USGS National Wildlife Health Center
Summer/Fall 2012 Bat Submission Guidelines

WNS Confirmed Area

(Appendix A Map – Pg. 8)

Unusual bat mortality (MAY-OCT) Pg. 3	Bats with suspicious fungus (MAY-AUG) Pg. 4	Bats with wing damage (WDI ≥ 2) (MAY-JUN) Pg. 5	Bats with suspicious WNS signs* (SEP-OCT) Pg. 5
<u>Priority Samples</u> • Any species • Any county • ≥ 5 dead bats at one location •For other situations- consult with NWHC	<u>Priority Samples</u> • Species not previously confirmed with WNS @ Gd contaminated site • Any species @ hibernaculum in county of unknown WNS status	<u>Priority Samples</u> • Species not previously confirmed with WNS in any county	<u>Priority Samples</u> • Species not previously confirmed with WNS @ Gd contaminated site • Any species @ site in county of unknown WNS status • Banded bat from Gd contaminated site @ site in county of unknown WNS status
<u>Samples to submit</u> (3-5 bats) • photos AND • fresh, intact carcasses	<u>Samples to submit</u> (1-5 bats) • photos AND • wing biopsies or fungal tape • fresh, intact carcass of non-T/E species (MAX 3)	<u>Samples to submit</u> (1-5 bats) • photos AND • wing biopsies • fresh, intact carcasses of non-T/E species (MAX 3)	<u>Samples to submit</u> (1-5 bats; MAX 3 euth) • photos AND • fresh, intact carcass of non-T/E species • wing biopsies from T/E species or banded bats

Species susceptible to WNS- *Myotis lucifugus, M. septentrionalis, M. sodalis, M. leibii, Perimyotis subflavus, Eptesicus fuscus*

* Signs include visible fungus, positive UV fluorescence on wings, WDI ≥2, suspicious behaviors (delayed arousal)

USGS National Wildlife Health Center
Summer/Fall 2012 Bat Submission Guidelines

Outside of the WNS Confirmed Area

(Appendix A Map – Pg. 8)

Unusual bat mortality (MAY-OCT) Pg. 3	Bats with suspicious fungus (MAY-AUG) Pg. 4	Bats with wing damage (WDI ≥ 2) (MAY-JUN) Pg. 6	Bats with suspicious WNS signs* (SEP-OCT) Pg. 7
Priority Samples • Any species • Any county • ≥ 5 dead bats at one location •For other situations- consult with NWHC	Priority Samples • Any hibernating species @ hibernaculum in any county	Priority Samples • Susceptible species in any county • Any hibernating species in any county	Priority Samples • Banded bat from Gd contaminated site @ hibernaculum in any county • Any hibernating species @ hibernaculum in any county
Samples to submit (3-5 bats) • photos AND • fresh, intact carcasses	Samples to submit (1-5 bats; MAX 3 euth) • photos AND • wing biopsies or fungal tape from any hibernating species • fresh, intact carcass of non-T/E species	Samples to submit (1-5 bats; MAX 3 euth) • photos AND • wing biopsies of any hibernating species • fresh, intact carcasses of non-T/E species	Samples to submit (1-5 bats; MAX 3 euth) • photos AND • fresh, intact carcass of non-T/E species or banded bat • wing biopsies from T/E species

Species susceptible to WNS- *Myotis lucifugus, M. septentrionalis, M. sodalis, M. leibii, Perimyotis subflavus, Eptesicus fuscus*

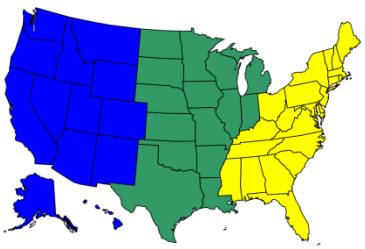
* Signs include visible fungus, positive UV fluorescence on wings, WDI ≥2, suspicious behaviors

Ver.5/7/2012

USGS

National Wildlife Health Center

Guidelines for Summer/Fall 2012 Bat Submissions (May-October)



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Purpose: The following sample submission guidelines are for use when surveying bat populations or evaluating unusual bat morbidity or mortality during Summer/Fall 2012. This document replaces the 2011/2012 Winter Submission Guidelines for Bats and all previous summer submission guidelines from the USGS- National Wildlife Health Center (NWHC).The guidelines are meant to assist with prioritizing appropriate field samples for laboratory submission based on geographic location, prior knowledge of WNS status in the area, and specific time periods. **The primary objectives of this targeted surveillance are to identify new geographic locations and bat species affected with WNS.** Because characteristic clinical signs observed during the winter do not readily apply to bats in summer and fall, surveillance/early detection of WNS during these periods is challenging. To date, WNS has not been diagnosed in bats sampled between June and August using available diagnostic methods. *Non-lethal sampling methods which test for the presence of Gd are therefore recommended in lieu of euthanasia.* **Furthermore, examination of wing tissues with long-wave ultra-violet (UV) light assists in targeting areas for diagnostic sampling.** The level of diagnostic evaluation depends on 1) the presence of unusual numbers of sick or dead bats, and 2) the distance from confirmed positive sites with greater emphasis on suspect WNS bats found outside the current disease boundaries and newly affected species located inside the disease boundaries. Limited evaluation of bats outside of these criteria will be considered on a case-by-case basis. All submissions require prior approval from the lab. There is an uncertain level of risk for fungal transmission during spring and fall trapping activities that should be considered in light of the diagnostic information to be gained and/or management action to be taken. As more observational data becomes available, summer and fall submission criteria may be revised. Please contact your regional FIT member with any questions, suggestions, or concerns (Eastern US: Anne Ballmann, 608-270-2445, aballmann@usgs.gov; Central US: LeAnn White, 608-270-2491, clwhite@usgs.gov; Western US: Barb Bodenstein, 608-270-2447, bbodenstein@usgs.gov).

Winter field signs associated with WNS in bats:

- White or gray powdery fungus seen around the muzzle, ears, wing/limbs, and/or tail;
- Excessive/unexplained bat mortality at the winter hibernaculum;
- Delayed arousal from torpor following disturbance;
- Aberrant bat behaviors (found on ground inside or outside the hibernaculum, roosting near hibernaculum entrance, increased bat activity outside the hibernaculum during cold weather) ;
- Thin body condition and/or dehydrated (wrinkled and flaky appearance of furless areas);
- Wing damage in cave bat species found outside the hibernaculum through May

WNS has been confirmed in the following species: Little brown bats (*Myotis lucifugus*); Tri-colored bats (*Perimyotis subflavus*); Northern long-eared bats (*M. septentrionalis*); Indiana bats (*M. sodalis*); Small-footed bats (*M. leibii*); Big brown bats (*Eptesicus fuscus*).

Be sure to comply with all Federal and State permits (or authorizations) when capturing and handling bats. These guidelines do not supersede permit requirements.

