Wild bat & rodent necropsy and sample collection

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Several emerging infectious diseases are currently recognized as threats to human health. A substantial number of these are zoonoses because animals are their natural reservoirs. One of the most crucial points of identifying and minimizing the impact of emerging infectious disease outbreaks is intensive and continuous surveillance. In the case of zoonoses, surveillance can be carried out in reservoir host populations where infection prevalence and population characteristics of reservoir species can be used to assess risk to humans and ameliorate or prevent outbreaks of EIDs. This manual is based on **Methods for Trapping and Sampling Small Mammals for Virologic Testing** by James N. Mills and published by the U.S. Department of Health & Human Services. It is intended as a guide for researchers performing ecologic and epidemiologic studies involving populations of potentially infected rodents. The procedures outlined are appropriate for work with any small-mammal capable of harboring infectious zoonotic agents. This protocol covers main points in which are addressed with greater detail in the original manual such as: selection of appropriate collection sites; trapping methods; handling, operation, and placement of traps for small mammals; safe and humane techniques for handling rodents; selection of sample fluids and tissues; proper storage, packaging and shipment of specimens to the laboratory; effective decontamination and cleaning of traps and other materials; safe disposal of infectious wastes; and careful collection and recording of all pertinent data.

Disclaimer

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General considerations

1) All personnel involved in capturing, handling, or sampling of bat and rodent samples are responsible for knowing and adhering to the institutional biosafety guidelines and procedures relevant to their tasks.

2) The principal investigator is responsible for ensuring that all personnel involved in the handling of bats or rodents and their organs have had the appropriate biosafety training.

3) All personnel handling bats or rodents or their biological specimens must be vaccinated against rabies virus before being involved in such activities. This should be documented in the workers medical follow-up history.

4) All personnel must agree to follow post-exposure guidelines for any injury obtained from handling bats that may represent a risk of exposure to rabies.

5) Current biosafety recommendations should be revised before the sample collection or organ harvest event to decide the level of personal protection required to prevent contamination of personnel. This risk assessment should be adapted for different biomes, geographic regions, biological specimen types, weather and season of the year.

6) Decontamination includes ***cleaning*** to remove organic material, dirt and grease as well as ***disinfecting*** to remove microbial contaminants using a suitable disinfectant. Thorough decontamination of equipment and *personal protective equipment* (PPE) are essential to protect personnel from pathogen exposure and to prevent the spread of pathogens to other wildlife **or human populations**.

7) First aid protocols for a bite, scratch or needle-stick injuries should include:

   a) Immediately cease work and notify principal investigator of accident.

   b) Wash lesion with household soap and water for full 5 minutes and then apply betadine (Povidone-iodine) or benzalkonium chloride to lesion. Benzalkonium chloride is known for its potency against rabies viruses. It is recommended that benzalkonium be kept readily available for such purposes.

   c) Post-exposure rabies vaccination should be applied immediately if bats, rodents, skunks, foxes, raccoons, coyotes or other feral canines caused lesion. The field team should carry refrigerated doses of rabies vaccine if working in a remote location so as to administer a booster dose immediately after exposure. Otherwise, exposed personnel should report to a medical clinic for administration of the booster doses according to published WHO recommendations. http://www.who.int/rabies/human/postexp/en/

Sample collection or harvest

1. If observed, place ectoparasites (flies, lice, fleas, etc.) in a pre-labelled 0.6 mL Eppendorf microcentrifuge tube previously loaded with 250 µL of 95% ethanol and stored at room temperature. Not all animals will harbour ectoparasites.

2. Take a sterile dental dacron or cotton swab and retrieve a sample of oropharingeal secretions, place in pre-labelled cryovial.

3. Take another sterile dental dacron or cotton swab and retrieve a rectal sample, place in pre-labelled cryovial.

4. Using a sterile 1 mL 27G insulin syringe previously anti-coagulated with 0.1 mL of EDTA retrieve as

much blood as possible through cardiac puncture. Place entire blood sample into pre-labelled cryovial (with both bat specimen ID and the word BLOOD). Small bats render between 0.1 mL on average, larger bats will provide around 1 mL.

5. Using strict aseptic technique take a sterile Adson dissecting tweezer and pinch the abdominal skin on the supra-pubical area and make a transverse cut using a sterile iris surgical scissor. Avoid cutting into abdominal organs!

6. Extend this incision in a caudo-cranial direction parallel to sagittal plane until the xiphoid process is reached.

7. Using a Metzenbaum scissor (or the same Iris scissors in small mammals) cut into rib cage vertically across the sternum. Open rib cage using tweezers, forceps or both BUT NEVER USING YOUR FINGERS! Gloves should remain BLOOD-FREE AT ALL TIMES. Any blood spill or stain or drop must be considered a biological hazard and immediately decontaminated.

8. Using same Adson tweezers and Iris scissors retrieve the heart and place into either a clean petri dish or P24 multi-well plate pre-filled with 0.2 mL of sterile PBS. The TEHCNICIAN will be the person responsible for breaking organs apart using scissors to allow them fit and be better preserved in the cryovials. Cryovials should be pre-labelled with animal ID and organ name as well as previously filled with either PBS (for immediate freezing) or Viral Transport Media / RNA preserving solution (for field-work harvested organs).

