertainty in the **Section "Notes"** of the data collection sheet).

c. Visual inspection for signs of nasal discharge, diarrhoea, and the presence and number of ectoparasites (i.e., ticks, mites, fleas, and/or lice). If any are present, remove a known number using forceps and place them into a green-top 1.5mL tube. Label the tube both at its top and side.

5. While restraining the animal, collect the following samples:
   a. One oral swab by inserting a large sterile polyester-tipped swab into the choana (**Figure 5.4**). Then, place the swab's tip into a blue-top 1.5mL tube and cut the swab's shaft using scissors wiped with 70% ethanol. Label the tube both at its top and side.
   b. One cloacal swab by inserting a large sterile polyester-tipped swab into the cloaca (**Figure 5.4**). Then, place the swab's tip into a brown-top 1.5mL tube and cut the swab's shaft using scissors wiped with 70% ethanol. Label the tube both at its top and side.

Figure 5.4. Swabbing the choana (A) and cloaca (B).

6. Then, collect blood samples from either the brachial or the jugular vein:
   a. Expose the ventral surface of either the left or right wing by gently pulling one wing from under the handler's palm and extending it. Locate the brachial vein dorsally to the elbow joint (it is situated under three small feathers in chickens (**Figure 5.5**)). Press the vein with the thumb proximally (i.e., towards the shoulder) to engorge it and, therefore, make it more visible. Clean the surface by spraying 70% ethanol. Insert a sterile 25g needle fitted to a 1mL syringe at a shallow angle and gently aspirate (**Figure 5.6**).
   b. Locate the jugular vein which lies longitudinally on the right side of the neck, between the trachea and the cervical vertebrae (it can easily be exposed since it lies in a featherless area of the chicken's neck). Press the vein with the thumb distally (i.e., at the base of the neck) to engorge it and, therefore, make it more visible. While keeping

pressure on the vein, elevate the neck slightly to stabilise the jugular vein. Clean the surface by spraying 70% ethanol. Insert a sterile 25g needle fitted to a 1mL syringe at a shallow angle and gently aspirate (**Figure 5.7**).

i. Immediately expel blood drops to fill a circle of the Whatman™ 903 card, then expel ≤100µL into a red-top 1.5mL tube. Label the tube both at its top and side. Label the blood spot.

ii. Ensure haemostasis by applying pressure to the site for 30sec to 1min using a cotton ball.

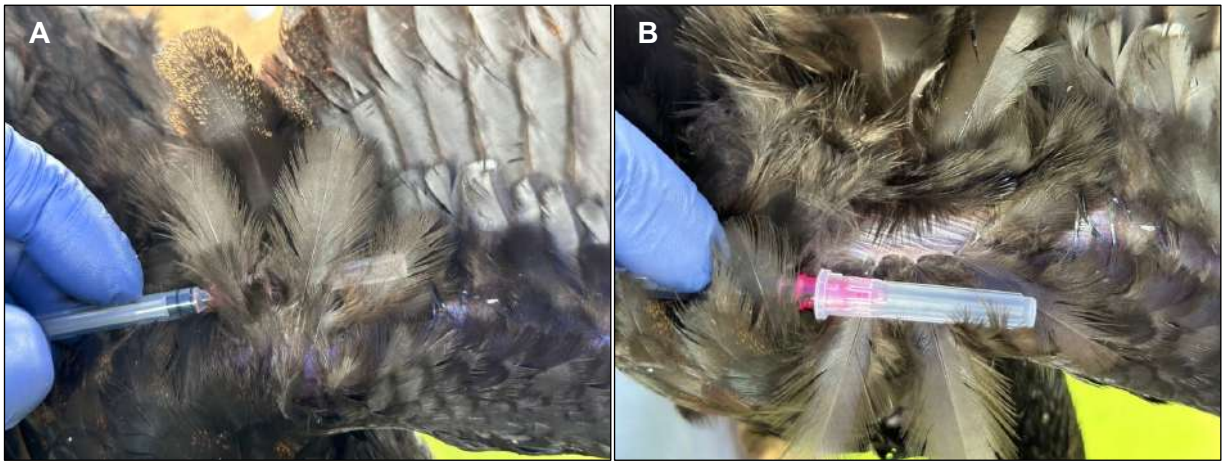

Figure 5.5. Locating the three feathers on the medial side of a chicken's wing to expose the brachial vein.

⚠ If unsuccessful, blood collection can be tried again (twice on each wing), but it will stop after the fourth trial or before then if the animal appears fatigued.

Figure 5.6. Blood collection from the brachial vein without bending the needle (A) and by doing so (B).

7. Check with the transcriber that the data collection is completed, then carefully hold the bird until its feet touch the ground and release it ensuring it appears active and alert.

a. If it does not, administer 20-40mL of sterile saline solution subcutaneously as described in **Section 9.1**.

8. Between one animal and the other, ensure to clean the processing station by using 70%

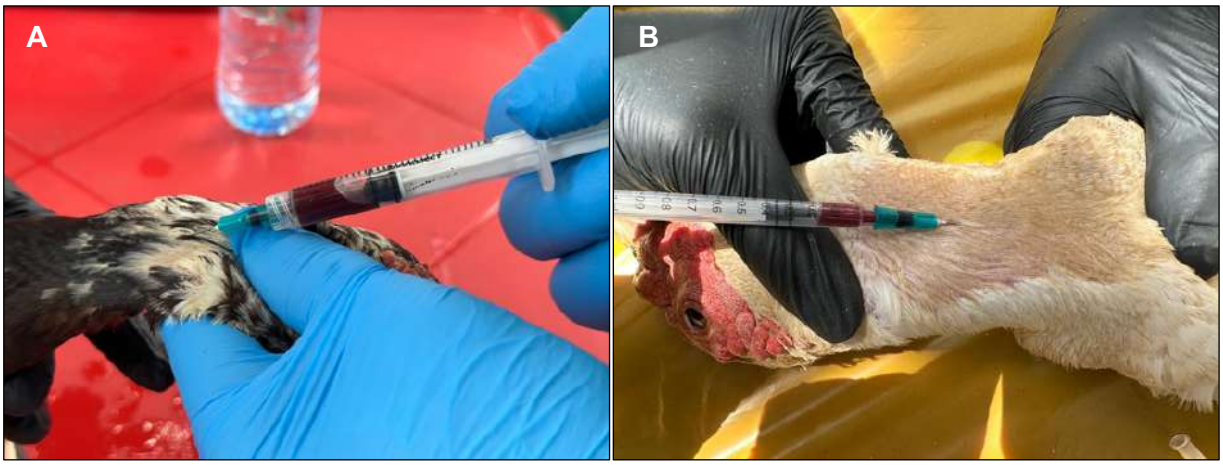

Figure 5.7. Blood collection from the jugular vein of domestic ducks.

ethanol on areas that have been in contact with the animal's body and its fluids. Also, use 70% ethanol to wipe the scissors used for cutting the swabs' shaft.

9. At the end of the sampling process, ensure to:
   a. Decontaminate the processing station and dispose of PPE as detailed in **Section 8.1** and **Section 8.2**.
   b. Take a photo of the completed data collection sheet as a digital record.
   c. Transport and store the collected specimens as described in **Section 10.2**.

Advice box

- After six weeks from their birth, poultry is fully grown and individuals are categorised as adults. Ask the animals' owner for clarifications in case of doubt.
- To safely handle the bird, one member of staff dedicates to controlling the bird's head, wings, and legs. With chickens, particularly roosters, use the right hand to securely restrain their talons since their spurs may cause serious injuries.

Figure 5.8. Detail of blood collection from the brachial vein of a chicken using a bent needle.

- The oral and cloacal swabs should be gently inserted without forcing since they are shallow. Once inside the choana or the cloaca, apply a rotational movement to the swab for 15sec.
- Avoid applying the oral swab to the lumen of the glottis and trachea; this procedure may inadvertently facilitate pathogen contamination of the respiratory tract.
- Blood collection from the brachial vein may be facilitated by bending the syringe's needle at a slight angle using its cap (**Figure 5.8**). Care must be taken when collecting blood from this vessel as it has the tendency to form haematomas; therefore, it is best practice to apply pressure to the area with the wing close to the animal's body to ensure haemostasis.
- The jugular vein is more superficial and mobile in chickens than ducks. Therefore, it can be more difficult to stabilise when collecting blood.
- The total amount of blood collected from each bird must not exceed 0.5% of its weight (e.g., ≤500µL blood for 100g individual).
- Further methodologies on capturing and manipulating domestic and wild birds are detailed by the Food and Agriculture Organization of the United Nations (FAO 2007).

6. DOMESTIC RUMINANT SAMPLING METHODS.

Herein, we outline the methodologies applied as part of the Fever Project to handle and sample domestic ruminants (i.e., cattle, goats, and sheep). We deliberately did not include procedures to mark the sampled ruminants because each individual may be re-sampled both during the dry and the rainy seasons. Before the sampling activities may start, informed consent from animal owners must be sought (see **Appendix B**). It is important to clarify with community leaders that revision and signature of animal owner consent forms are required before sampling and that the animal owner must be present to provide such consent. In case of a negative answer by the animal owner, sampling activities on domestic ruminants cannot proceed for that specific site. Animal owners are also asked to complete an animal health history questionnaire after providing their informed consent to do so.

6.1. PROTOCOL FOR HANDLING, SAMPLING, AND DATA COLLECTION.

☐ FBSL 2	☐ Clipboard/Folder	☐ Pipette tips
☐ Scissors	☐ Data collection sheet	☐ Pipette 100-1,000µL
☐ DNA/RNA Shield™	☐ Pencil	☐ Disinfection wipes
☐ Needles 21g	☐ Marker pen for tubes	☐ Swabs large
☐ Syringes 1mL	☐ Forceps for tick removal	☐ Whatman™ 903 cards
☐ Tubes 1.5mL	☐ Smartphone or camera	☐ Coloured tube stickers

Personnel directly executing the protocol apply PPE CAT II. The handling and sampling activities are performed on one individual animal at a time.

