

USGS National Wildlife Health Center  
Winter 2014/2015 Bat Submission Reference Chart

**Within the WNS Endemic Area**

(Appendix A Map – Pg. 10)

Unusual bat mortality/behavior  
not associated with WNS  
(NOV-MAY)  
Pg. 6

Bats with signs suggestive of WNS\*  
(NOV-MAY)  
Pg. 7-8

Priority Samples

- Any species
- Any county
- ≥ 5 dead/sick bats at one location
  
- For other situations- consult with NWHC

Priority Samples

- Species not previously confirmed with WNS at/near a contaminated hibernaculum in a confirmed county
  
- Any species at a hibernaculum of suspect or unknown status in an unconfirmed county

Samples to submit

- (5-8 bats)
- photos AND
  - fresh, intact carcasses
  - MAXIMUM of 3 euthanized non-T/E bats per site

Samples to submit

- (1-5 bats)
- photos AND
  - fresh, intact carcass or UV-guided wing biopsies of any species
  - combined wing/muzzle swab, or fungal tape if WNS confirmation is NOT required
  - Euthanasia of sick, live bats is not advised except for species not previously confirmed with WNS (MAXIMUM of 3 euthanized non-T/E bats per site )

Species confirmed with WNS- *Myotis lucifugus*, *M. septentrionalis*, *M. sodalis*, *M. leibii*, *M. grisescens*, *Perimyotis subflavus*, *Eptesicus fuscus*

\* WNS signs include visible fungus, UV fluorescence, WDI ≥2, suspicious behaviors (day flight activity, entrance roosting, delayed arousal)

USGS National Wildlife Health Center  
Winter 2014/2015 Bat Submission Reference Chart

**Outside of the WNS Endemic Area**

(Appendix A Map – Pg.10 )

Unusual bat mortality/behavior  
not associated with WNS  
(NOV-MAY)  
Pg. 6

Bats with signs suggestive of WNS\*  
(NOV-MAY)  
Pg. 7-8

Priority Samples

- Any species
- Any county
- $\geq 5$  dead/sick bats at one location
- For other situations- consult with NWHC

Priority Samples

- Species with confirmed susceptibility to WNS at a suspect positive hibernaculum
- Any hibernating species at/near a hibernaculum of unknown status in any county of unconfirmed status

Samples to submit

- (5-8 bats)
- photos AND
  - fresh, intact carcasses
  - MAXIMUM of 3 euthanized non-T/E bats per site

Samples to submit

- (1-5 bats)
- photos AND
  - fresh, intact carcass or of any species OR
  - combined wing/muzzle swab, UV-guided wing biopsies or fungal tape from T/E species or banded bats
  - MAXIMUM of 3 euthanized non-T/E bats per site

Species confirmed with WNS- *Myotis lucifugus*, *M. septentrionalis*, *M. sodalis*, *M. leibii*, *M. grisescens*, *Perimyotis subflavus*, *Eptesicus fuscus*

\* WNS signs include visible fungus, UV fluorescence, WDI  $\geq 2$ , suspicious behaviors (day flight activity, entrance roosting, delayed arousal)

USGS National Wildlife Health Center  
Winter 2014/2015 Pd Swab Surveillance Reference Chart

**WNS Management Areas**  
(Appendix A Map – Pg.10 )

ENDEMIC AREA (DEC-MAY)	INTERMEDIATE & AT-RISK AREAS Bats with no signs of WNS* (DEC-MAY) Pg. 9
<p><u>Priority Samples</u></p> <ul style="list-style-type: none"><li>• Any species with clinical signs from a hibernaculum or county of unknown WNS/Pd status</li><li>• Other research priorities identified in conjunction with the WNS Coordination Team/WNS Steering Committee</li></ul>	<p><u>Priority Samples</u></p> <ul style="list-style-type: none"><li>• Species with confirmed susceptibility to WNS at hibernaculum of unknown WNS/Pd status</li><li>• Species of unknown susceptibility co-roosting with susceptible species at a hibernaculum of unknown status</li><li>• Banded bats from contaminated hibernacula in Intermediate Area or Endemic Area</li><li>• Any species with suspect clinical signs of WNS when lethal take is not permitted</li></ul>
<p><u>Samples to submit</u> Requires prior arrangement with NWHC</p>	<p><u>Samples to submit</u> (15-25 bats); total 25 swabs per site</p> <ul style="list-style-type: none"><li>• wing/muzzle swabs (using NWHC kits)</li><li>• environmental substrates associated with roosting bats (supplemental)</li><li>• Requires prior arrangement with NWHC</li></ul>

Species confirmed with WNS- *Myotis lucifugus*, *M. septentrionalis*, *M. sodalis*, *M. leibii*, *M. grisescens*, *Perimyotis subflavus*, *Eptesicus fuscus*

\* WNS signs include visible fungus, UV fluorescence, WDI  $\geq 2$ , suspicious behaviors (day flight activity, entrance roosting, delayed arousal)