Unusual mortality of bats observed in any State or US Territory

1. Dead bats (typically 5 or more) of any species found in greater numbers than normally expected for the species, age class, location, or time of year are being accepted at the NWHC for diagnostic evaluation with prior approval. This includes investigating increased adult and/or pup mortalities at maternity colonies throughout the US. Single dead bats with unusual presentations will be considered on a case by case basis.

2. Collect 3-5 of the freshest carcasses (intact body, no evidence of scavenging, fur does not pull out easily, wings remain pliable, skin of face not dry or desiccated, little to no odor) which are representative of the affected species at a given site. Follow carcass collection and shipping instructions described in Appendix F. Keep individual carcasses chilled in separately labeled bags indicating:
    - date died & date collected (if different)
    - location name(nearest town, county, state)
    - collector's name & phone number
    - species
    - unique animal ID number (standard format: state, MMDDYY, collector, ###; ex: WI061610AEB001)
    - found dead or method of euthanasia

    Group all individually bagged carcasses destined for laboratory shipment into a second clean bag prior to placing samples into a field cooler with blue ice packs or traveling to additional sites.

3. If unable to ship chilled specimens within 48 hours of death for delivery to the lab no later than Thursday, freeze the carcasses and ship early the following week. ***NOTE:*** *The general public should be discouraged from handing any live bats due to the risk of rabies exposure and should be instructed to not directly contact any dead bats that they may wish to have examined. If willing, instruct public to use a disposable glove or a plastic bag placed over their hand as a protective covering to pick up the carcass if they are certain that the bat is dead. The bat carcass should then be double bagged in re-sealable plastic bag and packed on ice inside a cooler (not a food freezer) as soon as possible until you can arrive. Styrofoam coolers cannot be thoroughly disinfected and should be discarded after specimen retrieval while plastic coolers may be cleaned and decontaminated by following the current guidelines described in* "White-Nose Syndrome Decontamination Protocol-Version 03.15.2012". http://www.fws.gov/whitenosesyndrome/pdf/National_WNS_Decontamination_Protocol_v03.15.2012.pdf

4. Contact USGS-NWHC to arrange shipment and further instruction. (Eastern states: Anne Ballmann (608-270-2445; aballmann@usgs.gov); Central states: LeAnn White, (608-270-2491; clwhite@usgs.gov); Western states: Barb Bodenstein, 608-270-2447, bbodenstein@usgs.gov).

Be sure to comply with all Federal and State permits (or authorizations) when capturing and handling bats. These guidelines do not supersede permit requirements.

Any bat species with suspicious fungal growth on muzzle, ears, or wing membranes captured between May – August in any state

It is not anticipated to observe Geomyces destructans growth on bats during the summer or fall, however, we do not rule out the possibility ***particularly among bats that roost in contaminated caves or mines in WNS affected states over the summer****. To date, WNS has not been confirmed in bats examined nationwide between June and August. Non-lethal sampling methods which test for the presence of Gd are therefore recommended in lieu of euthanasia during this period (Jun-Aug). Screening wing tissues with long-wave UV light further assists with targeting suspicious areas for non-lethal sample collection. Please ensure any suspicious substance has a fuzzy or powdery, fungal-like appearance on hairless areas of the skin and is not dust, mud, cobwebs, roost-site substrate, ectoparasites, etc. If in doubt, e-mail close-up photos to your NWHC contact.*

Priority samples to submit for laboratory diagnostics:

- Species not previously confirmed with WNS with suspicious fungal growth roosting in a *G. destructans* contaminated hibernaculum
- Any species with suspicious fungal growth collected at a cave or mine located in a county of unknown status within a WNS confirmed state
- Any hibernating species with suspicious fungal growth collected at a cave or mine from outside of the WNS confirmed area (see map-APPENDIX A)

1. Photograph the affected individual prior to nonlethal sample collection and record information on data sheet (APPENDIX B). Next, collect a nonlethal sample for diagnostic evaluation. Options include wing biopsies from affected portions of the flight membranes only (APPENDIX D & E) or a fungal tape lift from the most prolific fungal growth on the bat, preferably the muzzle (APPENDIX C). Collect samples from up to 5 individuals per site. Marking sampled individuals with a wing band ID is advised prior to release. Contact NWHC prior to submission.

2. If the affected bat is a nonendangered or nonthreatened species and confirmation of WNS is desired, consider euthanasia to provide the whole carcass for diagnostic evaluation at NWHC. Take close-up photos of affected individuals prior to handling and record information on the datasheet (APPENDIX B). Collect up to 3 affected bats per location and contact NWHC prior to submission. Guidelines for humane bat euthanasia are available at: www.michigan.gov/documents/emergingdiseases/**Humane Euthanasia** of **Bats**-Final_244979_7.pdf

WNS CONFIRMED AREA ONLY- (see APPENDIX A)

Bat species not previously confirmed with WNS with evidence of wing damage (Wing Damage Index ≥ 2) detected between May-June ONLY

Protocols: Wing-Damage Index (http://www.fws.gov/WhiteNoseSyndrome/research.html)

Priority samples to submit for laboratory diagnostics:

- Species not previously confirmed with WNS with wing damage detected in any county between May-June

1. Any bat species (other than those listed on pg. 2) with evidence of moderate to severe wing damage collected between May-June from within the WNS confirmed area may be submitted. Photograph the affected individual prior to nonlethal sample collection and record information on data sheet (APPENDIX B). Wing damage is defined as multifocal depigmentation or increased translucency of flight membranes involving more than 50% of the wing surface area. Tears or holes in the membranes may also be present. Wing biopsies (2 per animal, maximum 5 bats) are acceptable (APPENDIX D & E). Marking sampled individuals with a wing band ID is advised prior to release. Contact NWHC prior to submission.

2. If the affected bat is not an endangered or threatened species and confirmation of WNS is desired, consider euthanasia to provide the whole carcass for diagnostic evaluation at NWHC. Take close-up photos of affected individuals prior to handling and record information on the datasheet (APPENDIX B). Collect up to 3 affected bats per location and contact NWHC prior to submission. Guidelines for humane bat euthanasia are available at: www.michigan.gov/documents/emergingdiseases/**Humane_Euthanasia**_of_**Bats**-Final_244979_7.pdf

Bats with suspicious physical signs of WNS between September –October

Priority samples to submit for laboratory diagnostics:

- Species not previously confirmed with WNS observed with suspicious signs (visible fungus, UV fluorescence of wings) at a contaminated hibernaculum
- Any species with suspicious signs observed at a hibernaculum in a county of unknown WNS status
- Banded susceptible species captured at a hibernaculum in a new county within a confirmed WNS positive state

1. If the affected bat is not an endangered or threatened species and confirmation of WNS is desired, consider euthanasia to provide the whole carcass for diagnostic evaluation at NWHC. Take close-up photos of affected individuals prior to handling and record information on the datasheet (APPENDIX B). Collect up to 3 affected bats per location and contact NWHC prior to submission. Guidelines for humane bat euthanasia are available at: www.michigan.gov/documents/emergingdiseases/**Humane_Euthanasia**_of_**Bats**-Final_244979_7.pdf

2. If the affected bat is an endangered or threatened species, collect a nonlethal for diagnostic evaluation. Options include wing biopsies from affected portions of the flight membranes only (APPENDIX D & E) or a fungal tape lift from the most prolific fungal growth on the bat, preferably the muzzle (APPENDIX C). Collect samples from up to 5 individuals per site. Contact NWHC prior to submission.