1. Place the different tube stickers (i.e., blue, brown, red, and green) on the 1.5mL tubes and pipette 500µL DNA/RNA Shield™ into each (see **Appendix C**).
2. Restraining techniques for small and large ruminants:
   a. Small ruminants can be straddled, firmly but gently, by placing the handler's knees immediately behind the animal's shoulders (**Figure 6.1**):
      i. While the body is restrained, also restrain the head by grabbing hold of the animal's horns or its jaw.

Figure 6.1. Small ruminant restraint before sampling procedures.

ii. Back the small ruminant against a fence/wall or into a corner to help control its hindquarters.

b. Cattle can be temporarily tethered to an anchor point (e.g., a tree trunk) using a rope harness around their horns (**Figure 6.2**):

i. While the head is restrained and the animal is standing, two staff members position themselves on either side of the animal at the height of the thigh.

ii. Alternatively, while the head is restrained, also restrain the hindquarters by hobbling them at the height of the metatarsus/phalangeal joints, then lay the animal to the ground in lateral recumbency.

Figure 6.2. Restraint of cattle before sampling procedures.

3. Record the following information on the data collection sheet:

a. Initials of the transcriber, date, species, and animal identification number.

b. Identify sex, reproductive status, and age (see **Table 6.1** and **Table 6.2**; if uncertain about age ask the animals' owner for clarifications, otherwise include "?" in the record and try explaining the uncertainty in the **Section "Notes"** of the data collection sheet).

c. Visual inspection for signs of nasal discharge, diarrhoea, and the presence and number of ectoparasites (i.e., ticks, fleas,

Figure 6.3. Ticks (highlighted by red arrows) tend to congregate near the anus and external genitalia of ruminants.

and/or lice). If any are present (**Figure 6.3**), remove a known number using forceps and place them into a green-top 1.5mL tube. Label the tube both at its top and side.

Table 6.1. Guide to estimating the age of small ruminants by their deciduous/permanent incisor teeth.

Teeth	Age of deciduous teeth	Age of permanent teeth
1st pair incisors	≤1 week	~15 months
2nd pair incisors	≤2 weeks	~22 months
3rd pair incisors	≤1 month	~32 months
4th pair incisors	≤2 months	~40 months

Table 6.2. Guide to estimating the age of cattle by their deciduous/permanent incisor teeth.

Teeth	Age of deciduous teeth	Age of permanent teeth
1st pair incisors	≤2 weeks	~21 months
2nd pair incisors	≤2 weeks	~27 months
3rd pair incisors	≤2 weeks	~36 months
4th pair incisors	≤2 weeks	~45 months

4. While restraining the animal, collect the following samples:
   a. One oral swab using a large sterile polyester-tipped swab. Then, place the swab's tip into a blue-top 1.5mL tube and cut the swab's shaft using scissors wiped with 70% ethanol. Label the tube both at its top and side.
   b. One rectal swab using a second large sterile polyester-tipped swab. Then, place the swab's tip into a brown-top 1.5mL tube and cut the swab's shaft using scissors wiped with 70% ethanol. Label the tube both at its top and side.
5. Then, collect blood samples:
   a. For small ruminants, while restraining the animal's body, hold its jaw to lightly twist its head to either side at 30° angle to access the jugular vein on the opposite side of the twist (i.e., collect blood from the left jugular vein when the head is twisted rightward and vice versa).Then, locate the jugular vein which lies longitudinally in the groove on either side of the trachea. Press the vein with the thumb distally to engorge it and, therefore, make it more visible. Clean the surface by spraying 70% ethanol. Insert a sterile 21g needle fitted to a 1mL syringe at a shallow angle and gently aspirate (**Figure 6.4**).

Figure 6.4. Blood collection from the jugular vein of small ruminants.

b. For cattle, while standing behind the animal, use the non-dominant hand to hold the tail approximately a third of its length from the base and lift it to expose its ventral aspect. Then, locate the coccygeal (tail) vein which lies longitudinally in the midline groove on the ventral aspect of the tail (approximately 10cm from the base of the tail). Clean the surface by spraying 70% ethanol. Insert a sterile 21g needle fitted to a 1mL syringe halfway along the body of a coccygeal vertebra and at 90° angle (i.e., perpendicularly) to a depth of approximately half the needle's length and gently aspirate (**Figure 6.5**).
   i. Immediately expel blood drops to fill a circle of the Whatman™ 903 card, then expel ≤100µL into a red-top 1.5mL tube. Label the tube both at its top and side. Label the blood spot.
   ii. Ensure haemostasis by applying pressure to the site for 30sec to 1min using a cotton ball.

Figure 6.5. Blood collection from the coccygeal (tail) vein of cattle.

⚠ If unsuccessful, blood collection can be tried again, but it will stop after the fourth trial or before then if the animal appears fatigued.

6. Check with the transcriber that the data collection is completed, then release the animal ensuring it appears active and alert.
7. Between one animal and the other, use 70% ethanol to wipe the scissors used for cutting the swabs' shaft.
8. At the end of the sampling process, ensure to:
   a. Decontaminate the processing station and dispose of PPE as detailed in **Section 8.1** and **Section 8.2**.
   b. Take a photo of the completed data collection sheet as a digital record.
   c. Transport and store the collected specimens as described in **Section 10.2**.

Advice box
• Tethering of cattle is not suitable for long-term confinement and must be done only on suitable grounds (i.e., a flat, shaded area that is clear of any obstructions). The rope

should be held, not tied, once it has been passed around the anchor point to allow for the rapid release of the animal if required.

- Shear the site where blood will be drawn before disinfection to minimise the chance of contaminating the needle during sampling.
- To open the mouth of domestic ruminants for swabbing their oral cavity, insert the thumb and index finger in both sides of the mouth (i.e., commissures). This manoeuvre can be executed on just one side of the mouth in cattle using the index and middle fingers (**Figure 6.6**).
- The oral and rectal swabs should be gently inserted. Once inside the mouth or the rectum, apply a rotational movement to the swab for 15sec.

Figure 6.6. Opening the oral cavity of cattle for swabbing.

- Further methodologies on handling livestock are detailed by the PREDICT Consortium (2019).

7. DOMESTIC DOG SAMPLING METHODS.

Herein, we outline the methodologies applied as part of the Fever Project to handle and sample domestic dogs. We deliberately did not include procedures to mark the sampled dogs because each individual may be re-sampled both during the dry and the rainy seasons by chance (however, specific individuals will not be sought out for re-sampling). Furthermore, after obtaining consent from the dog's owner or guardian, (see **Appendix B**), only owned and docile dogs will be sampled by Fever Project's personnel. Consequently, we did not include any techniques for physical restraint or chemical immobilisation of free-ranging and more aggressive dogs. As applied to the sampling of other domestic animals, clarify with community leaders that revision and signature of animal owner consent forms are required before sampling and that the animal owner must be present to provide such consent. In case of a negative answer by the animal owner, sampling activities on domestic dogs cannot proceed for that specific site. Animal owners are also asked to complete an animal health history questionnaire after providing their informed consent to do so.

7.1. PROTOCOL FOR HANDLING, SAMPLING, AND DATA COLLECTION.

☐ FBSL 2	☐ Clipboard/Folder	☐ Pipette tips
☐ Scissors	☐ Data collection sheet	☐ Pipette 100-1,000µL
☐ DNA/RNA Shield™	☐ Pencil	☐ Disinfection wipes
☐ Needles 25g	☐ Marker pen for tubes	☐ Swabs large
☐ Syringes 1mL	☐ Forceps for tick removal	☐ Whatman™ 903 cards
☐ Tubes 1.5mL	☐ Coloured tube stickers	☐ Muzzle
☐ Smartphone or camera		

Personnel directly executing the protocol apply PPE CAT II. The handling and sampling activities are performed on one individual animal at a time.

1. Place the different tube stickers (i.e., blue, brown, red, and green) on the 1.5mL tubes and pipette 500µL DNA/RNA Shield™ into each (see **Appendix C**).
2. Gently put a muzzle on the dog and restrain it by laying it down on either side (**Figure 7.1**):
    a. Two staff members position themselves on both sides of the dog. One person secures the animal's body by placing both hands under it.
    b. The second person holds the dog's limbs (i.e., those directly in front of the other staff member), with both hands at the height of the carpus/metacarpus and tarsus/metatarsus joints, pulling them towards her/him.

c. The first person follows the manoeuvre by gently laying the dog on its side, using both forearms to hold the animal down and hands to hold its limbs (i.e., those directly in contact with the ground or table) in order to prevent the dog from standing up.

3. While one member of personnel is restraining the animal, record the following information on the data collection sheet:
   a. Initials of the transcriber, date, species, and animal identification number.
   b. Identify sex, reproductive status, and age (see **Table 7.1**; if uncertain about age ask the animals' owner for clarifications, otherwise include "?" in the record and try explaining the uncertainty in the **Section "Notes"** of the data collection sheet).
   c. Visual inspection for signs of nasal discharge, diarrhoea, and the presence and number of ectoparasites (i.e., ticks, fleas, and/or lice). If any are present, remove a known number using forceps and place them into a green-top 1.5mL tube. Label the tube both at its top and side.

Figure 7.1. Restraint of a domestic dog using the muzzle and laying it down on its side for sampling.

4. While restraining the animal, collect the following samples:
   a. One oral swab using a large sterile polyester-tipped swab. Then, place the swab's tip into a blue-top 1.5mL tube and cut the swab's shaft using scissors wiped with 70% ethanol. Label the tube both at its top and side.
   b. One rectal swab using a second large sterile polyester-tipped swab. Then, place the swab's tip into a brown-top 1.5mL tube and cut the swab's shaft using scissors wiped with 70% ethanol. Label the tube both at its top and side.
5. Then, collect blood samples:
   a. Locate the lateral saphenous vein which lies across the lateral surface of the lower hind limb (i.e., from the cranial aspect of the tarsus to the caudal aspect of the stifle). Press the vein with the thumb distally to engorge it and, therefore, make it more visible. Clean the surface by

Figure 7.2. Detail of blood collection from the lateral saphenous vein of a domestic dog.

spraying 70% ethanol. Insert a sterile 25g needle fitted to a 1mL syringe at a shallow angle and gently aspirate (**Figure 7.2**).

b. Immediately expel blood drops to fill a circle of the Whatman™ 903 card, then expel ≤100µL into a red-top 1.5mL tube. Label the tube both at its top and side. Label the blood spot.
c. Ensure haemostasis by applying pressure to the site for 30sec to 1min using a cotton ball.