**Bat White-Nose Syndrome (WNS)/Pd Surveillance Submission Guidelines  
Winter 2014/2015 (November – May)**

<b>TABLE OF CONTENTS</b>	<b>PAGE</b>
Winter 2014/2015 NWHC Bat Submission Quick Reference Charts	
<b>Within the WNS Endemic Area</b> .....	<b>i</b>
<b>Outside the WNS Endemic Area</b> .....	<b>ii</b>
<b>Pd Swab Surveillance by WNS Management Area</b> .....	<b>iii</b>
Introduction and Methodology .....	<b>2-6</b>
Submission Guidelines by Geographic Areas	
<b>Unusual bat mortality/behavior not associated with WNS (all areas)</b> .....	<b>6</b>
<b>Bats with clinical signs suggestive of WNS</b>	
<input type="checkbox"/> <b>Within the WNS Endemic Area</b> (See Map in Appendix A).....	<b>7-8</b>
<input type="checkbox"/> <b>Outside of the WNS Endemic Area</b> (See Map in Appendix A).....	<b>7-8</b>
<b>Pd surveillance in absence of clinical signs of WNS</b> (Intermediate & At-Risk Areas).....	<b>9</b>
<b>Appendix A:</b> Map of current WNS Management Areas within the US (Dec 2014).....	<b>10</b>
<b>Appendix B:</b>	
B.1 USGS NWHC Wildlife Mortality Reporting and Diagnostic Services Request Form .....	<b>11</b>
<a href="http://www.nwhc.usgs.gov/services/Wildlife%20Mortality%20Reporting%20and%20Diagnostic%20Services%20Request%20Form.pdf">http://www.nwhc.usgs.gov/services/Wildlife%20Mortality%20Reporting%20and%20Diagnostic%20Services%20Request%20Form.pdf</a> (fillable PDF document)	
B.2 Hibernaculum/Individual Bat data collection sheet.....	<b>12-15</b>
<b>Appendix C:</b>	
Fungal tape-lift sampling instructions.....	<b>16-17</b>
Fungal tape-lift figures.....	<b>18</b>
<b>Appendix D:</b> Wing punch biopsy instructions.....	<b>19-20</b>
<b>Appendix E:</b> Bat wing swab protocol.....	<b>21-22</b>
<b>Appendix F:</b> Longwave ultraviolet (UVA) fluorescence screening of bat wing.....	<b>23</b>
<b>Appendix G:</b>	
USGS National Wildlife Center packaging and shipping instructions .....	<b>24-26</b>
<a href="http://www.nwhc.usgs.gov/mortality_events/reporting.jsp">http://www.nwhc.usgs.gov/mortality_events/reporting.jsp</a>	

The following sample submission guidelines are for use when surveying bat hibernacula or evaluating unusual bat morbidity or mortality during Winter 2014-2015. They are meant to assist with prioritizing appropriate field samples for laboratory submission based on geographic location and prior knowledge of WNS status at survey sites. **The primary objectives of this surveillance design are to identify range expansion of *Pseudogymnoascus (formerly Geomyces) destructans* and new bat species affected with WNS.** This document replaces all previous winter submission guidelines from the USGS- National Wildlife Health Center (NWHC). The level of diagnostic evaluation depends on 1) the presence of unusual numbers of sick or dead bats, 2) the distance from confirmed contaminated sites with greater emphasis on suspect WNS bats found at or beyond the current disease boundaries, and 3) the type of sample received.. These guidelines will be periodically reviewed to ensure that they meet the needs of the field and the laboratory. Please contact Anne Ballmann (608-270-2445, [aballmann@usgs.gov](mailto:aballmann@usgs.gov)) with any questions, suggestions, or concerns.

**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 or population decline 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 (membrane thinning, depigmented areas, holes, tears, flaky appearance) in cave bat species found outside the hibernaculum through May

**WNS has been confirmed in the following 7 species:**

- Little brown bat (*Myotis lucifugus*)
- Tri-colored bat (*Perimyotis subflavus*)
- Northern long-eared bat (*Myotis septentrionalis*)
- Indiana bat (*Myotis sodalis*)
- Small-footed bat (*Myotis leibii*)
- Big brown bat (*Eptesicus fuscus*)
- Gray bat (*Myotis grisescens*)

Potentially susceptible species (only *P. destructans* DNA detected):

- Southeastern myotis (*Myotis austroriparius*)
- Silver-haired bat (*Lasionycteris noctivagans*)
- Virginia big-eared bat (*Corynorhinus townsendii virginianus*)

## Key components of the diagnostic effort:

### 1. Hibernaculum data collection.

**Fill out the hibernaculum data collection sheet ([Appendix B.2](#)) whenever hibernacula are surveyed**, regardless of what state or county you are in and whether or not you see fungus on bats. These data will increase our understanding of the epidemiology of WNS and records of negative data (no fungus or abnormal behaviors observed) are important in this effort. Also complete the 2<sup>nd</sup> page (Individual Bat Specimen Collection Datasheet Winter 2013/2014) whenever samples are collected for laboratory analysis. If observed bat mortality does not appear to be related to WNS, please submit specimens with the NWHC Specimen History Form ([Appendix B.1](#)). E-mail or fax the appropriate completed datasheets to Anne Ballmann (NWHC-epi@usgs.gov; 608-270-2415 fax) when submitting samples to NWHC.