3. Individual bats previously banded at a *G. destructans* contaminated site that are recaptured at unconfirmed hibernacula with physical or behavioral evidence of WNS may be non-lethally sampled for evaluation. It is advised to work at the site entrance to minimize disturbance. Photograph the affected individuals prior to nonlethal sample collection and record information on data sheet (APPENDIX B). Next, collect 1 punch biopsy from an affected region of each wing membrane (APPENDIX D & E) for diagnostic evaluation. Collect samples from up to 5 individuals per site. Contact NWHC prior to submission. Information regarding band origination may be available by contacting the regional bat working groups or State WNS coordinators.

   Southeast Bat Diversity Network (Eric Britzke 864-634-3641 or Susan Loeb 864-656-4865)
   Midwest Bat Working Group: John.Whitaker@indstate.edu
   Northeast Bat Working Group: nebwg-l@list.wpunj.edu
   Western Bat Working Group: Angie McIntire 625-236-7574

Be sure to comply with all Federal and State permits (or authorizations) when capturing and handling bats. These guidelines do not supersede permit requirements.

OUTSIDE OF THE WNS CONFIRMED AREA- (see APPENDIX A)

Hibernating bat species with evidence of wing damage (Wing Damage Index ≥ 2) caught opportunistically between May-June ONLY

Protocols: Wing-Damage Index (http://www.fws.gov/WhiteNoseSyndrome/research.html)

Priority samples to submit for laboratory diagnostics:

- Susceptible species (list on pg. 2) with wing damage detected between May-June
- Any hibernating species with wing damage detected between May-June

1. Hibernating bat species (particularly *M. lucifugus, M. septentrionalis, P. subflavus*) with evidence of moderate to severe wing damage collected between May-June from states not previously known to have WNS may be submitted. Wing damage is defined as multifocal depigmentation or increased translucency of flight membranes involving more than 50% of the wing surface area. Tears or holes in the membranes may also be present. Wing biopsies (2 per animal, maximum of 5 bats) are acceptable (APPENDIX D & E). Photograph the affected individual prior sample collection and record information on data sheet (APPENDIX B).

2. If the affected bat is not an endangered or threatened species and confirmation testing for WNS is desired, consider euthanasia to provide the whole carcass for diagnostic evaluation at NWHC. Take close-up photos of affected individuals prior to handling and record information on the datasheet (APPENDIX B). Collect up to 3 affected bats per location and contact NWHC prior to submission. Guidelines for humane bat euthanasia are available at: www.michigan.gov/documents/emergingdiseases/**Humane_Euthanasia**_of_**Bats**-Final_244979_7.pdf

Bats with suspicious physical signs of WNS between September –October

Priority samples to submit for laboratory diagnostics:

- Banded susceptible species with suspicious signs of WNS (visible fungus, UV fluorescence of wings) captured at a hibernaculum outside of the WNS confirmed area
- Any hibernating species with suspicious signs captured at a hibernaculum outside of the WNS confirmed area

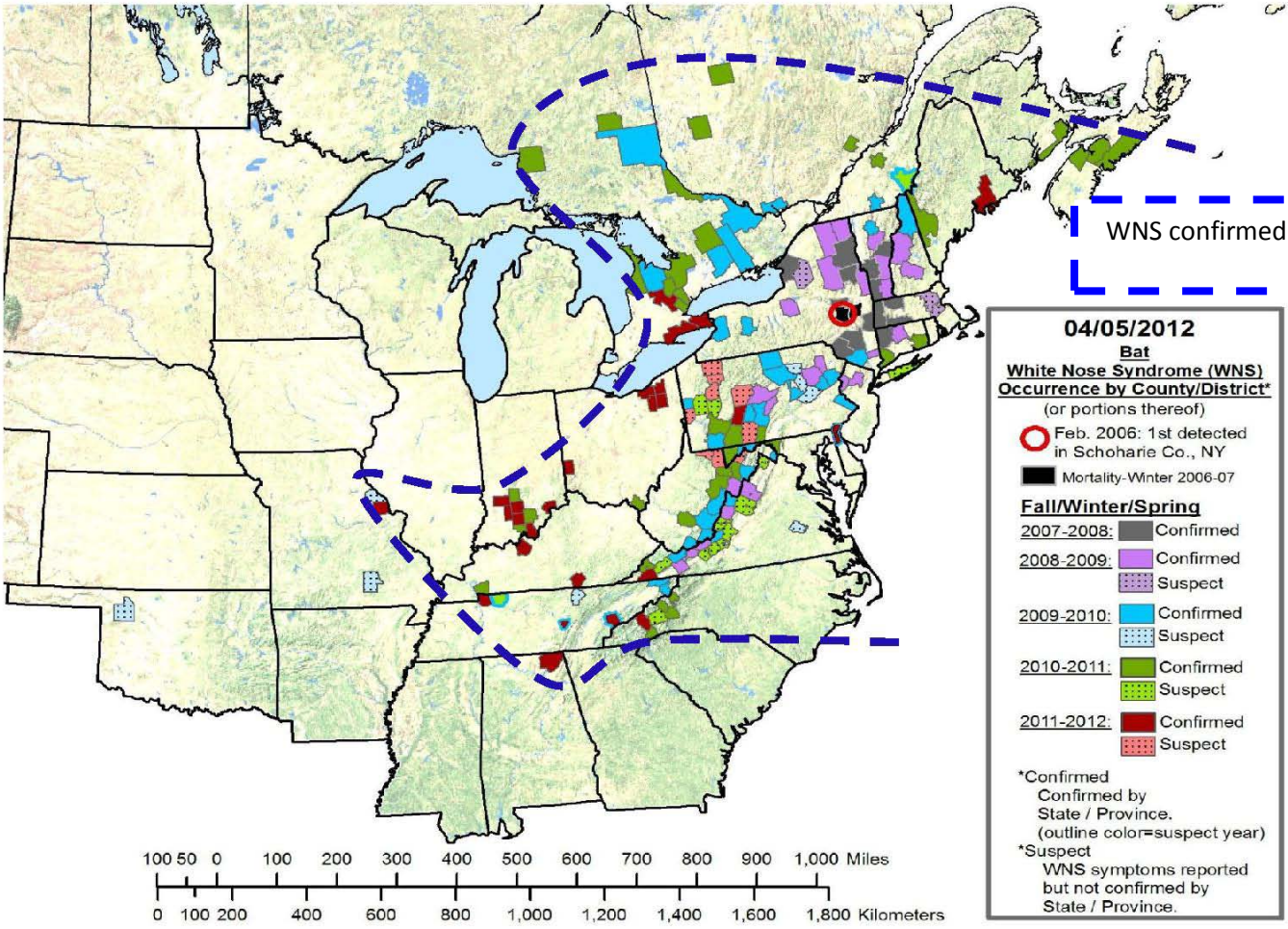
1. Individual bats previously banded at a *G. destructans* contaminated site that are recaptured at a hibernaculum outside the WNS confirmed area with physical or behavioral evidence of WNS may be submitted for evaluation. It is advised to work at the site entrance to minimize disturbance. Photograph the affected individuals prior to sample collection and record information on data sheet (APPENDIX B). A whole, fresh carcass is ideal if the affected bat is a nonthreatened or nonendangered species. Information regarding band origination may be available by contacting the regional bat working groups or State WNS Coordinator.

