Wild bat & rodent capture, handling and fieldwork.

Created: Nov 17, 2015; **Last modified**: Jul 14, 2017, **Version:** 2.0

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 harbouring 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

This document was made possible by the generous support of the Mexican Government through a grant (#264326) provided by the National Science and Technology Council (CONACYT) *"Apoyo complementario 2015 para infraestructura relacionada con seguridad, bioseguridad y certificación de laboratorios"* program. The document's contents are the sole responsibility of the authors and do not reflect the views of CONACYT or the Mexican Government.

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 the field team members have had the appropriate biosafety training.

3) All personnel handling bats 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.

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4) Field personnel must report to the principal investigator and 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 sampling event to decide what level of personal protection is required to prevent contamination of field 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) At least one member of the field team should have basic first aid, basic CPR training and be experienced with first aid procedures for injuries likely to be encountered in the field setting, not only bat bites.

8) 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 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/

Field team structure

Field team working with small mammals potentially bearing zoonotic pathogens should consider at least the following four participants:

1. COLLECTOR. Person in charge of collecting mammals from nets/traps and for handling live animals. Person in charge of placing animals in temporary cages or in vapour chamber for anaesthesia or euthanasia. This person should not handle animals after being dissected, nor instruments or

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biospecimens. If time and capture burden is low, can also assist in data collection by writing down parameters provided by the surgeon.

2. SURGEON. Highly trained scientist in charge of handling bats or rodents AFTER they have been anesthetized or euthanized only. Person in charge of restraining animals on dissection boards, measuring and calling out somatometric parameters to collector/helper. Provides organ and biospecimens for the technician to process. Helps TECHNICIAN in donning PPE, decontaminating, disinfecting, and doffing after fieldwork has stopped.

3. TECHNICIAN. Highly trained scientist in charge of processing organ samples as soon as harvested by the surgeon. Cuts organ samples into slices or blocks and places them in their corresponding transport vials and packaging. Helps surgeon in donning PPE, decontaminating, disinfecting, and doffing after fieldwork has stopped.

4. HELPER. Providing help with any activity NOT related to the handling of animals, specimens, or instruments. Helps set up and monitor mist nets or rodent traps as well as in setting up the field workstation, setting up a fire and providing logistics support to the remaining team members.

Personal protective equipment

1) While placing clean traps, a long-sleeved shirt, long pants, socks, and lace-up shoes should be worn. Coveralls which can be removed when the trapping/processing are completed may provide an additional measure of safety.

2) A pair of thick rubber gloves should be worn when handling traps with or without captured mammals.

3) The purpose of PPE is to protect personnel from contamination or exposure to biological agents as well as to prevent the contamination of other persons involved in downstream procedures with the samples and transmission of agents to other sampling locations, animals, populations, and regions.

4) PPE is an essential component of emerging infectious disease surveillance strategy and for work with zoonotic pathogens.

5) Planning and preparing for fieldwork should include estimating the number of sets of PPE that will be required by all personnel along with supplies for disinfecting personnel on site and containing contaminated PPE for disposal or decontamination/disinfection.

6) Personal protective equipment (PPE) varies according to the activities or field team member involved.

   a) The HELPER's activities do not require PPE as this member of the field team is not involved in animal/biospecimen handling and/or processing.

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b) The COLLECTOR's activities require basic PPE including plain long-sleeved clothing, N95 respirator and goggles or face shield and nitrile/latex gloves covered with heavy-duty electrician leather gloves to provide bite protection.

7) Both the SURGEON's and TECHNICIAN's activities require the donning of complete biosafety outfits. At minimum, both SURGEONS and TECHNICIANS should wear:

a) A disposable surgeon's gown which ties in the back or disposable coveralls,
b) Disposable shoe covers,
c) Outer and inner pairs of latex gloves, and
d) A powered air purifying respirator (PAPR) or a half-face respirator with safety goggles. Respirators should be equipped with high-efficiency particulate air (HEPA) filters.

8) Disposable clothing (gowns or coveralls, shoe-covers, and gloves) should be removed and placed in biohazard bags for disposal in accordance with state and local regulations after completion of processing and clean-up. If possible, complete on-site incineration should be considered.

Field-work technician (standing) and surgeon (seated) donning PPE, note inner surgical scrubs.