⚠ If unsuccessful, blood collection can be tried twice on each side, but it will stop after the fourth trial or before then if the animal appears fatigued.

Table 7.1. Guide to estimating the age of domestic dogs by their deciduous/permanent teeth.

Teeth	Age of deciduous teeth	Age of permanent teeth
Canines	≤4 weeks	~5.5 months
Incisors	≤6 weeks	~3.5 months
Premolars	≤6 weeks	~5 months
Molars	≤8 weeks	~6.5 months

6. Check with the transcriber that the data collection is completed, then release the animal ensuring it appears active and alert.
7. Between one animal and the other, use 70% ethanol to wipe the scissors used for cutting the swabs' shaft.
8. At the end of the sampling process, ensure to:
   a. Decontaminate the processing station and dispose of PPE as detailed in **Section 8.1** and **Section 8.2**.
   b. Take a photo of the completed data collection sheet as a digital record.
   c. Transport and store the collected specimens as described in **Section 10.2**.

Advice box

- The oral and rectal swabs should be gently inserted. Once inside the mouth or the rectum, apply a rotational movement to the swab for 15sec.
- The total amount of blood collected from each domestic dog must not exceed 0.6% of its weight (e.g., ≤6ml blood for 1kg individual).

8. DECONTAMINATION AND DOFFING.

After the daily activities for the sampling of animals are completed at each study site, it is pivotal to properly decontaminate supplies and doff all PPE to prevent the potential spread of any infectious agents and to preserve the integrity of fieldwork supplies. Before animal sampling can start, fieldwork staff members set up the donning/doffing station as detailed in **Section 2.2**, which allows for quick and easy decontamination and doffing. For decontamination purposes, animal sampling activities within the Fever Project applied sodium hypochlorite at 3% concentration (i.e., bleach). Virkon® at 1% solution (2% for decontaminating Sherman and Tomahawk traps) is an effective, safe, and widely used alternative to bleach. Virkon® solutions have the clear advantages to remain stable for up to seven days, to be compatible with metals and other materials without being corrosive, and to remain active when contaminated by soil and other organic matter. Nevertheless, the rationale behind our choice to use bleach was the impossibility to source Virkon® across Guinea or neighbouring countries.

8.1. DECONTAMINATION OF EQUIPMENT.

The layout of the donning/doffing station, including the doffing steps within the Fever Project's fieldwork activities, have been designed by using the knowledge gained during previous sampling trips: the contamination status of each item has been taken into consideration when planning the doffing flow. The steps to decontaminate equipment and materials after the end of fieldwork activities at a certain locality are detailed below.

8.1.1. Processing station and anaesthesia equipment.

1. Spray the external gloves with 70% ethanol.
2. Ensure that anaesthesia equipment is turned off.
3. Spray anaesthesia equipment, tubing, and adapters with 70% ethanol.
4. Spray the table, chairs, and non-metallic equipment with bleach (use 70% ethanol instead of bleach on metallic equipment (e.g., isoflurane's vaporiser) as bleach erodes the material over time).
5. Spray the items again every 2-3min to maintain a continuous 10min contact time for decontamination purposes.
6. Remove contaminated PPE as detailed by the doffing steps below (**Section 8.2**). Do not touch decontaminating equipment while wearing contaminated PPE.
7. Wipe decontaminated items down with paper towels if sprayed with bleach or let them air dry if sprayed with 70% ethanol.

8. Disassemble anaesthesia tubing and adapters, then pour the isoflurane remaining in the vaporiser back into its bottle.
9. Store items in their designated containers.

8.1.2. Uncontaminated Sherman and Tomahawk traps for rodents.

1. Follow these steps after the end of the last morning of trapping activities at a certain locality and wear PPE CAT II (see **Section 2.1**) while decontaminating the traps.
2. Dispose of bait and debris into the biohazard bag (red).
3. Spray the external gloves with 70% ethanol.
4. Scrub and wash each trap in a basin with water and soap (Sherman traps must be disassembled first).
5. Let the traps air dry.
6. Remove PPE as detailed by the doffing steps below (**Section 8.2**). Do not touch decontaminating equipment while wearing contaminated PPE.
7. Reassemble each Sherman trap before storage in their designated containers.

8.1.3. Contaminated Sherman and Tomahawk traps for rodents.

1. Follow these steps after the end of each morning of trapping activities and wear PPE CAT III (see **Section 2.1**) while decontaminating the traps.
2. Dispose of bait and debris into the biohazard bag (red).
3. Spray the external gloves with 70% ethanol.
4. Scrub and wash each trap in a basin with water and soap (Sherman traps must be disassembled first).
5. Spray the traps with 70% ethanol, then spray them again every 2-3min to maintain a continuous 10min contact time for decontamination purposes.
6. Let the traps air dry.
7. Remove PPE as detailed by the doffing steps below (**Section 8.2**). Do not touch decontaminating equipment while wearing contaminated PPE.
8. Reassemble each Sherman trap before storage in their designated containers.

8.1.4. Cloth drawstring bags used to handle bats, rodents, and shrews.

1. Follow these steps after the end of each session of trapping activities (i.e., night for bats and morning for rodents/shrews) and wear PPE CAT III (see **Section 2.1**) while decontaminating cloth bags.
2. Spray the external gloves with 70% ethanol.

3. Rinse with water all the dirty cloth bags contained in the basin with water and bleach to remove any bleach residues, then temporarily store the wet bags in a designated container.
4. Remove PPE as detailed by the doffing steps below (**Section 8.2**). Do not touch decontaminating equipment while wearing contaminated PPE.
5. The following morning, hang the wet bags outdoors to dry.

8.1.5. Triple high mist net pole system and mist nets for bats.

1. Follow these steps after the end of each night of trapping activities and wear PPE CAT III (see **Section 2.1**) while decontaminating the pole system and mist nets.
2. Spray the external gloves with 70% ethanol.
3. Remove the mist nets from the pole system and, while they are being folded for storage in their designated bags, spray liberally with 70% ethanol (alternatively, submerge them in 70% ethanol if available).
4. Wait 10min before pouring the remaining 70% ethanol out of each storage bag.
5. Disassemble the triple high mist net pole system, then spray it with 70% ethanol.
6. Wait 10min but spray the pole system again halfway, then wipe it down or let it air dry.
6. Remove PPE as detailed by the doffing steps below (**Section 8.2**) but keep internal gloves, N95 respirator, and fieldwork clothing on. Do not touch decontaminating equipment while wearing contaminated PPE.
7. Store the pole system in its designated bag.

8.2. DOFFING SEQUENCE.

The steps for doffing PPE after the end of fieldwork activities at a certain locality are detailed below. The doffing sequence varies based on the applied PPE CAT and a schematic summary for each PPE CAT is illustrated in **Figure 8.1**, **Figure 8.2**, and **Figure 8.3**. After removal, each item must be disposed of in the biohazard bag (red) unless indicated.

When doffing PPE CAT III and PPE CAT IV, one member of the fieldwork team must keep PPE on: this last member will tie the biohazard bag after the rest of the team has completed the doffing sequence (they may also support the rest of the team with removing the masking tape that holds gloves and sleeves together). This waste is placed into a second biohazard bag (red) which is also used to dispose of PPE worn by the above-mentioned last member. For further guidance on doffing procedures, consult *Field Biosafety Manual* (Valitutto, in preparation).