   Southeast Bat Diversity Network (Eric Britzke 864-634-3641 or Susan Loeb 864-656-4865)
   Midwest Bat Working Group: John.Whitaker@indstate.edu
   Northeast Bat Working Group: nebwg-l@list.wpunj.edu
   Western Bat Working Group: Angie McIntire 625-236-7574

2. If the affected bat is not an endangered or threatened species and confirmation of WNS is desired, consider euthanasia to provide the whole carcass for diagnostic evaluation at NWHC. Take close-up photos of affected individuals prior to handling and record information on the datasheet (APPENDIX B). Collect up to 3 affected bats per location and contact NWHC prior to submission. Guidelines for humane bat euthanasia are available at: www.michigan.gov/documents/emergingdiseases/**Humane_Euthanasia**_of_**Bats**-Final_244979_7.pdf

3. If the affected bat is an endangered or threatened species, photograph the affected individual prior to nonlethal sample collection and record information on data sheet (APPENDIX B). Next, collect 1 punch biopsy from affected region of each wing membrane (APPENDIX D & E) for diagnostic evaluation. Collect samples from up to 3 individuals per site. Contact NWHC prior to submission.

APPENDIX A



Most current map updates are posted at http://www.fws.gov/WhiteNoseSyndrome/maps.html

Modified from map by Cal Butchkoski, PA Game Commission

APPENDIX B – USGS NWHC Summer/Fall 2012 Bat Submission Datasheet

Date (MMDDYY):___________________
Location ID:_______________________
(*circle one*: maternity colony; bachelor colony;
day roost; hibernaculum; other_______________________)
County:________________
State:_______
Decimal degrees (NAD83): N_______________ E_______________
Collector:_____________________ e-mail: ___________________

Estimated number of live bats at site___________; Estimated number of dead bats__________
Bats present (G. species) & estimated % of total popn: ________________ (____%);________________ (____%);
________________ (___%); ________________ (____%); __________________ (____%)
Percent of total population affected by clinical signs: ______________
Percentage of each species affected: ________________ (____%);________________ (____%);
________________ (____%);________________ (____%);________________ (____%)
Distribution pattern of affected bats at site*:* solitary *vs.* clustered;
(circle one from each row) outer periphery *vs.* inner region *vs.* throughout site

ID or Band# (state, MMDDYY, collector, ###)	**Species** (4 letter code)	**Sex** *(circle one)*	**Status** (Live, Dead, Euth)	**Age Class** (Juv, Adult, Unknown)	**Weight (g)**	**Forearm length (mm)**	**Wing Damage Index** *(circle one)*	**Photo file ID**	**Disposition** (Released, Fungal Tape, Wing Biopsy, Whole Carcass, Archived)	**Comments/Notes Key**
		M F	L D E	J A U			0 1 2 3		R T B C A	
		M F	L D E	J A U			0 1 2 3		R T B C A	
		M F	L D E	J A U			0 1 2 3		R T B C A	
		M F	L D E	J A U			0 1 2 3		R T B C A	
		M F	L D E	J A U			0 1 2 3		R T B C A	
		M F	L D E	J A U			0 1 2 3		R T B C A	
		M F	L D E	J A U			0 1 2 3		R T B C A	
		M F	L D E	J A U			0 1 2 3		R T B C A	
		M F	L D E	J A U			0 1 2 3		R T B C A	
		M F	L D E	J A U			0 1 2 3		R T B C A	

Additional Notes/Diagrams:

APPENDIX C - Fungal tape-lift protocol for bats

Protocol: Tape-Strip Sampling of Bats for Identification of *Geomyces destructans* Fungal Infection

Authors: David S. Blehert and Anne Ballmann, USGS – National Wildlife Health Center

Date: 7 October 2009 (modified)

Purpose: The following procedure is designed to collect fungi from the skin of bats for later microscopic analyses while minimizing harm to the sampled bat.

Required materials:

NOTE- Neither the USGS nor the NWHC endorse these vendors as the only sources of these products. This information is provided only as a guideline.

1) Glass microscope slides with white label (25 mm (W) X 75 mm (L); 1 mm thick). Fisher Scientific Catalog #12-552. Fisher list price $58.34 pack (144/pack).

2) Fungi-Tape (25 yards X 1 inch; approximately 1 mm thick). Fisher Scientific Catalog #23-769-321 (Scientific Device Laboratory No. 745). Fisher list price $35.59 per box.

3) Plastic 5-slide transport mailers. (Maximum capacity is 10 slides per mailer – see instruction #9 below). Fisher Scientific Catalog #12-569-35 ($31.00 for pack of 25) or #12-587-17B ($185.35 for pack of 200).

4) Pencil

Procedure:

1) Wear new disposable gloves when handling each individual bat to reduce the risk of cross-contamination.

2) Label the end of a microscope slide in pencil with an animal ID number, date, and anatomical sample location.

3) Remove a precut piece of Fungi-Tape from the box being careful not to contaminate the adhesive surface.

4) Bend the tape-strip (without creasing), adhesive-side out, between your thumb and index finger so that the tape forms the shape of a “U” (Fig. 1).

5) Sample muzzles of bats with grossly visible blooms of fungal growth. When possible, avoid collecting samples from wing membranes as analyses of unfurred skin have not been reliable in detection of *Geomyces destructans*.

6) Lightly touch the adhesive surface of the tape-strip, at the bottom of the “U”, to an area of suspect fungal growth on bat surface (Fig. 2). DO NOT use your finger to press the tape down onto the bat’s muzzle. Attempt to maximize adherence of fungus to the tape adhesive while minimizing adherence of hair (Fig. 3).

7) If only a small area is transferred to the tape, use a different portion of the same tape "U" to touch another area of visible fungal growth on the bat. DO NOT attempt to obtain more than 3 lifts per tape strip. **Collect only 1 tape-strip per live bat.**

8) Align the tape-strip containing the fungal sample, adhesive-side down, over the microscope slide. Ensure that the edges of the tape-strip do not protrude beyond the edges of the microscope slide when laid flat, and do not remove any portion of the tape-strip from the glass slide once it has adhered (Fig. 4).