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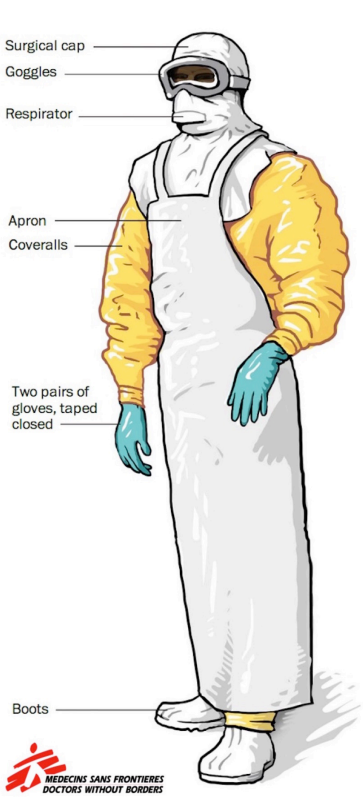
9) If coveralls or gowns are not disposable, they should be laundered on site in hot water and detergent or immersed in liquid disinfectant until they can be washed.

10) Ideal PPE for these both SURGEONS and TECHNICIANS should include personal underwear, long-sleeve water repellent but breathable (Tyvek®) or non-breathable (TyChem®) coverall with or without integrated hood, full face respirator fitted with at least N95 filter cartridges or purified air powered respirator (PAPR) fitted with at least N95 filter cartridges and an outer plastic apron. Using nitrile or latex inner gloves below coverall cuffs and either nitrile or rubber nitrile outer gloves taped above coverall cuffs as well as rubber boots (under the coverall preferred over taped over coverall).

11) At least four different respiratory protection configurations have been used in the past: 1) Goggles and N95 cup respirators (or duckbill respirator) as used by Médecins Sans Frontières (MSF) International; 2) Face shield with N95 cup respirator (or duckbill respirator) as recommended in the original Centers for Disease Control and Prevention (CDC) guidelines; 3) Full-face respirator fitted with N95 filter cartridges as recommended by US Military CBRN guidelines for field work; and 4) A powered, air-purifying respirator (PAPR) fitted with at least N95 filter cartridge (as used by CDC for field work with Hantaviruses).

12) Médecins Sans Frontières (MSF) International attire or the Centers for Disease Control and Prevention (CDC) outfit should be worn if pathogens of biological risk 3 or 4 are expected or when samples are to be obtained from animals of a previously unexplored region or for regions in which fatal, haemorrhagic, or encephalitic fever outbreaks have been recorded in the past.



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13) The MSF protective gear is considered "*the golden standard*" for clinical fieldwork with biological risk group 4 agents. Such attire is composed of inner surgical scrubs, a water repellent (Tyvek® or Tychem®) un-hooded coverall or jumpsuit, a separate water repellent hood, an outer impermeable synthetic apron or splashguard as well as dual gloves, rubber boots, face mask and goggles. The MSF attire should leave no exposed skin or hair whatsoever.

Field-station layout and PPE used by LGVH personnel for on-site bat processing.

14) The CDC recommends the use of water impermeable coveralls together with a single pair of gloves and a powered, air-purifying respirator (PAPR) for field-work with hantaviruses (see "Methods for Trapping and Sampling Small Mammals for Virologic Testing" available from http://www.cdc.gov/hantavirus/pdf/rodent_manual.pdf).

15) All reusable PPE used should be thoroughly decontaminated (meaning "***cleaned*** and ***disinfected"***) immediately after use.

16) Used PPE gear must be removed in a manner to avoid contamination of the personnel or the environment and disposed of appropriately and in a manner to avoid future contamination of other humans, animals, or the environment.

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17) Soiled laundry should be machine-dried on a high temperature setting or hung to air-dry in the sun, preferably within the laboratory.