**If there is no unexplained bat mortality and there is no evidence of fungal growth or unusual behaviors in live bats at the hibernaculum**, swab samples of bats (and/or the environment) can be submitted provided that prior arrangements have been made with the NWHC to participate in the expanded Pd surveillance program and NWHC swab materials and protocols are used. .

### 2. Field photographs.

Handling bats may cause much of the visible fungus to disappear before specimens arrive at the lab. Please take good quality field photographs of representative affected bats, particularly in regions where WNS has yet to be identified, to be included with all bat submissions. Digital photos can be e-mailed to Anne Ballmann for further submission consultation.

**When non-lethal swabs, tape-lift or biopsies samples are collected from bats with suspicious clinical signs**, we request close-up images of individual live bats to be sampled. E-mail photos to NWHC-epi@usgs.gov (608-270-2415 fax) along with the Hibernaculum/Bat data sheets ([Appendix B.2](#)) including the date photos were taken, site name, and the photographer's name.

### 3. Carcass collection.

Advised application- whenever laboratory confirmation of WNS is required (suspicious field signs of WNS in a species not previously confirmed with the disease or in a new geographic area).

Lethal take of a small number of affected animals may be necessary in the absence of natural mortality to confirm WNS. Ensure you have the proper permits or authorization for specimen collection. For guidance on acceptable methods of euthanasia in bats for WNS evaluation, contact [NWHC-epi@usgs.gov](mailto:NWHC-epi@usgs.gov) or visit [http://www.michigan.gov/documents/emergingdiseases/Humane\\_Euthanasia\\_of\\_Bats-Final\\_244979\\_7.pdf](http://www.michigan.gov/documents/emergingdiseases/Humane_Euthanasia_of_Bats-Final_244979_7.pdf).

**Once WNS has been confirmed in a federal or state-listed threatened or endangered species, only specimens of that species that are found dead or non-lethally sampled will be accepted for diagnostic testing except in extenuating circumstances where necessary permits allow.**

Collect the freshest carcasses (intact body, no evidence of scavenging, fur does not pull out easily) representing each affected species. If fresh carcasses are unavailable, mummified carcasses are preferable to wet, slimy carcasses and may be accepted upon consultation with NWHC. Follow carcass collection instructions described in [Appendix G](#). If carcasses are being submitted for diagnostic evaluation, keep individual carcasses chilled in separate bags with ID labels containing the following information:

- date died & date collected (if different)
- location (hibernaculum or nearest town, county, state)
- collector name & phone
- 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 in a 2<sup>nd</sup> clean bag upon exiting the hibernaculum but prior to traveling to another site. If you plan to visit additional sites on the same day, follow the current recommendations described in the USFWS Revised Decontamination Protocol (June 25, 2012)

[http://www.whitenosesyndrome.org/sites/default/files/resource/national\\_wns\\_revise\\_final\\_6.25.12.pdf](http://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_revise_final_6.25.12.pdf).

Contact NWHC-epi@usgs.gov to arrange shipping. If additional intact carcasses are being saved for future evaluation, triple-bag the labeled specimens, freeze carcasses and store locally. Keep record of frozen bat carcass inventory on datasheets ([Appendix B.2](#)).

**Please contact the NWHC prior to submitting samples. See [Appendix G](#) for NWHC shipping instructions.**

#### 4. Non-lethal sampling techniques:

*NOTE: Bats from WNS confirmed counties with visual evidence of WNS (white material on muzzle and wing membranes) are considered suspect positive for WNS. Disturbance of these bats that may compromise survival and further sampling is not advised unless there is a specific need. **Most current non-lethal sampling techniques cannot confirm WNS and may have a reduced reliability of Pd detection as compared to whole carcass evaluation.***

##### **Fungal tape-lift sample collection (see [Appendix C](#) for detailed instructions)**

Advised application- known susceptible bat species in an unconfirmed county within the WNS endemic region **with visible fungus**; any threatened/endangered bat species with visible fungus on muzzle; confirmation of the disease **is not** necessary.

Wear clean gloves to handle each bat to reduce the risk of cross-contamination of diagnostic samples. Collect tape-lifts only from visibly affected muzzles of bats (alive or dead) with fungal growth when carcasses cannot be submitted. E-mail hibernaculum/individual bat data collection sheet ([Appendix B.2](#)) to Anne Ballmann ([NWHC-epi@usgs.gov](mailto:NWHC-epi@usgs.gov)) and send fungal tape slides with a hard copy of the datasheet to the NWHC.