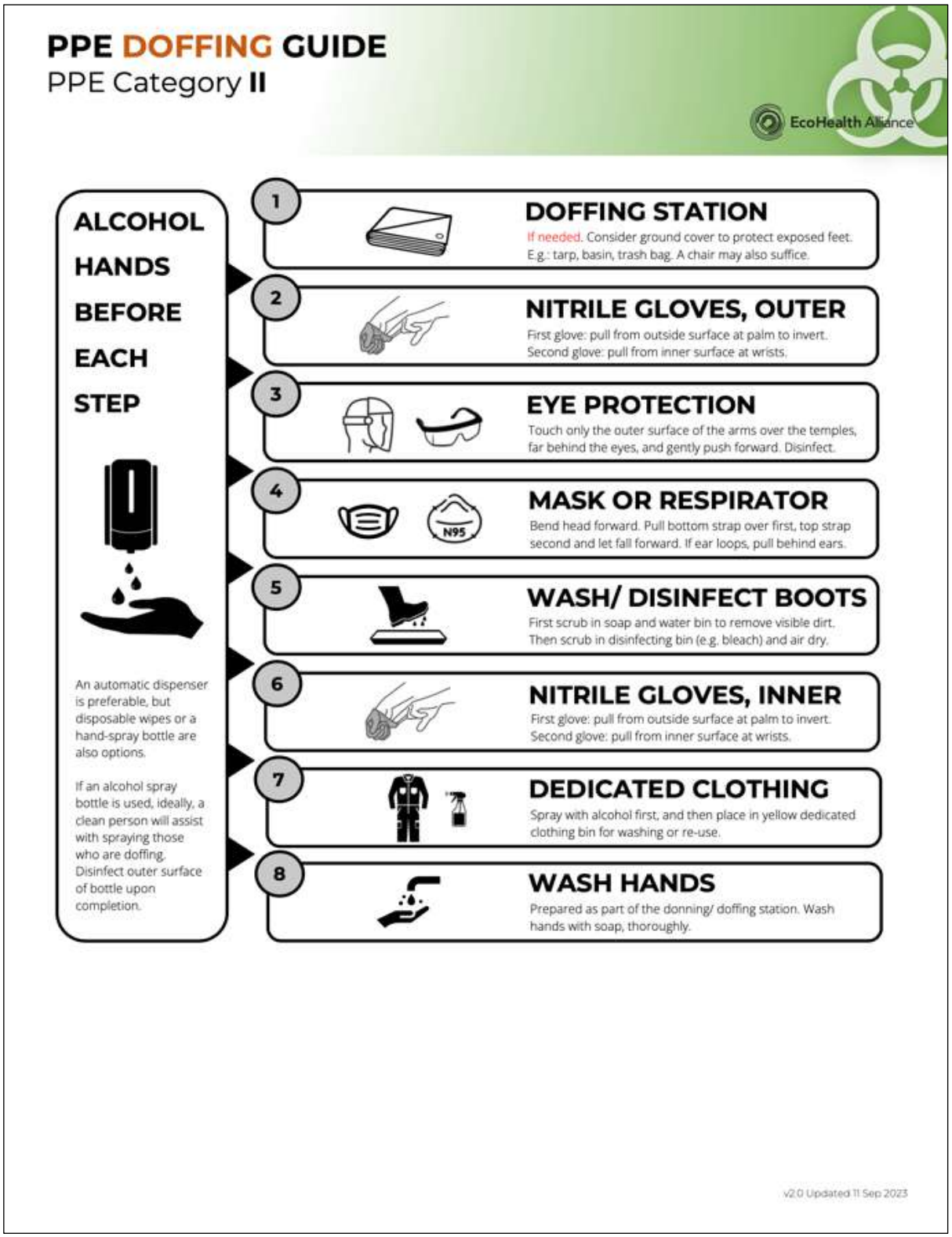

Figure 8.1. Step-by-step guide for doffing PPE CAT II (modified from: *Field Biosafety Manual* (Valitutto, in preparation)).

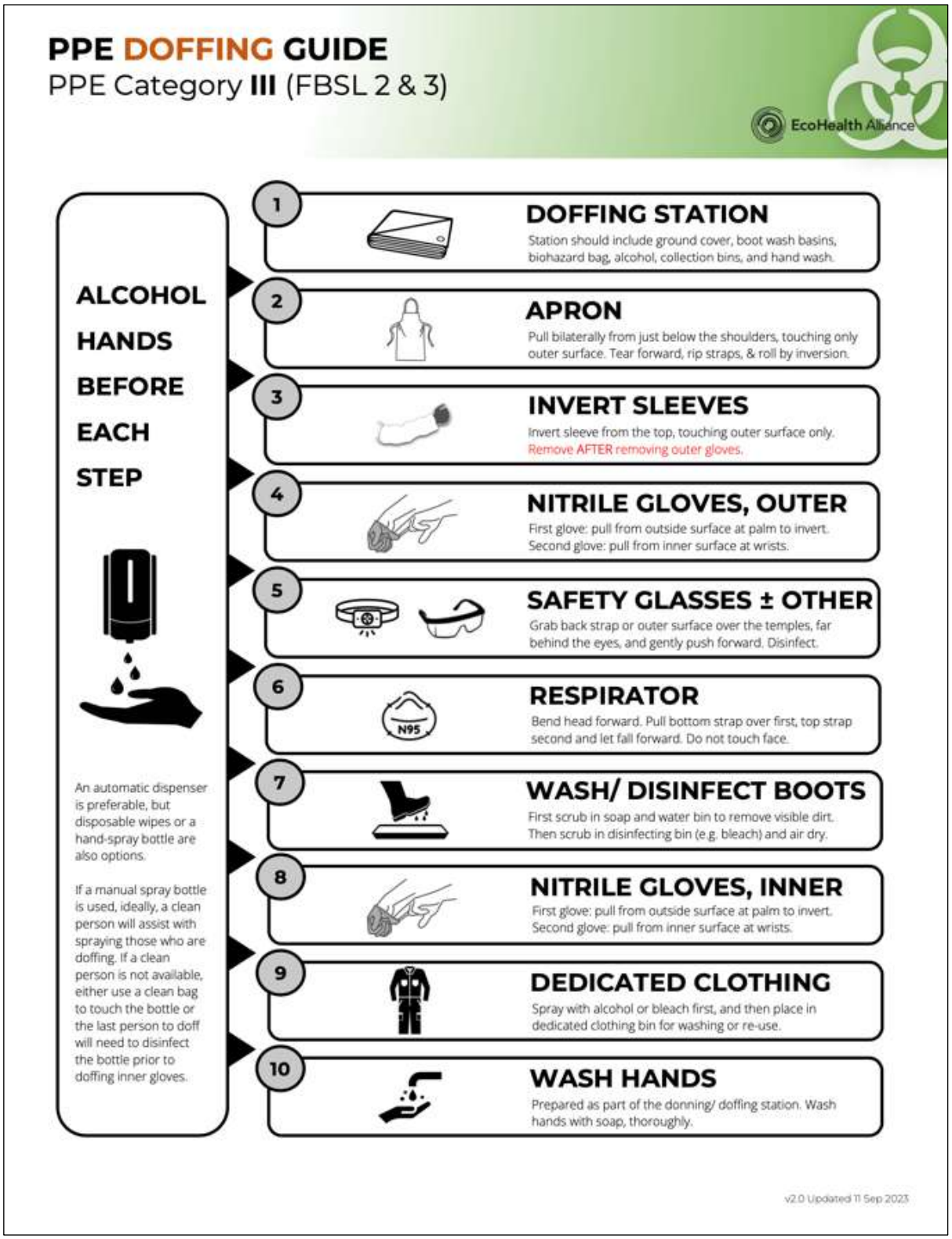

Figure 8.2. Step-by-step guide for doffing PPE CAT III (modified from: *Field Biosafety Manual* (Valitutto, in preparation)).

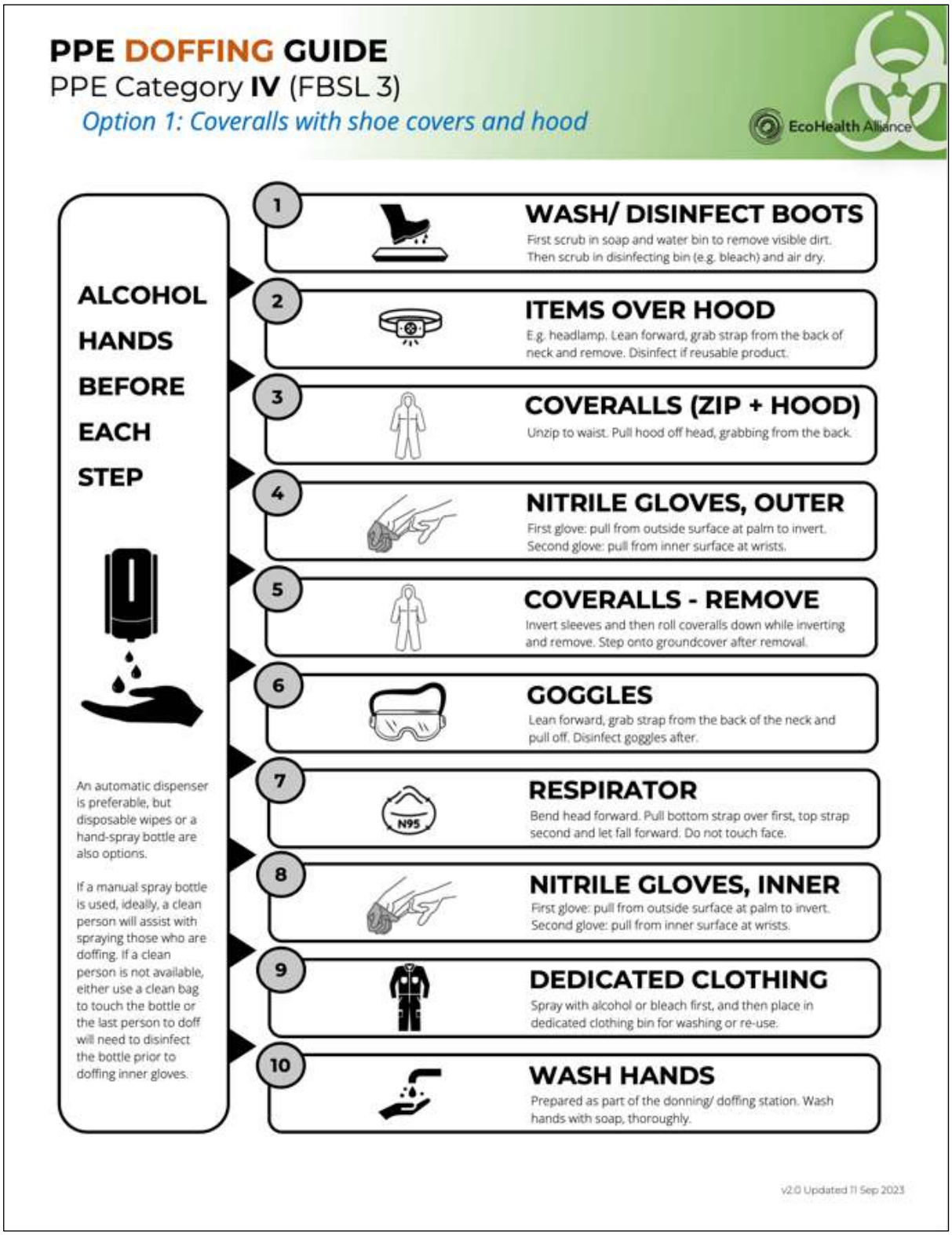

Figure 8.3. Step-by-step guide for doffing PPE CAT IV (modified from: *Field Biosafety Manual* (Valitutto, in preparation)).