Field-work preparations

1. All equipment must be checked and re-packaged at least one week prior to the scheduled field trip.

2. Equipment checklist used by our lab for on-site processing of captured bats includes:

    a. Basic outdoor survival kit (72 hr pack)

Equipment or material	Amount	Check
Tuna steak tins	3	☐
Instant meal noodles	3	☐
Beef jerky or dried meat	3	☐
Energy bars (per person)	3	☐
Dried-fruit portion (200 grs)	3	☐
Peanuts or grain mix (200 grs)	3	☐
Water bottle (1 liter)	3	☐
Tent, tube-tent or Beevac shelter	1	☐
Sleeping bag	1	☐
Sleeping bag under-foam	1	☐
Magnetic compass & local map	1	☐
GPS receiver unit	1	☐
Kestrel portable weather station	1	☐
Protective helmet or hardhat	4	☐
Hand lamp & spare batteries	1	☐
Headlamp & spare batteries	1	☐
Handheld transceiver radio (FRS/GMRS) & spare batteries	1	☐
Utility knife, multitool preferable	1	☐

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b. Team First Aid Kit (tFAK, can be replaced by several iFAKs)

Equipment or material	Amount	Check
Bandages low/medium compression 10 cm x 5m	3	☐
Bandages low/medium compression 7.5 cm x 5m	3	☐
Bandages low/medium compression 30 cm x 5m	3	☐
Sterile gauze 10 x 10 cm	10	☐
Sterile gauze 5 x 5 cm	10	☐
Sterile wound laparotomy compresses 45 x 70 cm	3	☐
Benzalkonium chloride solution (250 mL)	1	☐
Betadine (Povidone-iodine) 250 mL	1	☐
Sterile saline solution (1 L)	1	☐
Trauma shears	1	☐
Adhesive bandage and/or tape 5 cm wide	1	☐
Naproxen (500 mg tablets)	10	☐
Chlor-Trimeton antihistamine (12 mg tablets)	10	☐

c. Field-station equipment

Equipment or material	Amount	Check
Surgical drape (2 x 2 m)	2	☐
Rodent-prep foamy boards	10	☐
Ruler (washable) & Vernier micrometer	1	☐
Petri dishes (clean, covers included)	20	☐
Dissection scissors (Iris scissors)	5	☐
Toothed dissecting forceps (Adson forceps)	5	☐
Non-toothed dissecting forceps (DeBakey forceps)	5	☐
Cryovials (10/bat to be processed)	Variable	☐
Cryovial support rack	3	☐
Cryovial freezer box (holds 100 cryovials)	Variable	☐
Paper autoclave bags for carcasses (1 per bat)	Variable	☐
Red 2inch duct-tape roll	2	☐
NaOCl 0.5 stock solution (5 L)	1	☐
NaOCl 0.5% swan neck dispensing bottle	2	☐
NaOCl 0.1% mist spray dispensing bottle	2	☐
Ethanol 70% swan neck dispensing bottle	2	☐
Benzalkonium chloride disinfection tray (2 L)	3	☐
1 ml blood-drawing insulin syringe (1 per bat) 27G	Variable	☐
Plastic clipboard and paper sheets for annotations	2	☐

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Pen, pencil, and assorted permanent ink pens	1 each	☐
Box of medium nitrile gloves (200 gloves)	1	☐
Pair of thick rubber gloves for disinfection	2	☐
Pair of thick electrician gloves for animal handling	2	☐
Sharp's container (1 L)	1	☐
5x5 cm sterile gauze packs (pack of 3)	20	☐
Chloroform for anaesthesia/euthanasia (100 ml)	1	☐
Vapour chamber (p1000 pipette tip box)	2	☐
Disinfection toothbrush and large brush	1 each	☐
Disposable paper towel (pack of 500)	3	☐
Lighter and/or matches	1 each	☐
Printed bat capture forms	Variable	☐

(Continued from field-station equipment)

Equipment or material	Amount	Check
5 mm paracord rope (10 m)	2	☐
Mist nets (2 x 6 m)	2	☐
Mist nets (2 x 12 m)	2	☐
Flexible bat cage (nylon)	2	☐
Red biohazard bags (autoclavable) 60 x 90 cm	5	☐
Black rubbish bags 60 x 90 cm	5	☐
Digital camera, extra batteries and SD cards	1	☐
Laptop computer	1	☐
Folding plastic field-work table (1 x 1 m) minimum	1	☐
Folding plastic field-work table (1 x 3 m) optimum	1	☐
Folding plastic chairs	4	☐
Portable balance for weighing mammals	1	☐
Sterile pre-packaged cotton swabs (pack of 3)	Variable	☐
Ice-cooler for samples and dry ice or -80ºC ice packs	1	☐
N95 cup respirator	10	☐

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Field sampling station setup

1. Proper station set-up reduces time & stress to which both scientist and small mammals are subjected to by easing handling, avoiding contamination, exposure to infectious agents and minimizing unnecessary or time-consuming procedures.