□ **Ultraviolet light (UVA) screening of wing membranes (see [Appendix F](#) for detailed instructions)**

Advised application- any dead bat or live bat with physical or behavioral signs suggestive of WNS but lacking visible fungal growth examined mid-winter through spring. **This is a screening technique with unknown specificity outside the WNS endemic area.**

This technique requires handling individual bats to examine extended wings and thus results in hibernation disturbance as well as unknown safety risks to bats. Detection of pale yellow-orange fluorescence spots on wings **IS NOT** definitive for diagnosing WNS and therefore should be used in conjunction with other techniques for targeted sample collection. Absence of fluorescence does NOT equate with absence of infectious Pd on the bat. **UVA screening is not currently recommended for use in apparently healthy bat populations beyond the Intermediate Area.**

□ **Wing punch biopsy (see [Appendix D](#) for detailed instructions)**

Advised application- any threatened/endangered bat species **with visible fungus or characteristic fluorescence on wing membranes under UVA light**; known susceptible species in an unconfirmed county within the WNS endemic area with physical evidence (visible fungus, wing damage). This non-lethal sampling is the preferred, more sensitive method to fungal tape lifts for diagnostic evaluation when fungus is present on both flight membranes and muzzle as PCR and/or histopathology may be performed.

To reduce the risk of cross-contamination among bats, all equipment (i.e.: gloves, tissue punches, biopsy boards, and forceps) should be cleaned or changed between each sampled bat. Collect wing biopsies only on live bats with visible fungal growth or characteristic UV fluorescence when whole carcasses cannot be submitted. Biopsy punches should be collected from portions of the wing membrane that exhibit fungal growth or other types of visible lesions ([Appendix D&F](#)). E-mail hibernaculum/individual bat data collection sheet ([Appendix B.2](#)) to Anne Ballmann (NWHC-epi@usgs.gov) and overnight ship samples to the NWHC.

□ **Bat wing swab (see [Appendix E](#) for detailed instructions)**

Advised application- known susceptible species observed in a hibernaculum of unknown WNS status within the Intermediate Area or At-Risk Area when clinical signs of WNS are rare or absent; known susceptible species in an unconfirmed county within the WNS endemic area with clinical signs; any bat species (including threatened/endangered species) from new geographic regions **with visible fungus or suggestive fluorescence on wing membranes under UVA light** when lethal sampling is not permitted.

Torpid bats within arm's reach can be sampled using this technique without removing them from roost locations to minimize disturbance. **For Winter 2014/2015, bat swab sampling kits provided by NWHC are available for approx. 5 -10 sites per state within the Intermediate Area or approx. 3-5 sites per state within the At-Risk Area for Pd surveillance.** Contact Anne Ballmann (608-270-2445, [aballmann@usgs.gov](mailto:aballmann@usgs.gov)) for details.



*Note: Non-lethal sampling techniques are meant to serve as adjunct or alternative diagnostic methods to evaluate for the presence of P. destructans among suspect bats at a particular location. The maximum number of individuals (in any sample combination of carcasses, tape lifts, or wing biopsies) per site that will be accepted for WNS/Pd diagnostic evaluation is 10 per season unless prior arrangements have been made with the lab. Not all submitted samples may be tested; this will be at the discretion of the lab. For expanded Pd swab surveillance at hibernacula where winter bat populations lack clinical signs of WNS, the target sample size is 15-25 bats.*

**5. Biosecurity concerns:**

A site contaminated with *P. destructans* retains this designation indefinitely regardless of the presence of affected bats. Follow the most current **protocols for containment and decontamination of field gear and personnel** described in “National White-Nose Syndrome Decontamination Protocol Version 06.25.2012)”

[\[http://www.whitenosesyndrome.org/sites/default/files/resource/national\\_wns\\_revise\\_final\\_6.25.12.pdf\]](http://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_revise_final_6.25.12.pdf) prior to leaving each survey site. If you plan to visit a potentially uncontaminated hibernaculum after conducting survey work at a contaminated hibernaculum, use clothing, footwear, gear, and vehicles dedicated for use at clean sites.



## **UNUSUAL BAT MORTALITY/BEHAVIOR NOT ASSOCIATED WITH WNS**

**Before entering hibernacula of endangered Indiana bats or any other listed bat species, appropriate Federal and State permits (or authorizations) must be obtained. For listed species, authorization is needed to collect and possess dead specimens, to handle live bats, or to euthanize sick bats.**

Priority samples to submit for laboratory diagnostics:

1. Any species in any county nationwide where 5 or more dead or sick bats are observed at one location over a short time period (approx.1-2 weeks).
- **If no fungal growth on live bats is observed at the site where unexplained bat mortalities are detected**, collect 5 - 8 freshly dead bats, chill and ship to NWHC as soon as possible for evaluation according to packaging and shipping instructions in Appendix G. A maximum of 3 affected non-T/E species may be euthanized per site for submission if the quality of available carcasses is questionable. Complete a specimen history form (Appendix B.1).

## BATS WITH CLINICAL SIGNS SUGGESTIVE OF WNS

Before entering hibernacula of endangered Indiana bats or any other listed bat species, appropriate Federal and State permits (or authorizations) must be obtained. For listed species, authorization is needed to collect and possess dead specimens, to handle live bats, or to euthanize sick bats.