9) Lightly wipe over the top surface of the tape-strip using a clean paper or cloth towel to consistently adhere the strip to the slide. Circle the area of tape used to transfer the fungus with a permanent marker.

10) Place each slide into a slide mailer for safe transport. If 2 slides are placed per slot, ensure that the tape surfaces of each slide are facing outwards (only the non-tape sides should be in contact so as not to crush the tape). Seal the slide mailer shut with standard tape or rubber bands prior to shipment.

11) Place slide mailer(s) into a clean Ziploc bag and seal closed to transport from the hibernaculum. Place in a second Ziploc bag

12) The slide mailers can now be held at ambient temperature and shipped to the NWHC for microscopic examination. Ship mailers in a padded envelop with a completed specimen history form. If including slide mailers in a cooler shipment with bat carcasses, ensure that the slide mailers are not in contact with the blue ice. Send an electronic copy of the completed specimen history form to LeAnn White (clwhite@usgs.gov) or Anne Ballmann (aballmann@usgs.gov). Contact Anne (608-270-2445) or LeAnn (608-270-2491) if you have any additional questions.

APPENDIX C. Illustrations – Fungal tape-lift protocol for bats

-Photographs by D. Berndt and D. Johnson, USGS – NWHC

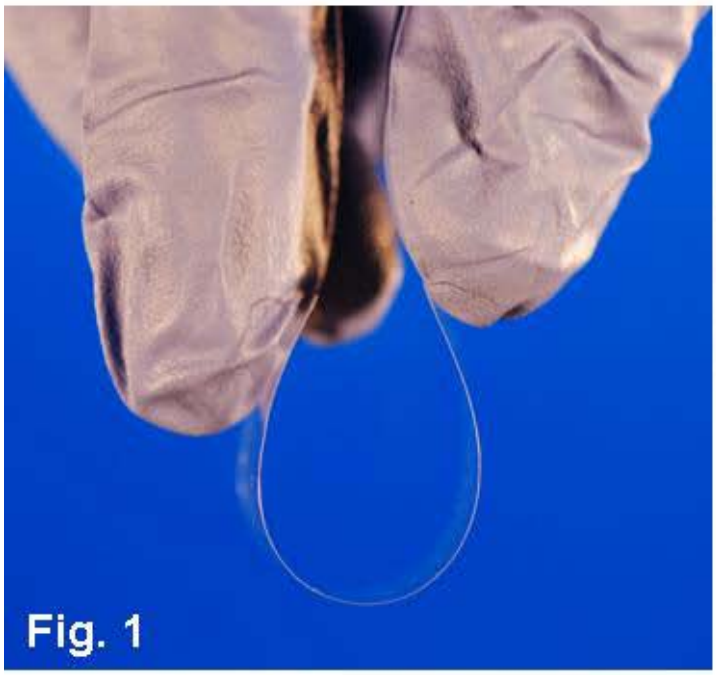


Fig. 1

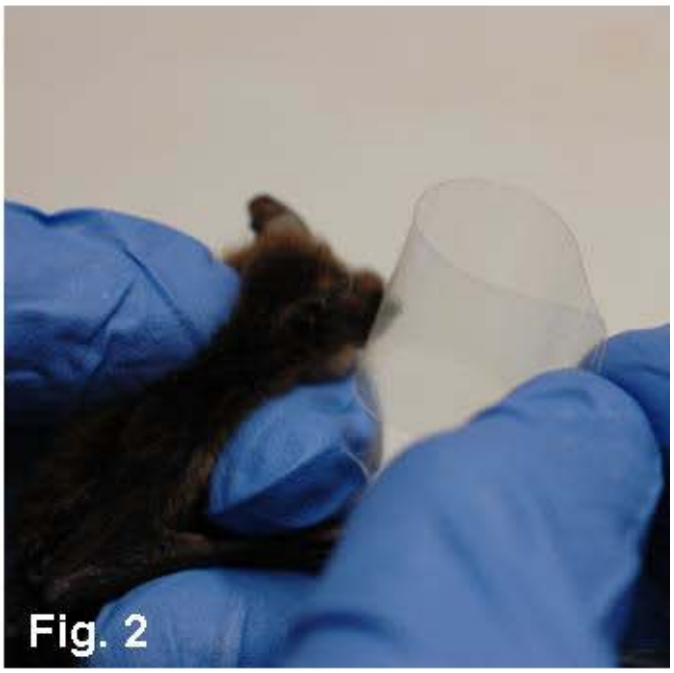


Fig. 2

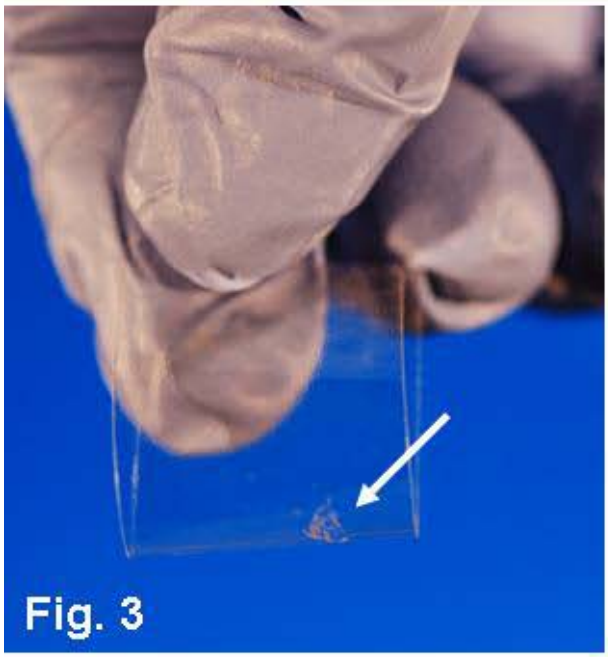


Fig. 3

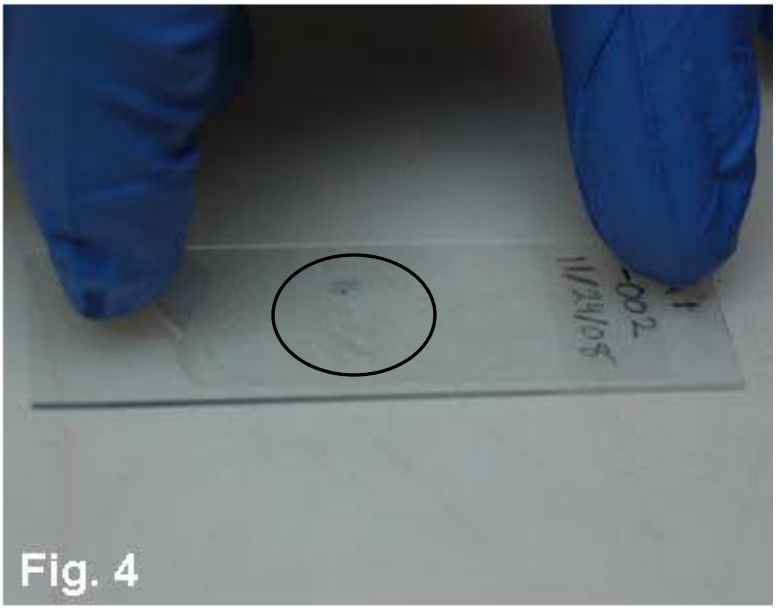


Fig. 4

Appendix D - Instructions for Taking a Wing Membrane Biopsy

Updated by Pat Ormsbee and Jan Zinck 5/14/09 (original: Shonene Scott, Portland State University 5/2003)
Modified by Anne Ballmann 4/28/12