2. Sampling materials should be located at a designated sampling station where investigators agree sampling will take place. A good field sampling station should be:

    a. An area that is accessible and protected from wind and wildlife.

    b. An area that is easy to disinfect.

    c. Away from public view and human interference.

    d. A location where, if decontamination efforts fail, is not likely to represent a risk for humans, livestock, or the environment.

3. Materials should be organized in a manner that allows easy access and swift processing of animals, to minimize animal stress and the handling time. Sampling materials must also be arranged for easy access by the individual doing the specific sampling technique (i.e., venipuncture, swabbing), and with sufficient space to avoid cross-contamination.

Collection and transport of captured small mammals

1. Traps or mist nets containing mammals should be handled using thick rubber gloves, as they can be easily decontaminated with disinfectant.

2. Captured mammals that have died are to be placed in their corresponding paper bags and then inside a -4ºC cooler (clearly marked with biohazard signs to avoid use for the transportation of human foodstuff).

3. Mist nets should be inspected once every 30 minutes in the search for captured specimens.

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Vampire bat (*Desmodus rotundus*) entangled in mist net.

4. Live captured bats should be retrieved from mist nets upon discovery by experienced and rabies-vaccinated personnel and immediately placed in gas permeable transport tube nets with other collected specimens. Animals destined for on-site field processing should be placed in chloroform vapour chambers for euthanasia. Those destined for lab processing should be transported as is.

5. If a pickup truck is available, the bags containing captured rodents should be transported in the bed of the truck and never in the passenger section. On hot days, the traps should be covered with a light-coloured tarp to prevent overheating of the animals. If a pickup truck is not available, animals should be transported in the trunk or roof of the vehicle. Animals and cages should not be subjected to wind drafts, direct sunlight, or rain.

6. Live mammals or those intended for release should be kept in bat cages or gas-permeable cotton bags and kept in cool dry place until sampling but for no more than 6 hours. These cages should be placed at least 10 meters away from the field-station.

7. Empty traps which have been visited by animals (as evidenced by feces, urine, or nesting) should be decontaminated before reuse or storage by submerging in disinfectant or soapy water or disinfectant.

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Vampire bat (*Desmodus rotundus*) being handled by collector fitted with heavy-duty electricians' gloves.

Data collection

1. A single team-member should be assigned the task of recording data (HELPER).

2. Cryovials, microcentrifuge tubes and other specimen vials must be labelled with a unique identifier before the fieldwork is scheduled.

3. Consistent data recording is an essential component to establishing biological specimen collections. The data collection, bat handling and bat processing members of the team should share the same criteria for subjective terms such as sample quality, health status, etc.

4. Our ***bat capture form*** consists of five sections:

    a. Capture site: includes information on date and time of sampling, location (both colloquial and Geographic Positioning System (GPS) coordinates (Latitude, longitude and altitude), weather conditions and moon phase.

    b. Specimen: includes taxonomic information, sex, reproductive and health status, feeding habits if known as well as capture motive and modality.

    c. Morphometry: includes different anatomical variables such as weight, wingspan, Cephalo-caudal, fronto-occipital, tibial, radial, and other lengths.

    d. Sample inventory: Includes information on the type of samples obtained from each