### □ Sites within the WNS endemic area (see [Appendix A](#))-

#### Priority samples to submit for laboratory diagnostics:

1. Bat species not previously confirmed with WNS with suspicious fungal lesions or aberrant behavior in a confirmed county
2. Any bat species with suspicious signs at/near a hibernaculum of suspect or unknown status in an unconfirmed county

#### Site prioritization recommendations:

Only hibernacula of critical biological or management significance that require conclusive laboratory confirmation of WNS should be surveyed for clinically affected bats within the WNS confirmed area. **Notification of need for diagnostic confirmation at sites within this region should be communicated to the laboratory prior to collection of bats.** Take field photos and submit 3-5 bats (fresh dead or euthanized) with physical or concurrent behavioral evidence suggestive of WNS along with a completed Hibernaculum/bat datasheets ([Appendix B.2](#)). Once WNS is confirmed in the county, only bat species of unknown susceptibility will typically be accepted for WNS diagnostic evaluation from that county. Surveillance swabs, however, may be submitted from 3-5 clinically affected bats at sites of unknown Pd status within a WNS confirmed county if laboratory confirmation of Pd is desired.

### □ Sites outside the WNS endemic area (see [Appendix A](#))-

*Note:* It is recommended that all previously identified *P. destructans* contaminated hibernacula outside the WNS endemic area be surveyed between late February-March for the development of WNS. Do not submit samples if signs of WNS are absent in the bat population without prior consultation with NWHC.

#### Priority samples to submit for laboratory diagnostics:

1. Species with confirmed susceptibility to WNS at a suspect positive hibernaculum
2. Any cave bat species with suspicious fungal lesions or aberrant behavior at/near a hibernaculum of unconfirmed status

#### Site prioritization recommendations:

To be determined by the wildlife management agency. Please consult the National Surveillance Implementation Plan (Dec 2011) for prioritization recommendations.

The following sample collection descriptions apply to bats with clinical signs suggestive of WNS regardless of the area they are detected. Consult the NWHC Bat Submission Quick Reference Charts (pg. i-ii) for a summary of sample prioritization recommendations.

- **If fungus, wing damage or characteristic UV fluorescence on wing membranes is observed on dead bats**, fill out hibernaculum/bat datasheets ([Appendix B.2](#)) and e-mail to NWHC-epi@usgs.gov (608-270-2415 fax). Submit 3-5 fresh carcasses of new bat species of unknown susceptibility only that appear affected from a confirmed county. If county is of suspect or unknown WNS status, submit 3-5 carcasses of any affected species. (See pg. 2 for list of confirmed susceptible species).

- **If live bats have behavioral or physical evidence of suggestive of WNS but no mortality is observed AND**

**WNS confirmation IS required**, follow one of the methods below:

1. Euthanize up to 3 bats (representative of affected non-T/E species) with evidence of fungus for submission to NWHC. For guidance on acceptable methods of euthanasia in bats for WNS evaluation, contact [NWHC-epi@usgs.gov](mailto:NWHC-epi@usgs.gov) or visit [http://www.michigan.gov/documents/emergingdiseases/Humane\\_Euthanasia\\_of\\_Bats-Final\\_244979\\_7.pdf](http://www.michigan.gov/documents/emergingdiseases/Humane_Euthanasia_of_Bats-Final_244979_7.pdf).
2. Perform UV-guided wing punch biopsies on 3-5 individuals (2 biopsies per each individual – See Appendices D&F) per field site from an affected portion of the flight membranes only. Photograph the bat prior to biopsy and record associated geographic, demographic, and physical data ([Appendix B.2](#)). *NOTE: The diagnostic reliability for WNS/Pd detection in wing punch biopsies may be reduced as compared to whole carcass evaluation. Thus, negative results do not rule out the possibility of an animal being infected.*

Submit photos (clusters and live individuals) and bat carcasses to NWHC ([Appendix G](#)). Include completed hibernaculum/bat datasheets ([Appendix B.2](#)).

**WNS confirmation is NOT required**, follow one of the methods below:

1. Collect fungal tape-lifts of grossly visible white fungal growth on the muzzles of 3 - 5 affected live bats (See [Appendix C](#) for detailed instructions). A new tape strip and gloves should be used for each individual bat. Tape-lift slides can be stored and shipped at room/ambient temperature. Follow packaging and shipping instructions for slides only in Appendix C. Include the completed datasheets from [Appendix B.2](#). *NOTE: The sensitivity of tape-lift samples to detect P. destructans is highly dependent on the slide quality; thus, negative results do not rule out the possibility of an animal being infected.*
2. Collect combined wing/muzzle swabs from 3 - 5 visibly affected live bats using kit materials provided NWHC (See [Appendix E](#) for detailed instructions). Photograph the bat prior to swabbing and record associated geographic, demographic, and physical data on hibernaculum/bat datasheet ([Appendix B.2](#)).