NOTE: If punch biopsies are the only sample type to be submitted to the lab for PCR testing of *G. destructans* in a particular case, it is highly recommended that 2 biopsies per bat be collected (from different wings). Additional population genetic sampling should not be attempted in these individuals to reduce the number of holes in the wings.

1. When taking biopsies it is important to reduce the potential for cross-contamination between bats. In order to do this, use a small clean piece of sturdy cardboard that can be discarded after each animal, a new tissue punch for each sample, sterilized forceps, and disposable gloves.

2. Label a sterile vial: Use a black ultra-fine Sharpie permanent marker and a sticky paper label. Be careful that once the label is adhered to the tube the entire identifier is visible. Use the following naming convention to uniquely identify the bat:

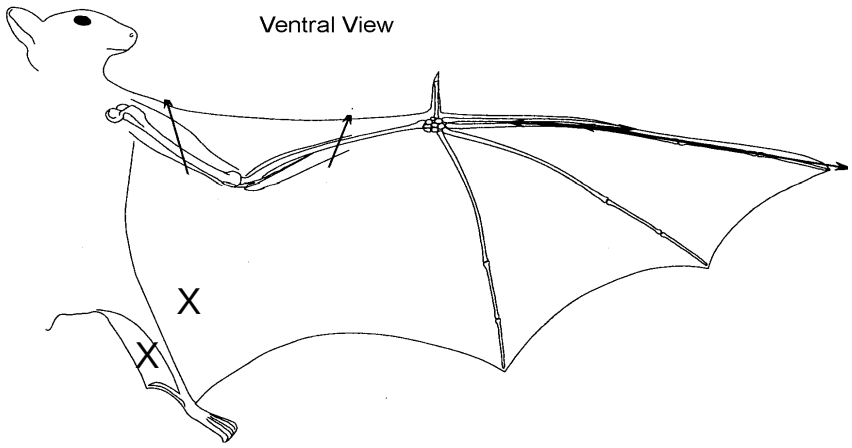
3. State, Date (MMDDYY), Collector initials, bat number (ex: WI061609AEB001)

4. Have a fresh cardboard square, a labeled tube, a new tissue punch, and a sterilized forceps ready. Do not touch (contaminate) the end of the punch, the forceps, or the inside of the tube lid with fingers or environmental debris.

5. Identify 2 representative lesions to biopsy on the affected wings/tail of the bat. Place the bat on the cardboard on its back and extend one wing membrane (Avoid sampling from bats with large wing tears). For people inexperienced in this technique, it works best when one person holds the bat and another person collects the biopsy.

6. When collecting wing tissue biopsies, avoid bones and major blood vessels. (Figure 1). ***Long-wave UV light can optimize biopsy placement and allows for additional histopathological evaluation (target areas exhibit faint yellow-orange fluorescent spotting-See APPENDIX E).*** <u>If possible, locate an affected area near the body wall</u> within the lower half of the wing membrane or uropatagium. Press the punch firmly through the membrane and twist the punch slightly to ensure a complete punch. Apply direct pressure to biopsy site for several minutes if bleeding occurs.

Figure 1: "X" marks ideal sample locations for collecting tissue biopsies from flight membranes.



6. Carefully lift the bat off the biopsy board and look for the tissue sample. It should either be on the board or inside the tip of the punch. Be careful on windy days since the wind can blow the tissue off of the board. A new 25 ga needle or sterile forceps can be used to pick up the tissue and transfer each biopsy to separate storage vials which contain no storage media.

7. Release the bat only after tissue samples have been placed into the tubes, the tubes have been closed, and any bleeding has stopped. The number of biopsies has been limited to 2 per bat to prevent compromising flight.

8. While in the field, sample tubes should be stored on ice. Subsequently, samples should be frozen until submitted for fungal PCR analysis.

9. Dispose of the used biopsy punch after each animal. DO NOT reuse the same biopsy punch on multiple bats. The punches are very sharp. Be careful to not cut yourself. Change into new gloves before handling each bat.

10. Before reusing forceps while in the field, follow the flame sterilization protocols described in "Disinfection Protocol for Bat Field Research/Monitoring, June 2009" (http://www.fws.gov/northeast/wnsresearchmonitoring.html). Upon returning to the office, perform a more thorough cleaning and disinfection of nondisposable biopsy equipment with detergent washing followed by soaking in a 10% bleach solution for 10 min with a thorough clean water rinse. Once dry, forceps can be placed into a clean hard surface container (not plastic bags), free of contaminates, marked for cleaned forceps, and with handles all pointing in the same direction.

11. Ship wing tissues to NWHC: ensure that all cryovials are labeled and lids are secured in place to prevent cross-contamination of samples. Wrap lid of cryovials in parafilm and place in a Ziploc bag. If parafilm is not available double-bag specimens before placing in cooler. Specimens should be chilled and shipped overnight in a cooler with blue ice. If samples cannot be shipped overnight freeze them and ship as soon as possible.
Send an electronic copy of the completed specimen history form or datasheet to the appropriate NWHC contact . Specimen history form, shipping address, and examples of appropriate shipping materials are in Appendix F. Contact Anne Ballmann (aballmann@usgs.gov , 608-270-2445) if you have any additional questions.

SUPPLIES: NOTE- Neither the USGS nor the NWHC endorse these vendors as the only sources of these products. This information is provided only as a guideline

- 3-5mm biopsy punches Fisher Scientific Catalog # NC9515874 ($106.73/pack of 50) for PCR or histopathology
- Forceps **OR** 25 gauge needles and sharps collection container
- 10% bleach solution (can be made fresh each time, or can be stored in opaque containers for 24 hours, it begins to break down after this)
- Sterile rinse water
- 2 ml sterile plastic vials with caps
- 95% ethanol and flame source such as cigarette lighter (for sterilizing metal sampling equipment)
- Fine point permanent marker
- Vial labels
- Disposable gloves
- Paper towels/gauze
- Nonporous cutting board
- Ziploc bags and cooler with blue ice

APPENDIX E

Protocol: UV fluorescence screening of bat wings
Authors: Anne Ballmann, Carol Meteyer (modified from G. Turner & J. Gumbs 2011)
Date: 5/7/2012

Purpose: To examine bat wings with little to no visible fungal growth for evidence of yellow-orange fluorescence areas suggestive of an infection by *Geomyces destructans*

Equipment:
NOTE- Neither the USGS nor the NWHC endorse these vendors as the only sources of these products. This information is provided only as a guideline.