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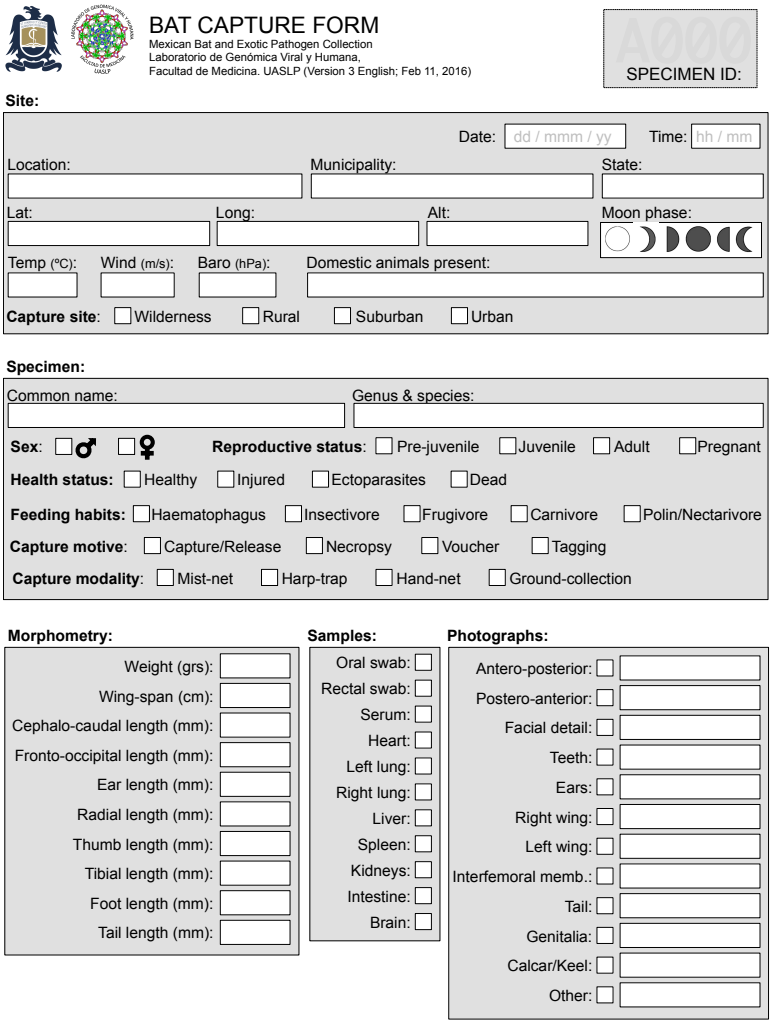
specimen.

e. Photographic record: Includes a quick checklist of photographs taken from each captured bat specimen.

5. Form information is transferred to a MS excel® sheet which so original data forms can be autoclaves and discarded.



BAT CAPTURE FORM
Mexican Bat and Exotic Pathogen Collection
Laboratorio de Genómica Viral y Humana,
Facultad de Medicina. UASLP (Version 3 English; Feb 11, 2016)

SPECIMEN ID: A000

Site:

Date: dd / mmm / yy Time: hh / mm

Location: Municipality: State:

Lat: Long: Alt: Moon phase:

Temp (°C): Wind (m/s): Baro (hPa): Domestic animals present:

Capture site: ☐ Wilderness ☐ Rural ☐ Suburban ☐ Urban

Specimen:

Common name: Genus & species:

Sex: ☐♂ ☐♀ **Reproductive status**: ☐ Pre-juvenile ☐ Juvenile ☐ Adult ☐ Pregnant

Health status: ☐ Healthy ☐ Injured ☐ Ectoparasites ☐ Dead

Feeding habits: ☐ Haematophagus ☐ Insectivore ☐ Frugivore ☐ Carnivore ☐ Polin/Nectarivore

Capture motive: ☐ Capture/Release ☐ Necropsy ☐ Voucher ☐ Tagging

Capture modality: ☐ Mist-net ☐ Harp-trap ☐ Hand-net ☐ Ground-collection

Morphometry:

Weight (grs):
Wing-span (cm):
Cephalo-caudal length (mm):
Fronto-occipital length (mm):
Ear length (mm):
Radial length (mm):
Thumb length (mm):
Tibial length (mm):
Foot length (mm):
Tail length (mm):

Samples:

Oral swab: ☐
Rectal swab: ☐
Serum: ☐
Heart: ☐
Left lung: ☐
Right lung: ☐
Liver: ☐
Spleen: ☐
Kidneys: ☐
Intestine: ☐
Brain: ☐

Photographs:

Antero-posterior: ☐
Postero-anterior: ☐
Facial detail: ☐
Teeth: ☐
Ears: ☐
Right wing: ☐
Left wing: ☐
Interfemoral memb.: ☐
Tail: ☐
Genitalia: ☐
Calcar/Keel: ☐
Other: ☐

English version of our bat capture form is also available for download from: www.genomica.uaslp.mx/Protocolos/ROBA_Bat_Coll_ENG.pdf.