## ***Pd* SURVEILLANCE IN ABSENCE OF CLINICAL SIGNS OF WNS (INTERMEDIATE & AT-RISK AREAS)**

**Before entering hibernacula of endangered Indiana bats or any other listed bat species, appropriate Federal and State permits (or authorizations) must be obtained. For listed species, authorization is needed to collect and possess dead specimens, to handle live bats, or to euthanize sick bats.**

### Priority swab samples to submit for laboratory diagnostics:

1. Species with confirmed susceptibility to WNS at hibernaculum of unknown WNS/*Pd* status
2. Species of unknown susceptibility to WNS co-roosting with species of confirmed susceptibility at hibernaculum of unknown WNS/*Pd* status
3. Bats banded at contaminated hibernacula within the WNS Intermediate Area or any location within the WNS Endemic Area found at a site of unknown WNS/*Pd* status

### Site prioritization recommendations:

To be determined by the wildlife management agency and should not include hibernacula participating in similar projects for early detection of *P. destructans* (*Pd*) in hibernating bat populations. Broad spatial distribution of selected hibernacula within the state is desirable for a surveillance program. Hibernacula known to contain winter populations of little brown bats, tri-colored bats, and/or northern long-eared bats are encouraged as *Pd* has more commonly been detected on these species. Please consult the National Surveillance Implementation Plan (Dec 2011) for prioritization recommendations.

Additionally, winter surveys of neighboring hibernacula (up to 4 sites) located within a 20 mile radius from a subset of 1<sup>st</sup> or 2<sup>nd</sup> year contaminated sites are requested to better model the rate of *Pd* dispersal and evaluate site prioritization criteria assumed to have higher risk of contamination. Please consult with Anne Ballmann ([aballmann@usgs.gov](mailto:aballmann@usgs.gov); 608-270-2445) to assist with site selection.

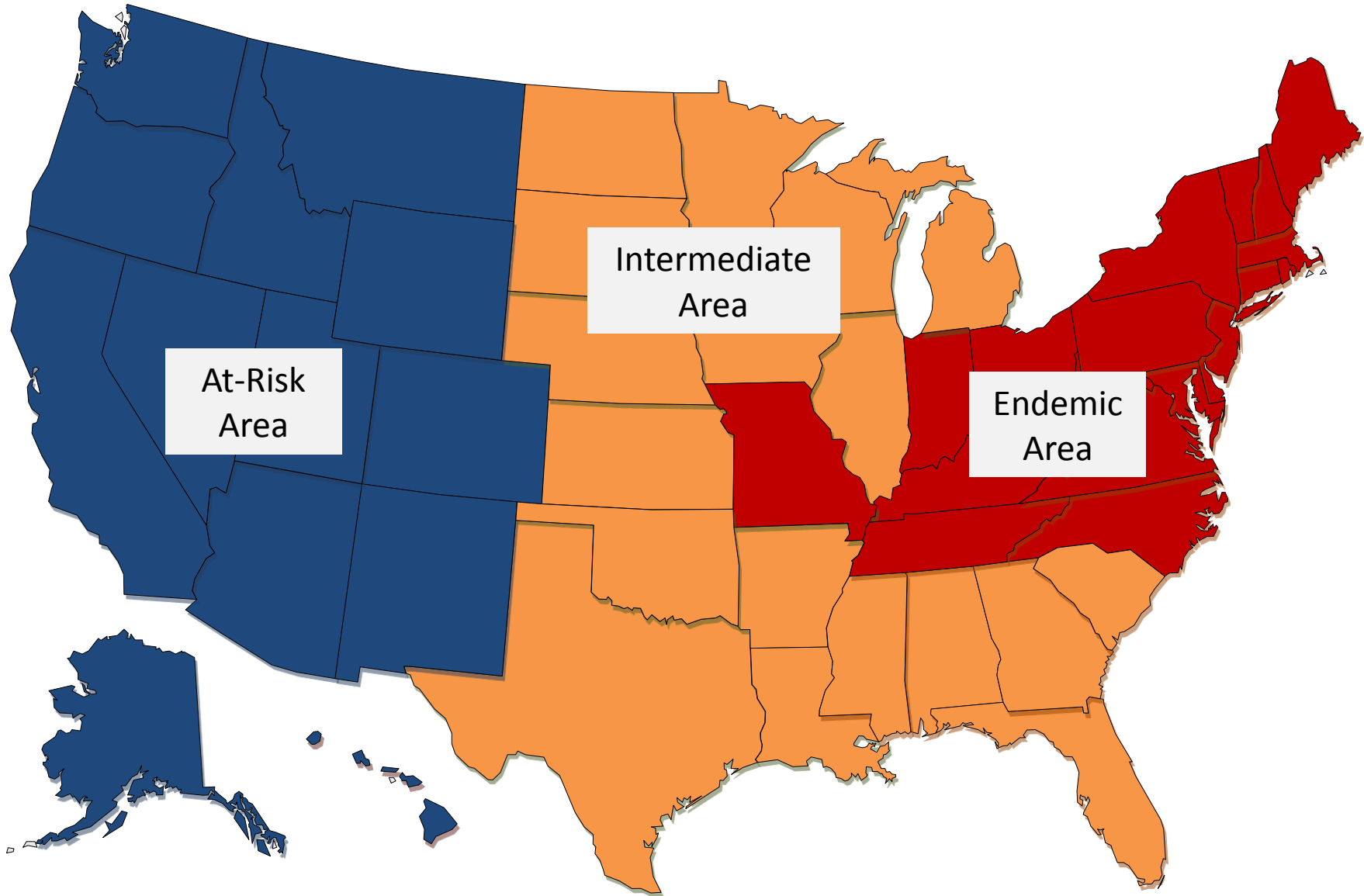
Swab samples from a total of 25 bats (minimum sample size = 15 bats) per hibernaculum are requested. Collect swabs from bats roosting within arms' reach and from representative roosting areas throughout the hibernaculum. This may include bats roosting individually or in separate clusters (depending on natural behavior of the species). Complete the hibernaculum/bat datasheets ([Appendix B.2](#)) to include with submission.