8.2.1. Doffing PPE CAT II.

1. Spray the external gloves with 70% ethanol before removing them, then spray the internal gloves with 70% ethanol (do not remove gloves if wearing only one pair).
2. If wearing safety glasses, remove them by touching only the outer surface of their sides near the temples and gently pushing forward (**Figure 8.4**). Place the glasses in a dedicated small basin for their decontamination by spraying them with bleach.
3. Spray the internal gloves again with 70% ethanol.
4. If wearing a respirator, remove it by bending the head forward, pulling first the bottom strap over the head, then the top strap, and finally letting the respirator fall forward (do not touch the skin to avoid contact with contaminated material) (**Figure 8.5**).
5. Spray the internal gloves again with 70% ethanol.
6. Open arms and legs to spray fieldwork clothing with 70% ethanol.
7. Scrub the impermeable boots in the basin with water and soap, then move to the basin with water and bleach to scrub boots a second time.
8. Remove the impermeable boots while they are immersed in the basin and step on the tarp.
9. Lay the impermeable boots off the tarp to air dry, the spray the internal gloves again with 70% ethanol before removing them.
10. Spray your bare hands with 70% ethanol.
11. Doff fieldwork clothing and place it in the biohazard bag (yellow) for the dedicated clothing.
12. Wear your personal shoes.
13. At the handwashing station, wash your hands thoroughly with soap and water.

Figure 8.4. Removal of safety glasses.

Figure 8.5. Removal of respirator.

8.2.2. Doffing PPE CAT III.

1. Spray the external gloves with 70% ethanol.
2. Remove the disposable apron by pulling bilaterally from below the shoulders, touching only the outer surface of the apron; the ties behind the neck and back will break (do not

pull the apron over the head as this gesture increases the risks of inhalation and skin contact with contaminated material).

3. Spray the external gloves again with 70% ethanol.
4. Remove the masking tape that holds gloves and sleeves together, then remove the disposable sleeves by inverting them from their proximal part while touching only their outer surface.
5. Spray the external gloves again with 70% ethanol before removing them, then spray the internal gloves with 70% ethanol.
6. Remove the safety glasses (and headlamp if wearing one) by touching only their outer surface and gently pushing forward (**Figure 8.4**). Place the glasses in a dedicated small basin for their decontamination by spraying bleach (spray 70% ethanol for the headlamps).
7. Spray the internal gloves again with 70% ethanol.
8. Remove the N95 respirator by bending the head forward, pulling first the bottom strap over the head, then the top strap, and finally letting the respirator fall forward (do not touch the skin to avoid contact with contaminated material) (**Figure 8.5**).
9. Spray the internal gloves again with 70% ethanol.
10. Open arms and legs to spray fieldwork clothing with 70% ethanol.
11. Scrub the impermeable boots in the basin with water and soap, then move to the basin with water and bleach to scrub boots a second time.
12. Remove the impermeable boots while they are immersed in the basin and step on the tarp.
13. Lay the impermeable boots off the tarp to air dry, the spray the internal gloves again with 70% ethanol before removing them.
14. Spray your bare hands with 70% ethanol.
15. Doff fieldwork clothing and place it in the biohazard bag (yellow) for the dedicated clothing.
16. Wear your personal shoes.
17. At the handwashing station, wash your hands thoroughly with soap and water.

8.2.3. Doffing PPE CAT IV.

1. Spray the external gloves with 70% ethanol (if heavily contaminated, a clean field assistant can offer their removal and replacement with a new pair of gloves).
2. Scrub the impermeable boots in the basin with water and soap, then move to the basin with water and bleach to scrub boots a second time.
3. Remove the impermeable boots while they are immersed in the basin but step off the tarp.
4. Lay the impermeable boots off the tarp to air dry.
5. Spray the external gloves again with 70% ethanol.
6. If wearing a headlamp over the hood, place it in a dedicated small basin for its decontamination and spray it with 70% ethanol.

7. Spray the external gloves again with 70% ethanol.
8. Unzip the coveralls to the waist, then remove their hood (carefully remove the hood by grabbing it from behind the head and pulling down).
9. Spray the external gloves again with 70% ethanol.
10. Remove the masking tape that holds gloves and sleeves together.
11. Spray the external gloves again with 70% ethanol before removing them, then spray the internal gloves with 70% ethanol.
12. Doff the coveralls by inverting and rolling down its upper part and sleeves to the waist, then fully by inversion.
13. Spray the internal gloves again with 70% ethanol.
14. Remove the goggles by grabbing the straps from behind the head while leaning forward. Place the goggles in a dedicated small basin for their decontamination by spraying bleach.
15. Spray the internal gloves again with 70% ethanol.
16. Remove the N95 respirator by bending the head forward, pulling first the bottom strap over the head, then the top strap, and finally letting the respirator fall forward (do not touch the skin to avoid contact with contaminated material).
17. Spray the internal gloves again with 70% ethanol.
18. Open arms and legs to spray fieldwork clothing with 70% ethanol.
19. Spray the internal gloves again with 70% ethanol then remove them.
20. Spray your bare hands with 70% ethanol.
21. Doff non-contaminated fieldwork clothing, then place them in their designated container.
22. Wear your personal shoes.
23. At the handwashing station, wash your hands thoroughly with soap and water.

9. FIRST AID PROCEDURES FOR ANIMALS AND PERSONNEL.

Prior to fieldwork activities, all staff directly involved in handling and sampling has received pre-exposure vaccination against rabies virus and has been fit tested to identify an adequate N95 or equivalent respirator. Personnel directly involved in sampling procedures has achieved veterinary qualifications and, for the purposes of the Fever Project, has undergone specific training and has been deemed as fully competent on all biosafety, animal handling, and sampling methodologies. Furthermore, it has been agreed that two qualified staff members are always needed to safely perform handling and sampling procedures on each animal. Staff has also reviewed the safety data sheet for each potentially hazardous product used within the Fever Project and printed copies were made readily accessible to all fieldwork personnel.

9.1. ANIMAL WELFARE.

Before animal handling, particularly wildlife, each subject must be assessed for signs of ill health and reproductive status. The purpose is to quickly identify any individuals that may need to be immediately released or that may require appropriate therapeutic response prior to release. Therefore, animals that are deemed unable to withstand handling will not be part of the study; in other words, they will be released without sampling. Pregnant and nursing females must be handled with particular care to avoid disrupting the developing foetus or displace the neonate(s), respectively. These females (but not their pups) may be sampled but be sure to offer oral hydration upon completion.

Hypothermia may be observed in the captured bats, rodents, and shrews, although it is an unlikely event during activities within the Fever Project; however, it may be more likely during the rainy season (from June to October). Lethargy and/or dehydration can be more common events during activities within the Fever Project, particularly after sampling blood. If an animal appears hypothermic and/or lethargic before, during, or after sampling procedures, place it immediately into a cloth bag and under a warm blanket to reduce stress due to handling (we also advise the use of an instant heat pack for first aid). Then, check after 5min for signs of recovery. If the animal shows signs of recovery, gently restrain its body while only exposing the head and offer 20µL/1g fluids (i.e., fruit juice for frugivorous bats and rodents, saline solution for insectivorous bats and shrews) using a syringe without needle. Release at point of capture only if the animal appears clearly active and record the event in the **Section "Notes"** of the data collection sheet, including whether sampling was incomplete or not done. If the animal does not show signs of recovery or appears dehydrated (tented skin, sticky mucous membranes, and sunken eyes are signs attributable to dehydration), evaluate the subcutaneous administration of aseptic fluid:

1. Restrain as described previously, then select a body region with loose skin for subcutaneous injection (i.e., the flank for smaller bats, rodents, and shrews; the neck for larger bats, rodents, and dogs; the inguinal region for avian species).
2. Calculate a dosage of 0.02mL/g sterile saline solution (e.g., 1mL of solution subcutaneously for 50g individuals or 20mL for 1kg individuals).
3. Aspirate warm sterile saline solution (ideally at a temperature between 27-37°C) using a sterile 25g or 27g needle fitted to an appropriately sized syringe.
4. Clean the animal's body surface with a disinfection wipe, pinch the skin to elevate it from the underlying tissues, then insert the needle at a shallow angle. Massage the injection site while delivering the fluids if the skin appears tight and absorption slow.
    a. Notice that lack of resistance during the injection is indicative of the needle being subcutaneously.
    b. Ensure that there is no leakage of aseptic fluid from the injection site.
    c. Insert the needle for ≤1cm when rehydrating a chicken or duck to avoid the risk of reaching the air sacs.
5. Repeat the previous step several times to administer the adequate volume of fluids.
6. When treating bats, rodents, or shrews, place the rehydrated wild small mammal into a cloth bag and under a warm blanket to reduce stress due to handling, checking after 5min for signs of recovery. When treating domestic animals, keep them under observation in a shaded area and away from other animals.
7. Immediately release (at point of capture for wild small mammals) only if the animal appears clearly active and alert.
8. The subcutaneous administration of sterile saline solution must be recorded in the **Section "Notes"** of the data collection sheet, including if sampling was incomplete or not executed.

9.2. ANIMAL EUTHANASIA.

In the case of unlikely recovery, the individual must be euthanised. Any adverse events (i.e., accidental death before/during sampling and anaesthesia) must be recorded in the **Section "Notes"** of the data collection sheet. The following procedures adhere to guidelines on euthanasia standards set by the American Veterinary Medical Association for domestic animals and wildlife (Underwood & Anthony 2020) and to protocols approved by the CNERS in Guinea (064/CNERS/23) and IRB of Georgetown University in the USA (STUDY00002481).