The Spanish version of the same form is available from: www.genomica.uaslp.mx/Protocolos/ROBA_Bat_Coll_SPA.pdf.

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Morphometric data documentation

1. As soon as bats have been anesthetized or euthanized place animal in a pre-labelled paper bag (with animal ID and tare weight) to collect and contain ectoparasites.

2. Weigh captured animal within the bag and write net weight on bag as well as weight differential (empty bag versus bag with animal) to register animal weight.

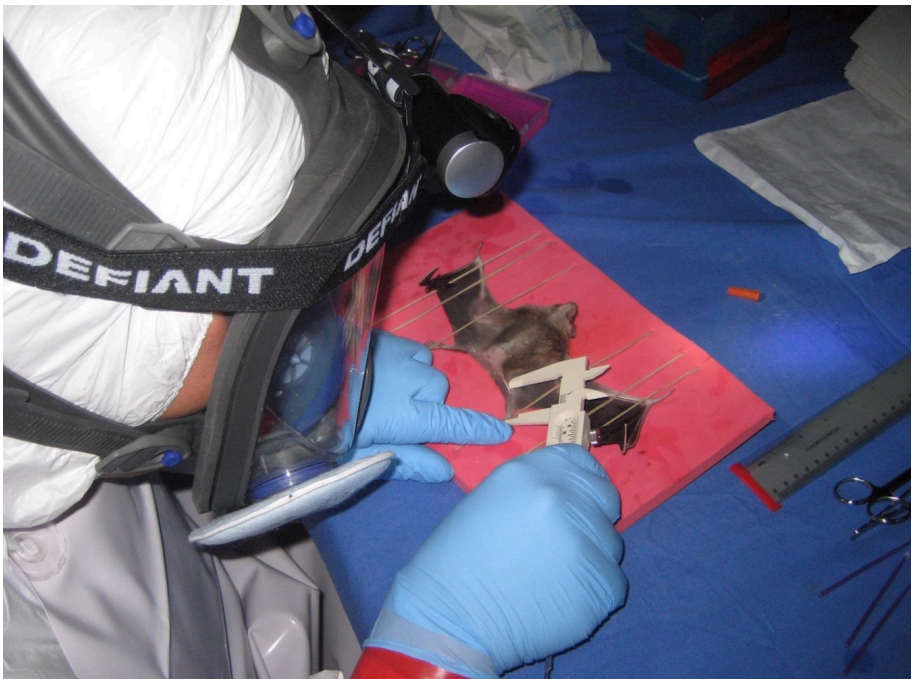
3. Place bag with euthanized animal in cooler at -4ºC until processing in BSL2+ biological containment lab.

4. Once all captured animals have been euthanized, weighed, and placed in labelled bags, introduce cooler into BSL2+ biocontainment laboratory.

5. Once within BSL2+ laboratory and using the grid etched into the main workstation make and document the necessary measurements as suggested in the previously mentioned "bat capture form".

6. After measuring animals, place in their corresponding bags and register information in bat capture form.

7. The SURGEON will request bags with euthanized animals periodically to harvest biological samples of interest (see below). These will be surgically processed within a class II type A2 biological safety cabinet located within the BSL2+ biocontainment laboratory only.



Collecting morphological information (tibial length in this case) with micrometer.

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Site decontamination

1. After all animals have been processed, place all contaminated materials including paper towels, plastic bags, gauze, cotton, table coverings, and carcass bags in a biohazard bag. Close and seal the bag with autoclave tape.

2. Prepare a fire large enough to burn all disposable materials with enough wood being made available for complete incineration of materials.

3. Wipe down all re-usable materials, working surfaces, table, chairs, and other instruments or equipment (balance, markers, even the disinfectant spray bottle themselves) with freshly prepared 0.5% NaOCl disinfectant.

4. Allow contact time of at least 15 minutes (20 recommended) for proper disinfection.

5. Spray 0.5% NaOCl disinfectant unto PPE and coverall WHILE STILL BEING WORN! Allow 15 to 20 minutes of contact time for proper disinfection.

6. Place all contaminated materials that are not disposable in fire and allow for proper incineration. Do not leave fire unattended, do not light fire until al staff have doffed from PPE. Gloves SHOULD NOT be worn while lighting or maintaining the fire.

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.

2.0 Changes to document format only.

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