Hibernacula surveys conducted in areas outside the known range of *Pd* where 1 or more bats with suspicious physical or behavioral signs suggestive of WNS are identified should submit fresh, whole affected bat carcasses for diagnostic evaluation in lieu of swab samples whenever possible. Should detection of clinical bat(s) occur after initiation of swab sample collection but prior to sampling 25 bats, discontinue collection of remaining swabs and follow guidelines for sample collection in bats with clinical signs outside the WNS endemic area (pg. 7-8).

Contact Anne Ballmann ([aballmann@usgs.gov](mailto:aballmann@usgs.gov); 608-270-2445) to discuss alternative options for *Pd* surveillance in bats not associated with winter hibernacula.

APPENDIX A

MAP A: Designated WNS Management Areas within the United States (December 2014)



APPENDIX B.1



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 contact the NWHC Field Epidemiology Team before shipping.  
Alaska, continental US, or Puerto Rico: [NWHC-epi@usgs.gov](mailto:NWHC-epi@usgs.gov), 608-270-2480  
Hawaii/Pacific Islands: [thierry\\_work@usgs.gov](mailto:thierry_work@usgs.gov), 808-792-9520

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)

Investigator Name(s): \_\_\_\_\_

Date: \_\_\_\_\_

Phone /e-mail: \_\_\_\_\_

<b>State:</b>	<b>County:</b>	<b>Site Name:</b>	
<b>Latitude:</b>		<b>Longitude:</b>	Datum:
Nearest Pd contaminated site (name):		Distance (miles):	Direction:

**Observations at the hibernaculum entrance (within area impacted by daylight)**

# of bats observed flying at entrance in 5 minutes \_\_\_\_\_

Bat species	Bands observed	# live <sup>1</sup>	# dead <sup>1</sup>	# moribund <sup>1</sup>	# with fungus visible <sup>1</sup>	Distribution of affected bats (Solitary, Clustered <sup>2</sup> )	Photo #(s) of affected bats
						S C	
						S C	
						S C	
						S C	
						S C	
						S C	
						S C	

<sup>1</sup>Indicate if number is an estimate count; <sup>2</sup>Cluster defined as ≥2 bats in direct contact

**Bat observations inside the hibernaculum** (check one: \_\_\_ Full survey \_\_\_ Partial survey)

Bat species	Bands observed	# live <sup>1</sup>	# dead <sup>1</sup>	# moribund <sup>1</sup>	# with fungus visible <sup>1</sup>	Distribution of affected bats (Solitary, Clustered <sup>2</sup> )	Photo #(s) of affected bats
						S C	
						S C	
						S C	
						S C	
						S C	
						S C	
						S C	
						S C	

Comments: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

PLEASE ATTACH A MAP OF THE HIBERNACULUM WITH LOCATIONS OF SAMPLED BATS WITHIN THE SITE MARKED & COMPLETE THE INDIVIDUAL BAT DATASHEET FOR ALL SPECIMEN COLLECTIONS