9.2.1. Euthanasia of wildlife.

Euthanasia following non-recoverable injuries of wildlife is a rare event during animal sampling activities within the Fever Project. However, the following protocol must be applied when euthanasia of bats and small terrestrial mammals becomes necessary:

1. Use a clean plastic resealable bag (e.g., Ziploc®) of 1L for smaller bats, mice, and shrews, or of 4L for larger bats and rats (the only exception being giant pouched rats (*Cricetomys* spp.) which will undergo a second round of anaesthesia as detailed in **Section 4.3**).
2. Add isoflurane to a cotton wool ball and place it in the resealable bag (i.e., 0.4mL for 1L bag and 1.6mL for 4L bag).
3. Place the animal in the resealable bag, seal it, then wait approximately 5min to induce the animal into anaesthesia.
4. Calculate a dosage of 1-2mEq/kg potassium chloride, also expressed as 75-150mg/kg, at 20mEq/10mL concentration (e.g., 50µL of solution for 50g individuals or 1mL for 1kg individuals).
5. Aspirate the potassium chloride using a sterile 25g or 27g needle fitted to a 1mL syringe.
6. Rapidly, remove the mammal from the bag and perform intracardial injection of the dose of potassium chloride:
   a. Locate the heart by palpation at sternum level.
   b. Insert the needle immediately left to the sternum and exactly halfway of its length (i.e., between the sternal notch and the top of the xiphoid process), perpendicularly to the animal's body (**Figure 9.1**).
   c. Before the lethal injection, aspire blood into the syringe to provide evidence of correct insertion of the needle into the heart (if blood is not aspirated, retract the needle slightly and re-position it).
   d. Check the heartbeat by palpation to confirm cessation of cardiac function and death.
7. Place the carcass in the resealable bag, seal it, then dispose of it in the biohazard bag (red).

9.2.2. Euthanasia of livestock.

Euthanasia following non-recoverable injuries of domestic animals has never occurred during animal sampling activities within the Fever Project. Nevertheless, guidelines have been set in the unlikely event that sampled cattle, small ruminants, poultry, or dogs may require euthanasia:

1. Seek to obtain a clear agreement of the owner's will on whether they decide to slaughter the animal following routine practices or agree to euthanise the animal following the procedure detailed below.
   a. Should the owner opt to slaughter the animal, this must occur immediately and under the supervision of the veterinary team to minimise suffering.
   b. Should the owner agree that the veterinary team executed chemical euthanasia of the animal, the owner will acknowledge via written consent that the animal will not be used for human consumption after euthanasia.

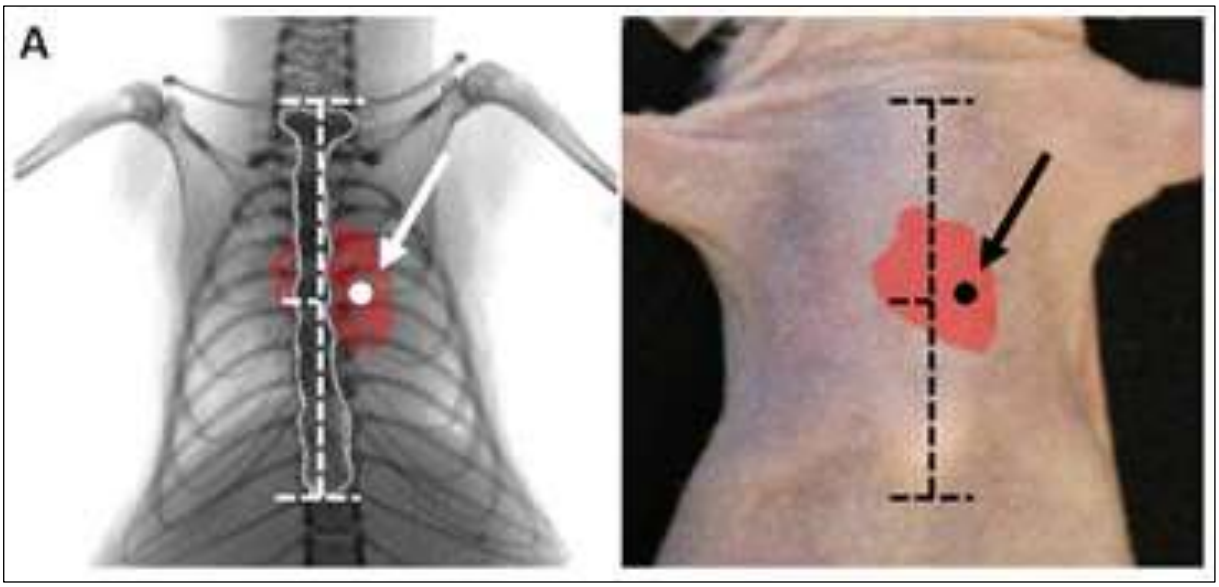

Figure 9.1. Ventral radiographical view of a laboratory mouse's thorax showing the correct location for intracardiac injection. Anatomical landmarks (i.e., sternal notch, fourth intercostal space, and xiphoid process) are highlighted by horizontal dashes (modified from: Campbell et al. (2012)).

2. Administer DEXDOMITOR® (i.e., dexmedetomidine hydrochloride) by intravenous or intramuscular injection using a sterile 21g or 25g needle fitted to a 3mL syringe:
   a. At 50mcg/kg either into the jugular vein or thigh muscles of cattle, small ruminants, and poultry.
   b. At 5-15mcg/kg either into the jugular vein, lateral saphenous vein, or thigh muscles of dogs.
3. Allow the animal to rest quietly for 15min following the injection of DEXDOMITOR®.
4. Calculate a dosage of 1-2mEq/kg potassium chloride, also expressed as 75-150mg/kg, at 20mEq/10mL concentration (e.g., 10mL of solution for 10kg individuals).
5. Aspirate the potassium chloride using a sterile 21g or 25g needle fitted to a 3mL syringe.
6. Perform intravenous injection of the dose of potassium chloride using the same vein as detailed above.
7. Check the heartbeat by auscultation and palpation to confirm cessation of cardiac function and death.
8. Ensure disposal of the carcass following biosafety protocols and local legislation.

9.3. HEALTH AND SAFETY OF PERSONNEL.

If injury to a staff member occurs during fieldwork, they must stop activities to immediately apply first aid. Contusions, scratches, and bites are the most common injuries that could happen. Personnel should not put themselves at risk of biting if an animal attempts to escape; in these circumstances, minimise the chance of injuries to both the animal and personnel by

allowing the animal to escape. Nevertheless, in the event of bites immediately wash and protect the wound, then seek medical care.

1. Wash immediately the wound for 15min with soap and water first, then with Betadine® (a topical aqueous solution of 10% povidone-iodine) or benzalkonium chloride. Pat dry with a clean towel, then apply antibiotic ointment (e.g., Neosporin® or Polymycin™) and cover the wound with bandage. The member of personnel providing first aid must wear clean, sterile disposable gloves to protect themselves.
2. After first aid, seek medical care for all bites. Wound infections following bites by domestic and wild animals include rabies, tetanus, pasteurellosis, and various viral, bacterial, and fungal agents (Abrahamian & Goldstein 2011). Since work within the Fever Project involves sampling of bats and domestic dogs, post-exposure rabies treatment strategy must be agreed with the closest health centre prior to fieldwork activities. The World Health Organization recommends a course of rabies vaccine and/or immunoglobulin as post-exposure prophylaxis (https://www.who.int/news-room/fact-sheets/detail/rabies).

9.3.1. First aid kit assembly.

For the purposes of immediate care following injury during fieldwork, a first aid kit is always kept stocked and readily available inside the vehicle used by personnel. Fever Project's first aid kits includes:

- Rabies virus vaccines for post-exposure prophylaxis stored at +4°C.
- Benzalkonium chloride wipes.
- Betadine® 10% solution.
- Hydrogen peroxide 3% solution.
- Triple antibiotic ointment (i.e., bacitracin, neomycin, and polymyxin B).
- Cold gel for relief from sprains.
- Eyewash station.
- Eyewash solution.
- Plasters, bandages, and sterile dressings.
- Sticky tape.
- Sterile nitrile gloves.
- Paper towels.

9.3.2. Needlestick injury prevention.

Personnel directly involved in sampling procedures has also undergone training on needlestick injury prevention. Briefly, needlestick and sharps injuries are of particular concern because they may result in the inoculation of pathogens and infected material. Needlestick

injury prevention aims to tackle potentially hazardous actions (e.g., handling, recapping, and disposing of syringe needles) by implementing specific procedures to safely handle syringes and needles. Nevertheless, should a needlestick or sharps injury occur, immediately wash the wound, then seek medical care as detailed for animal bites in **Section 9.3**.

1. Uncap the needle: pull the cap to expose the needle with a firm movement and never leave a syringe uncapped lying around the processing station.
2. Do not recap the needle: it is not advised to recap a needle before disposal. However, in the event of a needle needing its cap, apply the one-hand scoop technique (**Figure 9.2**):
   a. Leave the cap on the table and guide the tip of the used needle into it using only one hand.
   b. Lift the syringe and needle vertically and, once the tip is covered, use the other end to lock the cap into place (alternatively, apply an upright push against a stable surface to lock the cap and decontaminate the surface with 70% ethanol).
3. Do not remove the needle: uncapped needles must never be unplugged from the syringe by hand. However, in the event this is necessary in order to transfer the sample for storage, or to use the same syringe again, recap the needle as described above then twist the base of the needle in its cap to unplug it from the syringe.
4. Dispose of the syringe and its needle: immediately after use, place a used syringe with the attached needle in a container suitable for sharp disposal.
5. Spray the gloves with 70% ethanol.

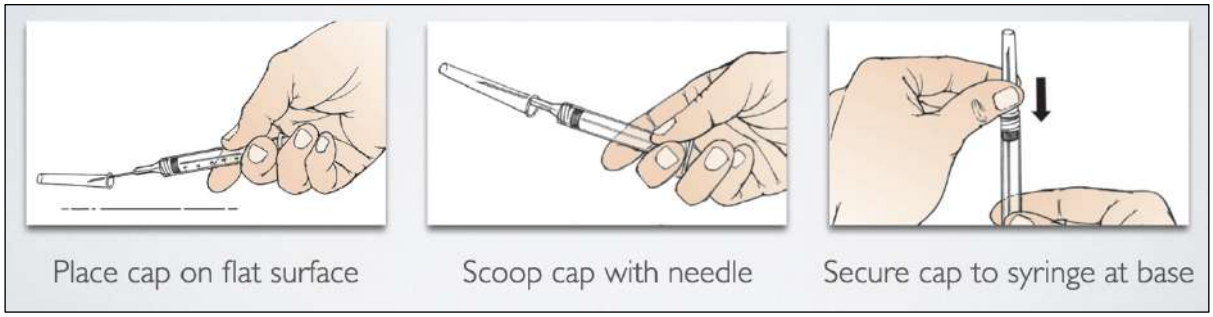

Figure 9.2. One-hand scoop needle recap technique.