9. Harvest both lungs in a similar manner.

10. Harvest entire liver.

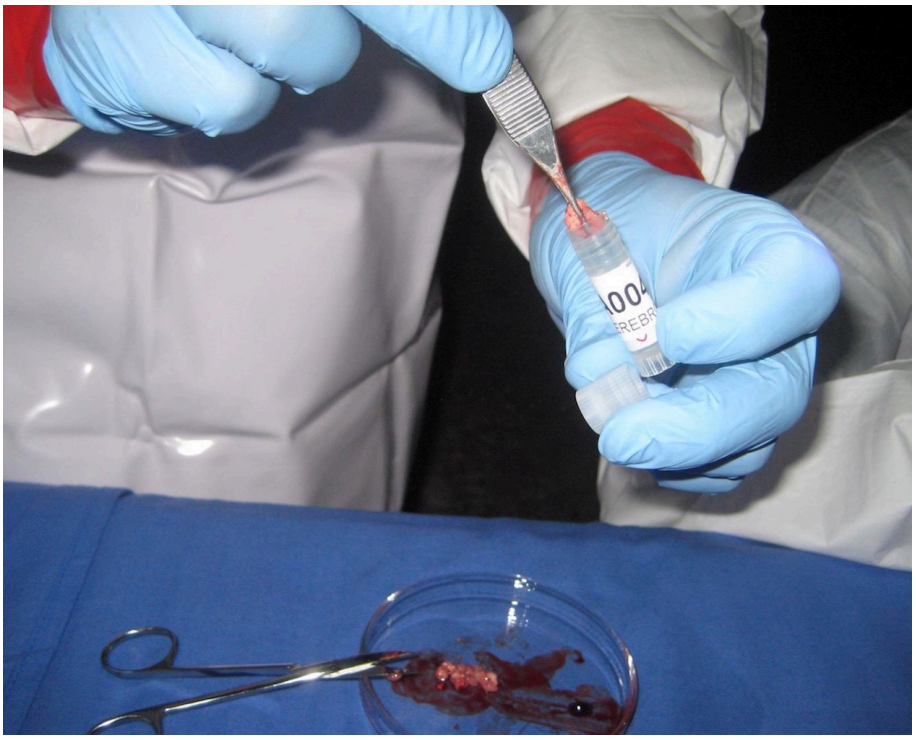
11. Harvest spleen.

12. Harvest both kidneys.

13. Harvest as much of distal intestine as will fit into cryovial.

14. Place as much paper towels as required inside the abdominal cavity of animal and flip around for brain harvest procedure.

15. Using an Iris scissor introduce a tip of the scissor into foramen magnum and make a transverse plane cut around the cranium to expose brain. Harvest as much brain as possible using non-toothed tweezers.



Brain sample being placed into correspondingly labelled cryovial.

16. Samples destined for nucleic acid based molecular analysis should be placed in cryovials having either PBS (when processed within BSL2+ lab setting) or RNA preservation solution (See http://www.genomica.uaslp.mx/Protocolos/Mol_RNA_Presv.pdf) when field processed and kept frozen at least at -10 ºC until placed on long-term -80 ºC storage.

17. Samples destined for virological analysis should be placed in cryovials having 1 mL of Viral Transport Media (see http://www.genomica.uaslp.mx/Protocolos/Viro_VTM.pdf) and kept frozen at a temperature of –20 ºC until placed on either -80 ºC or -196 ºC long-term storage.

18. Samples destined for histopathological analysis should be placed in cryovials having 1 mL of 10% buffered formalin and kept at room temperature.

19. After processing, bat carcasses should be enveloped with dry paper towels and placed, along with all biological waste material, plastics used for their processing and other paper towels, into their corresponding bag. Carcasses and these materials should not be doused with NaOCl as they will be autoclaved for decontamination.

20. Used instruments should be placed in a disinfectant bath for a minimum of 15 minutes, after which they should be scrubbed with a brush in fresh disinfectant and dried using 70% ethanol-

21. Autoclaving protocol consists of placing paper bags with bats and their contaminated materials inside the autoclave and setting it for 90 minutes at 121ºC and 15 psi (excluding exhaust time) and using a drying step of 150ºC for 32 minutes.

22. After autoclaving carcasses are placed into red biohazard bags and destined for incineration along with institutional biological waste.

References

1. James N. Mills *et al*, U.S. Department of Health & Human Services, Centers for Disease Control and Prevention. Methods for Trapping and Sampling Small Mammals for Virologic Testing. 1995.

2. Blehert, David S. White-Nose Syndrome Diagnostic Laboratory Network. Ver. 1.3 (1 Mar 2015) www.whitenosesyndrome.org/.

3. V. Shelley, S. Kaiser, E. Shelley, T. Williams, M. Kramer, K. Haman, K. Keel, and H.A. Barton - Evaluation of strategies for the decontamination of equipment for Geomyces destructans, the causative agent of White-Nose Syndrome (WNS) Journal of Cave and Karst Studies, v. 75, no. 1, p. 1-10.

Revision history

1.0 Original document.