- 380-385 nm wavelength UV 51 bulb LED flashlight (LED Wholesaler #7202UV385-$35) or 368 nm wavelength 9 V UV box (Contact Greg Turner [gturner@pagc.gov] for more details on UV box system)
- Disposable exam gloves
- Digital camera
- Permanent marker
- PPE: UVA blocking safety glasses, SPF30+ sunblock on exposed human skin

Optional equipment for non-lethal sample collection-

- 2 ml sterile vials with screw cap lids
- 10% buffered neutral formalin
- 3-5 mm sterile punch biopsies

Procedure: ***(To reduce potential cross-contamination, use clean exam gloves when handling each bat.)***

1. In complete darkness, shine the UV flashlight facing down approximately 3-5 inches (7.5-12.5 cm) above the extended ventral surface of the flight membranes (Fig. 1A). If using a UV box, place the bat on its back and extend the wing and corresponding foot over the UV light source to transilluminate the wing surface. Disinfect surface of UV box between bats. Avoid shining the light into the unprotected eyes of the bat or people or exposing bat skin to UV light for more than 3 minutes.

2. Examine wing membrane for circular areas of yellow-orange fluorescence (Fig. 1B). Fluorescence will be faint when viewed with the naked eye using a hand-held UV flashlight. Visualization is greatly enhanced by examining a digital photograph of the UV-illuminated wing surface when using the UV box. Photography does not improve visualization with the UV flashlight.

3. If the bat is to be euthanized, use a permanent marker to circle representative areas of fluorescence on the wing membrane to target sampling in the laboratory. Place marks outside of the fluorescent border.

4. If live-sampling techniques are used, collect paired wing punch biopsies (3-5 mm diameter, See Appendix D) that incorporate areas of UV fluorescence. Place one wing biopsy into a 2ml vial containing 1.5 ml of 10% buffered neutral formalin for histology. Place the second wing biopsy into an empty vial for PCR and keep chilled in the field. Label vials with the unique bat ID number.

5. Submit samples along with any digital photos of fluoresced wings to the appropriate FIT contact at NWHC.

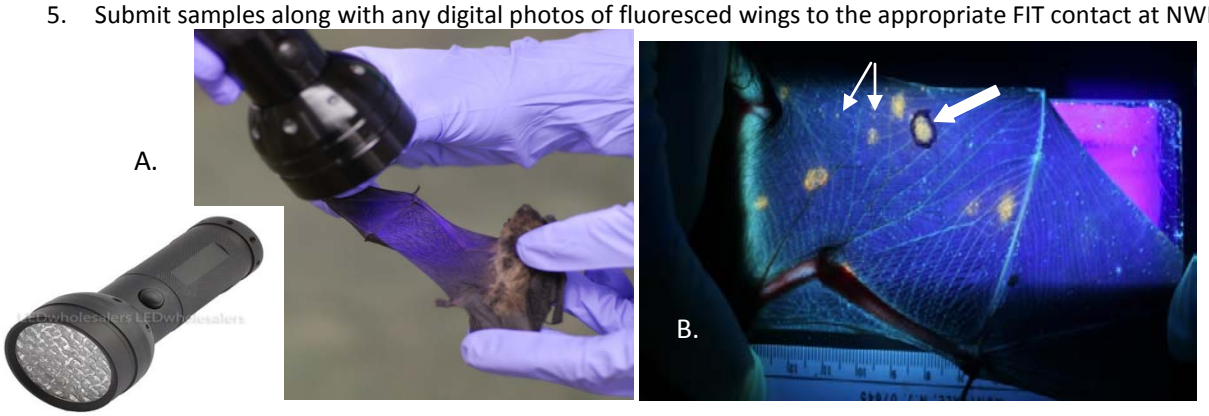


Figure 1. A) UV flashlight examination of ventral bat wing to be conducted in total darkness. B) Digital photo of backlit extended wing held over 368 nm UV light box. Arrows identify yellow-orange fluorescent areas of various diameters associated with suspect *G. destructans* infection.

Appendix F

National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711
Phone: 608.270.2400
FAX: 608.270.2415

SPECIMEN HISTORY FORM

For mortality events please e-mail a USGS Field Investigation Team member before shipping
Eastern States: Anne Ballmann aballmann@usgs.gov , 608-270-2445
Central States: LeAnn White clwhite@usgs.gov, 608-270-2491
Western States: Barb Bodenstein bbodenstein@usgs.gov, 608-270-2447
For single animal cases, Nationwide: Jennifer Buckner jbradsby@usgs.gov, 608-270-2443

Submitter's name: **Telephone:**
Address:

E-mail:

Collector's Name: **Affiliation:**

Telephone:

E-mail:

Date collected:
Method of animal collection: ☐ **Found Dead,** ☐ **Died in Hand,** ☐ **Euthanized**
Method of euthanization:
Species:
Number Submitted: **Condition:** ☐ **Chilled,** ☐ **Frozen,** ☐ **Preserved Tissues**

Specific die-off location (refuge unit, pond, address, intersection, park, etc):

State: **County:** **Nearest City:**

Latitude/longitude (Decimal degree in WGS 84): **Zone:**

Disease onset date: (Best estimate) **Disease end date:** (best estimate)

Species affected: (The diversity of species affected may provide clues to the disease involved.)

Age/sex: (Any pattern noticed that is related to age and sex?)

Known dead: (Actual number counted) **Known sick:**

Estimated dead: **Estimated sick:**
(Consider removal by scavengers or other means, density of vegetation, etc.)

Clinical signs: (Any unusual behavior and physical appearance.)

Population at risk: (Number of animals in the area that could be exposed to the disease.)

Population movement: (Recent changes in number of animals on area and their source or destination, if known.)

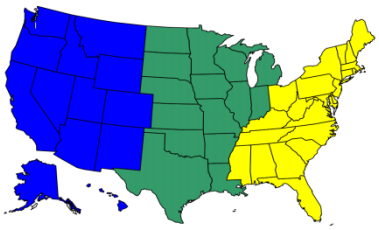
Problem area description: (Land use, habitat types, and other distinctive features.)