10. STORAGE OF SAMPLES.

This section details our plan for sample storage to ensure that specimens are exposed to identical and adequate conditions until their arrival at laboratories of reference for long-term storage prior to sample processing and diagnostics testing.

10.1. IMPLEMENTATION OF A COLD CHAIN.

A cold chain is a set of procedures that ensures a temperature-controlled storage of specimens after collection, during their distribution to laboratories, their storage prior to testing, and subsequent long-term archival. Cold chain guidelines, spanning from coolants of choice to temperature gauges and record keeping, have the overarching objective to monitor that all biological specimens are stored and transported at optimum temperatures for unbiased detection of pathogens based on the storage medium used, and that any deviations are recorded and addressed prior to downstream analysis. Requirements set by the PREDICT Consortium (2019) are a relevant reference to consult before planning cold chain workflows.

Within the context of the Fever Project, specimen storage sampling and transport options have been chosen to minimise cold chain requirements and ensure that specimens can remain robust and viable even if a cold chain cannot be maintained consistently. To this end, specimens are typically able to be transported for up to seven days at ambient temperature but should be transferred as soon as feasible to a +4°C fridge or -20°C freezer (ISSMV for specimens collected within the prefecture of Dalaba, and the Guéckédou prefectural hospital for specimens collected within the prefecture of Guéckédou). Repeated freezing and thawing must be avoided as it degrades nucleic acids and proteins. Periodically, specimens may be transferred from Guéckédou to ISSMV for longer-term storage, and all specimens may also be transferred to Conakry for final testing and archiving, depending on availability of freezers and testing capabilities. Specimen transfer must follow the national policy (Standley et al. 2019) for safe and secure transportation of biomedical specimens and should be conducted using cool boxes or in-build refrigeration. This is particularly important for specimens that have been temporarily stored at -20°C; these must not thaw during transit, so every effort should be made to ensure that they remain frozen for the duration of transportation, and immediately placed back into -20°C or -80°C until testing occurs.

10.2. STORAGE OF ANIMAL SPECIMENS WITHIN THE FEVER PROJECT.

The choice to preserve each animal specimen (i.e., ≤100µL whole blood, oral swabs, rectal swabs, and ticks) into a 1.5ml tube containing 500µL DNA/RNA Shield™ (manufactured by Zymo Research) derives from the objective difficulty to guarantee a temperature ≤-20°C

immediately after sampling and throughout transportation. We selected DNA/RNA Shield™ reagent due to its ability to stabilise nucleic acids, preserve their genetic integrity, inactivate infectious agents contained in the sample, and integrate many workflows for nucleic acid extraction and downstream applications using molecular diagnostics and next-generation sequencing technologies. The guaranteed stability of samples at ambient temperature (for up to seven days at >30°C), including the validated inactivation of highly pathogenic viral agents, are features that we found particularly applicable to our fieldwork conditions.

Nevertheless, the unsuitability of whole blood in DNA/RNA Shield™ for serological testing required an additional storage medium and specimen type: dried blood spots on Whatman™ 903 Protein Saver Card (manufactured by Cytiva). Whatman™ 903 Protein Saver Card can store ≤80µL whole blood for each circle contained in the sample collection area and maintain the integrity of antibodies and other proteins. After drying time of approximately 2h at ambient temperature, cards are archived into foil-barrier resealable bags including one 1-10g indicating silica gel sachet as desiccant. However, being the processing station installed outdoors during fieldwork as part of the Fever Project, we observed that the drying time can be heavily dependent on the weather conditions. Therefore, when sampling in adverse weather conditions such as during the rainy season (from June to October), we advise that these cards should also dry indoors before archival. The result is a stabilised sample suitable for both molecular and serological testing that does not need refrigeration, although long-term storage at -20°C is recommended particularly for RNA recovery (Arca-Lafuente et al. 2022; Keck et al. 2022).

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APPENDIX A. LIST OF MATERIALS.

The following list of materials has been assembled with the sole intent of transparency about the equipment employed during animal sampling activities within the Fever Project. Our intention is that the items listed below, including their manufacturers, may serve as guidance for future users rather than indicating a prescriptive choice. Similar equipment manufactured by various companies is available in different countries.

Equipment	Manufacturer	Catalogue no.*
Anaesthesia		
Anaesthetic mask exotic	Jorgensen	173271
Anaesthetic mask feline	Midmark Corporation	007296
Anti-spill funnel filler	Vetland Medical	520-11-22
Induction chamber small	MWI Animal Health	180084
Isoflurane Fluriso™	VetOne	501017
Ophthalmic lubricant	Optixcare	142423
Adapter 15mm, 15mm ID x 22mm OD	Vetamac	1422
Adapter, 15mm OD x 22mm OD	Vetamac	KC-2119
Adapter, FS-2A vaporiser inlet	MidMark	92305072
Adapter, FS-2 vaporiser outlet	MidMark	92305073
Silicone tubing 3m, 6mm ID x 10mm OD	Amazon.com	Not available
Oxygen concentrator Eclipse 5™	CAIRE Inc.	Not available
Protector Case 1650	Pelican Products	1650-020-110
Vaporizer D20 Vetland	Vetland Medical	Not available
First aid		
Eyewash solution	PhysiciansCare	24-201
Eyewash station portable	MAASTERS	Not available
DEXDOMITOR® (dexmedetomidine hydrochloride)	Zoetis	052922
Potassium chloride 20mEq/10mL	Hospira	0409-6651-06
Rabies virus post-exposure vaccine VERORAB	SANOFI PASTEUR	Not available
Saline solution sterile 0.9g/100mL	VetOne	510223
PPE		
Aprons disposable	BAYMRO	BDP461
N95 respirators	Indiana Face Mask	A105
Sleeves disposable	ULINE	S-17928
Tyvek® 400 coverall	DuPont	TY122S
Sampling		
BCM triple high mist net pole system complete	Bat Conservation and Management	Not available
Cloth drawstring bags 25x25cm	Not applicable, handmade	Not applicable
DNA/RNA Shield™	Zymo Research	R1100
Foil-barrier resealable bags	Cytiva	10534321
Fridge/Freezer portable	SnoMaster	SMDZ-LS28

GPS handheld GPSmap 60CSx	Garmin	010-00422-01
Mist nets nylon 9m x 60mm mesh	Avinet	RT09
Muzzles fabric	Coppthinktu	Not available
Sherman traps 9x3x3.5in	H.B. Sherman Traps	LFATDG
Silica gel sachets 10g	Manutan	A329277
Callipers sliding vernier 150mm	Ultrassist	Not available
Spring balances 100g	PESOLA	10100
Spring balances 500g	PESOLA	10500
Swabs sterile	Thermo Fisher Scientific	22-363-170
Tomahawk traps 19x6x6in	Tomahawk Live Trap	202
Whatman™ 903 Protein Saver cards	Cytiva	10534612

* The catalogue number of each item has been included only when unequivocally available.

APPENDIX B. CONSENT FORM FOR ANIMAL OWNERS.

Study number: STUDY00002481 Principal Investigator (s): Dr. Claire Standley, Msc PhD, Georgetown University; Dr. Alpha Mahmoud Barry, Santé Plus Organisation (Guinea)

Title: Quelle fièvre? Reducing the threat from pathogens causing febrile illness in Guinea

SITE: _____ PREFECTURE: _____

CONSENT COVER LETTER – ANIMAL SAMPLING

Dear Participant,

My name is Mr./Ms. _____, and I work for the University of Dalaba, in collaboration with the Ministry of Livestock, in collaboration with Santé Plus and Georgetown University, to conduct a research study: "Quelle fièvre? Reducing the risk of pathogens causing fever in Guinea". The general objective of the study is to identify high-consequence etiologies of acute fever illness in humans. The project is funded by the U.S. Defense Threat Reduction Agency (DTRA).

The specific objectives of the research are:

1. To identify new and/or high-risk pathogens that can cause unusual fever sicknesses in animals or people in Guinea.

2. To identify if there are signs of a group of known, concerning viruses that we know come from animals.

3. To identify factors that put people at risk of getting and/or spreading diseases from animals in Guinea.

The study includes taking samples from domestic animals, including cattle, sheep, goats, dogs, pigs and chickens, as well as some wild animals such as rodents and bats.

We are coming to you to obtain your consent to participate in our study. If you agree to participate, we will ask you to complete a short questionnaire about your animal, and collect some samples (blood, saliva, stool and urine). If your animals have ticks on them, we might also collect some for further analysis. The considerations for your participation are:

- Your participation in this research project is completely voluntary.
- You may decline to participate altogether, and you are free to stop your participation at any time.
- Your responses and any personal information collected by the study team will not mention your name.
- Data from this research will be kept under lock and key and reported only as a collective combined total. No one other than the researchers will know your individual answers to this questionnaire.
- Furthermore, we are aware of the ethics and professionalism involving the importance and the welfare of your animals and will therefore collect samples with care and in the best way possible.
- The results from the questionnaires and tests from animals sampled in the community will be grouped together.
- Feedback will be provided in line with the Ministry of Livestock for the improvement of the health of your community's animals. Any data presented or published will have all names and identifying information removed first.
- There is little overall risk to your animals during collection of samples. Your animal may experience some bruising at the site of blood collection. If needed to reduce your animal's stress or agitation for collection of samples, we may use some medication that will temporarily calm them down. This medication is short-acting and will not harm the animal or affect the meat or milk. Removing ticks may cause brief discomfort for your animal.

Study number: STUDY00002481 Principal Investigator (s): Dr. Claire Standley, Msc PhD, Georgetown University; Dr. Alpha Mahmoud Barry, Santé Plus Organisation (Guinea)

Title: Quelle fièvre? Reducing the threat from pathogens causing febrile illness in Guinea

- Samples collected from your animals will be tested for a variety of pathogens which may cause disease in animals or people. In addition to this study, they may be archived for further testing in the future to help improve understanding of human and animal health.
- All the procedures will be performed by qualified veterinarians and animal health professionals, and have been approved both by the Georgetown University Institutional Animal Care and Use Committee as well as the National Committee for Research Ethics in Guinea.

If you agree to participate in this project, please respond to questions in the attached form and also allow us to collect the needed samples from your animals. It should take approximately 30 minutes to complete both sampling and filling out the form.

If you have any questions about this project, feel free to contact Dr. Alpha Mahmoud Barry, co-lead investigator in Guinea (contact Information: (+224) 622 646480 or alphaguinea@gmail.com).

Thank you for your assistance in this important endeavor.

Please sign this form if you accept to participate in this project.

Signature of the participant: _____ Date_____

Community ID:_____ Animal owner ID: _____

Animal Owner:

Responder signature or thumbprint: _____

Date:

Person Explaining the Research:

Your signature below means that you read the entire consent form, word-for-word, to the participant and you have answered any questions about the research and that the participant provided consent to participate in the study.

_____	_____	_____
Signature of Researcher	Date	Printed Name

Witness to Explaining the Research (if needed):

Your signature below means that you witnessed the consent process performed correctly.

_____	_____	_____
Signature of Witness	Date	Printed Name

APPENDIX C. LABELLING ANIMAL SAMPLES AND DATA COLLECTION SHEETS.

The data collection sheets included herein (i.e., domestic animals, bats, and rodents/shrews) have been designed to suit animal sampling workflows as part of the Fever Project. The font coloured in light grey has been used to provide examples. We chose not to label the type of specimen collected into 1.5mL tubes containing 500µL DNA/RNA Shield™ (i.e., oral swab, rectal swab, whole blood, and ectoparasites) since different colours of tube stickers (i.e., blue, brown, red, and green) have been applied to the top of each tube for specimen recognition.

FEVER PROJECT: DATA COLLECTION SHEET FOR DOMESTIC ANIMALS													
Prefecture: Dalaba		**Sub-prefecture (sub-prefecture ID):** Mitty (MI)			**Site:** Seboury			**GPS:** 10.783, -12.309			**Environment type*:** Rural settlement		
Identification			**Physical status**					**Specimens collected**					**Notes**
Initials operator	**Species**	**Animal ID****	**Age†**	**Sex (status)††**	**Nasal discharge (yes/no)**	**Diarrhoea (yes/no)**	**Ecto‡**	**Oral swab (500µL Shield)**	**Rectal swab (500µL Shield)**	**Whole blood (500µL Shield)**	**Whole blood (903 card)**	**Ecto (500µL Shield)**	
SC	Goat	DMI-G1-4.9.23	A	F (N)	Yes	No	Ticks	Yes	Yes	Yes	Yes	7 ticks	-
SC	Chicken	DMI-K1-4.9.23	J	M	No	No	No	Yes	Yes	Yes	Yes	No	Rehydrated 20mL
SC	Dog	DMI-D1-4.9.23	A	F (E)	No	Yes	Fleas + Ticks	Yes	Yes	No	No	2 fleas 4 ticks	Nervous dog, blood not collected
CJS	Duck	DMI-U1-5.9.23	A	M	No	No	No	Yes	Yes	Yes	Yes	No	-
CJS	Chicken	DMI-K1-5.9.23	A	M	No	No	No	Yes	Yes	Yes	Yes	No	-
CJS	Dog	DMI-D1-5.9.23	J	F (N)	No	No	Ticks	Yes	Yes	Yes	Yes	2 ticks	-

* Examples of environment types are rural settlement, crop field, town centre, farm, and forest.

**** Animal ID: DMI-G1-4.9.23 = Dalaba Mitty Goat 1 Date (cattle = C; chicken = K; dog = D; duck = U; goat = G; sheep = S).**

† Animal age: adult = A; juvenile = J.

†† Animal sex and reproductive status: female = F (pregnant = E; lactating = L; not pregnant/lactating = N); male = M.

‡ Ectoparasites: ticks, fleas, lice, and mites.

FEVER PROJECT: DATA COLLECTION SHEET FOR BATS														
Prefecture: Dalaba		**Sub-prefecture (sub-prefecture ID):** Mitty (MI)			**Site:** Seboury			**GPS:** 10.783, -12.309				**Environment type*:** Forest	
Date trapping night no. 1: 04.09.23					**Time mist nets up:** 19.45							**Time mist nets down:** 00.15	
Date trapping night no. 2: 05.09.23					**Time mist nets up:** 19.20							**Time mist nets down:** 21.00	
Identification			**Physical status**					**Specimens collected**					**Notes**
Initials operator	**Species**	**Animal ID****	**Age†**	**Sex (status)††**	**Weight bat+bag (bag only) in grams**	**Length (mm) forearm**	**Ecto‡**	**Oral swab (500µL Shield)**	**Rectal swab (500µL Shield)**	**Whole blood (500µL Shield)**	**Whole blood (903 card)**	**Ecto (500µL Shield)**	
SC	R. aegyp.	DMI-B1-4.9.23	A	F (N)	130 (12)	94	Ticks	Yes	Yes	Yes	Yes	3 ticks	-
SC	R. aegyp.	DMI-B2-4.9.23	A	M (SC)	163 (12)	100	No	Yes	Yes	Yes	Yes	No	-
SC	Unknown	DMI-B3-4.9.23	A	F (E)	55 (13)	66	No	Yes	Yes	No	No	No	Released, weak
SC	H. monst.	DMI-B4-4.9.23	J	F (N)	270 (15)	123	Ticks	Yes	Yes	Yes	Yes	5 ticks	Anaesthetised
CJS	Unknown	DMI-B1-5.9.23	J	M (AB)	42 (12)	58	No	Yes	Yes	Yes	Yes	No	Juice refused
CJS	Unknown	DMI-B2-5.9.23	A	F (L)	58 (12)	65	Ticks	Yes	Yes	Yes	Yes	1 tick	-
CJS	H. monst.	-	J	F (N)	270 (12)	-	-	No	No	No	No	No	Released, re-capture

* Examples of environment types are rural settlement, crop field, town centre, farm, and forest.

**** Animal ID: DMI-B1-4.9.23 = Dalaba Mitty Bat 1 Date (bat = B).**

† Animal age: adult = A; juvenile = J.

†† Animal sex and reproductive status: female = F (pregnant = E; lactating = L; not reproducing/pregnant/lactating = N); male = M (testes abdominal/not fully descended = AB; testes scrotal/clearly visible = SC).

‡ Ectoparasites: ticks, fleas, and lice.

FEVER PROJECT: DATA COLLECTION SHEET FOR RODENTS AND SHREWS

Prefecture:	**Sub-prefecture (sub-prefecture ID):**	**Site:**	**GPS:**	**Environment type*:**
Dalaba	Mitty (MI)	Seboury	10.783, -12.309	Farm

Type and number of traps indoors per night: 60 Sherman		**Type and number of traps outdoors per night:** 40 Sherman + 15 Tomahawk	
Date trapping night no. 1: 04.09.23	**Time traps set:** 18.45	**Time traps inspected:** 06.15	**Type and number of misfires:** 3 Sherman + 1 Tomahawk
Date trapping night no. 2: 05.09.23	**Time traps set:** 18.30	**Time traps inspected:** 06.30	**Type and number of misfires:** 5 Sherman + 0 Tomahawk
Date trapping night no. 3: 06.09.23	**Time traps set:** 18.10	**Time traps inspected:** 06.10	**Type and number of misfires:** 1 Sherman + 2 Tomahawk
Date trapping night no. 4: not done	**Time traps set:** -	**Time traps inspected:** -	**Type and number of misfires:** -

Identification				**Physical status**					**Specimens collected**					
Initials operator	**Species**	**Animal ID****	**Indoors/Outdoors**	**Age†**	**Sex (status)††**	**Weight animal+bag (bag only) in grams**	**Length (mm) body (tail)**	**Ecto‡**	**Oral swab (500µL Shield)**	**Rectal swab (500µL Shield)**	**Whole blood (500µL Shield)**	**Whole blood (903 card)**	**Ecto (500µL Shield)**	**Notes**
SC	Mastomys	DMI-R1-5.9.23	Indoors	A	F (N)	80 (12)	135 (125)	Ticks	Yes	Yes	Yes	Yes	2 ticks	-
SC	Mastomys	DMI-R2-5.9.23	Outdoors	J	M (AB)	50 (12)	100 (98)	No	Yes	Yes	No	No	No	Escaped
SC	Cricetomys	DMI-R3-5.9.23	Outdoors	A	F (L)	>500g	-	Ticks + Earwigs	Yes	Yes	Yes	Yes	1 tick	Anaesthetised
CJS	Crocidura	DMI-M1-6.9.23	Outdoors	J	F (N)	40 (15)	82 (65)	Fleas	Yes	Yes	Yes	Yes	2 fleas	Anaesthetised
CJS	Unknown	DMI-R1-6.9.23	Outdoors	A	M (SC)	67 (12)	95 (110)	No	Yes	Yes	Yes	Yes	No	-
CJS	Cricetomys	-	Outdoors	A	F (L)	>500g	-	-	No	No	No	No	No	Released, re-capture
CJS	M. musc.	DMI-R1-7.9.23	Indoors	A	M (SC)	38 (15)	100 (98)	Ticks	Yes	Yes	No	No	No	Released, weak

* Examples of environment types are rural settlement, crop field, town centre, farm, and forest.

**** Animal ID: DMI-R1-4.9.23 = Dalaba Mitty Rodent 1 Date (rat and mouse = R; shrew = M).**

† Animal age: adult = A; juvenile = J.

†† Animal sex and reproductive status: female = F (pregnant = E; lactating = L; not reproducing/pregnant/lactating = N); male = M (testes abdominal/not fully descended = AB; testes scrotal/clearly visible = SC).

‡ Ectoparasites: ticks, fleas, lice, and earwigs.