Environmental factors: (Record conditions such as storms, precipitation, temperature changes, or other changes that may contribute to stress.)
Comments: (Additional information/observations of value such as past occurrences of disease in area, photographs or videos)

Appendix F **USGS – National Wildlife Health Center**

INSTRUCTIONS FOR COLLECTION AND SHIPMENT OF BAT CARCASSES

Contact your USGS Field Investigation Team (FIT) member first!
Eastern states – Anne Ballmann aballmann@usgs.gov 608-270-2445
Central states - LeAnn White clwhite@usgs.gov 608-270-2491
Western states – Barb Bodenstein bbodenstein@usgs.gov 608-270-2447
Single animal cases, Nationwide: Jennifer Buckner jbradsby@usgs.gov,
Emergency Contact Number 608-270-2400



The following instructions should be used for collecting and shipping wildlife carcasses, carcass parts, and samples extracted from animals to the National Wildlife Health Center (NWHC) to insure adequate and well preserved specimens. Freezing/thawing impedes isolation of some pathogens and damages tissues. NWHC prefers unfrozen specimens if they can be sent within 24-36 hours of collection or death. We will provide guidance on freezing samples on a case-by-case basis. As a general guideline: if you cannot call or ship within 24-36 hours, freeze the animal(s).

☐ Contact FIT to get shipping approval and discuss shipping arrangements. Typically, ship specimens by 1-day (overnight) service, Monday through Wednesday, to guarantee arrival at NWHC before the weekend. If specimens are fresh and need to be shipped on Thursday or Friday, special arrangements can be made.

☐ Email/fax history and tracking number to FIT. Packages will not be opened if history does not arrive first!

☐ Use rubber, vinyl, or nitrile gloves when picking up sick or dead animals. If you do not have gloves, insert your hand into a plastic bag.

☐ More than one disease may be affecting the population simultaneously. When possible, collect both sick and dead animals. Note behavior of sick animals before euthanizing.

☐ Collect specimens that are representative of all species affected and geographic areas.

☐ Collect the freshest dead specimens. Decomposed or scavenged carcasses are usually of limited diagnostic value. If you plan to collect animals in the field, take along a cooler containing ice to immediately chill carcasses.

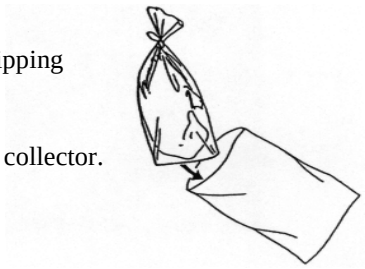
☐ Contact NWHC for assistance when collecting samples from animals that are too large to ship.

☐ Collect animals under the assumption that an infectious disease or toxin is involved and other animals may be at risk. Protect yourself as some diseases and toxins are hazardous to humans.

☐ Place each animal in a plastic bag, close, and seal the bag. Twist non-zipper bags closed, fold over on itself, and secure with package strapping or duct tape. Label the outside of this bag with the following information in waterproof ink:

- Date collected
- Location (specific site, town, county, state)
- Collector (name/address/phone)
- Species
- -Found dead or euthanized
- -Your reference #

☐ Place 1st bag inside a 2nd bag, close and seal. More than one individually bagged animal can be placed in the 2nd bag. This prevents cross-contamination of individual specimens and leaking shipping containers.



☐ Tag the outside of 2nd bag and number of animals and type, date collected, location, and name of collector. Reminder order: TAG, BAG, BAG, TAG.

- □ Use a hard-sided cooler in good condition for shipment. Close the drain plug of cooler and tape over inside. Line cooler with a thick bag (1 mil thickness, 3rd layer of bags).
- □ Place absorbent material in the 3rd plastic bag to absorb any liquids that might leak during shipping.
  See appendix for examples of bags and absorbent materials.
- □ Pack the individually bagged animal(s) that are contained within the 2nd sealed bag into the 3rd bag with enough FROZEN BLUE ICE PACKS or similar coolant to keep carcasses cold. Use enough coolant to keep samples chilled if there is a delay in delivery.
  - Blue ice (unfrozen) can be obtained at hardware, sporting goods, or grocery stores.
  - Wet ice can be used if frozen in a sealed plastic container (i.e., soda or water bottle).
  - DO NOT USE DRY ICE.
- □ Seal the 3rd bag with methods described for 1st bag.
- □ Place the completed specimen history and return shipping label in a ziplock bag and tape to the inside lid of the cooler (if you want theplastic cooler returned). NWHC CANNOT PAY FOR SHIPPING.
- □ Using packing or duct tape, tape the cooler shut around the lid and at each end using a continuous wrap around the cooler.
- □ Attach the shipping document (airbill) with the DOT information below to the outside of each cooler in a resealable pouch:

  Address:

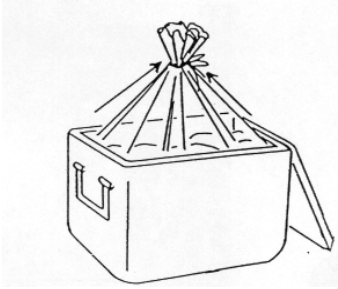
  National Wildlife Health Center
  Necropsy Loading Dock
  6006 Schroeder Road
  Madison, WI 53711

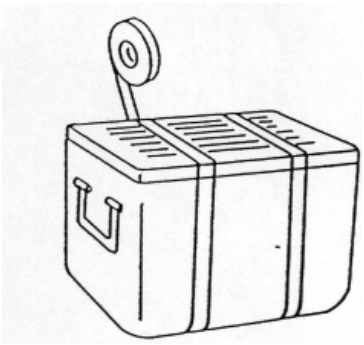
  Emergency Contact:

  NWHC FIT emergency
  608-270-2400

  Supplementary Labels**:**

  Keep Cold





- □ Mark the cooler with the appropriate information:

  (See Pg. 3 for printable marking labels)

  - Carcasses of animals that died of unknown causes:

    BIOLOGICAL SUBSTANCE, CATEGORY B and **UN 3373.**
  - Blood and tissue samples from apparently healthy animals (hunter-killed, live captured):

    EXEMPT ANIMAL SPECIMENS.
  - Blood and tissue samples from dead or sick animals:

    BIOLOGICAL SUBSTANCE, CATEGORY B and **UN 3373**.

. Note the tracking number in case packages are delayed.

- □ These instructions cover federal shipping regulations for commercial carriers.

Appendix:

Example of bags available at large supermarkets (list not all inclusive):

Inner and second layer bags:

Hefty Big Bag – 22 gal	Ziplock Freezer – 1 gallon
Hefty Freezer – 1 gal	Ziplock Big Bag – 20 gallon
Hefty Jumbo – 2.5 gal	Glad Freezer – 1 qt, 2 qt, 1 gal

Third layer for cooler liner:

Hefty Cinch Sak (1.1 mil) – 33 and 39 gal	Glad Force Flex (1.05 mil) – 25 gal
Hefty Lawn and Leaf (1.1 mil) – 33 and 39 gal	Hefty Ultra Flex (1.3 mil) – 30 gal
House brand large trash (1.1 mil) – 30 gal	House Lawn - Leaf (1.2 mil) – 39 gal

Absorbent material:

Super absorbent packet or pads for water	Cellulose wadding
Paper towels	Cotton batting or cotton balls

Do not use packing peanuts or shredded paper.

. .

BIOLOGICAL SUBSTANCES, CATEGORY B

EXEMPT ANIMAL SPECIMENS