North American Bat Species Codes

Common Name	Genus sp.	Code	Life Strategy
Big brown	<i>Eptesicus fuscus</i>	EPFU	hibernator
Brazilian (Mexican) free-tailed	<i>Tadarida brasiliensis</i>	TABR	hibernator
California myotis	<i>Myotis californicus</i>	MYCA	hibernator
Cave myotis	<i>Myotis velifer</i>	MYVE	hibernator
Eastern small-footed	<i>Myotis leibii</i>	MYLE	hibernator
Fringed myotis	<i>Myotis thysanodes</i>	MYTH	hibernator
Gray	<i>Myotis grisescens</i>	MYGR	hibernator
Indiana	<i>Myotis sodalis</i>	MYSO	hibernator
Little brown	<i>Myotis lucifugus</i>	MYLU	hibernator
Long-legged myotis	<i>Myotis volans</i>	MYVO	hibernator
Keen's bat	<i>Myotis keenii</i>	MYKE	hibernator
Mexican long-eared	<i>Myotis auricolus</i>	MYAR	hibernator
Northern long-eared	<i>Myotis septentrionalis</i>	MYSE	hibernator
Occult bat	<i>Myotis occultus</i>	MYOC	hibernator
Rafinesque's big-eared	<i>Corynorhinus rafinesquii</i>	CORA	hibernator
Southeastern myotis	<i>Myotis austroriparius</i>	MYAU	hibernator
Townsend's big-eared	<i>Corynorhinus townsendii</i>	COTO	hibernator
Tri-colored (E. pip)	<i>Perimyotis subflavus</i>	PESU	hibernator
VA big-eared	<i>C. townsendii virginianus</i>	COTOv	hibernator
Western long-eared myotis	<i>Myotis evotis</i>	MYEV	hibernator
Western small-footed	<i>Myotis ciliolabrum</i>	MYCI	hibernator
Yuma myotis	<i>Myotis yumanesis</i>	MYYU	hibernator
Big free-tailed	<i>Nyctinomops macrotis</i>	NYMA	non-hibernator
California leaf-nosed	<i>Macrotus californicus</i>	MACA	non-hibernator
Canyon bat	<i>Parastrellus hesperus</i>	PAHE	?
Eastern red	<i>Lasiurus borealis</i>	LABO	non-hibernator
Evening	<i>Nycticeius humeralis</i>	NYHU	non-hibernator
Ghost-faced	<i>Mormoops megalophylla</i>	MOME	non-hibernator
Greater long-nosed	<i>Leptonycteris nivalis</i>	LENI	non-hibernator
Greater mastiff	<i>Eumops perotis</i>	EUPE	non-hibernator
Hoary	<i>Lasiurus cinereus</i>	LACI	non-hibernator
Lesser long-nosed	<i>Leptonycteris yerbabuenae</i>	LEYE	non-hibernator
Mexican long-tongued	<i>Choeronycteris mexicana</i>	CHME	non-hibernator
Northern yellow	<i>Lasiurus intermedius</i>	LAIN	non-hibernator
Pallas' mastiff	<i>Molossus molossus</i>	MOMO	non-hibernator
Pallid	<i>Antrozous pallidus</i>	ANPA	non-hibernator
Seminole	<i>Lasiurus seminolus</i>	LASE	non-hibernator
Silver-haired	<i>Lasionycteris noctivagans</i>	LANO	non-hibernator
Spotted	<i>Euderma maculatum</i>	EUMA	non-hibernator
Southern yellow	<i>Lasiurus ega</i>	LAEG	non-hibernator
Underwood's mastiff	<i>Eumops underwoodi</i>	EUUN	non-hibernator
Western red	<i>Lasiurus blossevillii</i>	LABL	non-hibernator
Western yellow	<i>Lasiurus xanthinus</i>	LAXA	non-hibernator







## APPENDIX C - Fungal tape-lift protocol for bats

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

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

**Date:** 6 Dec 2010; 27 Dec 2013; 1 Mar 2015 (modified)

**Purpose:** The following procedure is designed to collect visible fungi from the muzzles of bats for later microscopic analyses while minimizing harm to the sampled bat. **This technique will NOT confirm White-nose Syndrome (WNS) on bats and should not be used as the sole sampling methodology in areas where WNS has not been previously confirmed in the bat population.**

### 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 and permanent marker

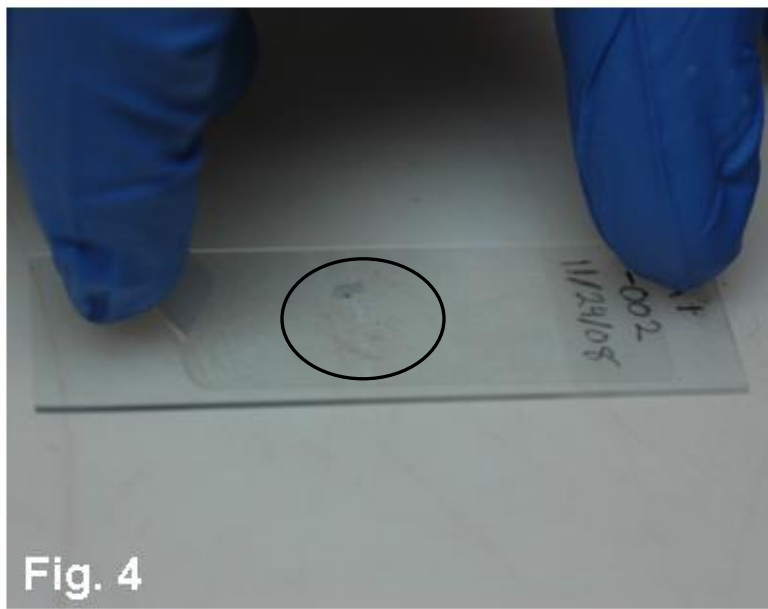
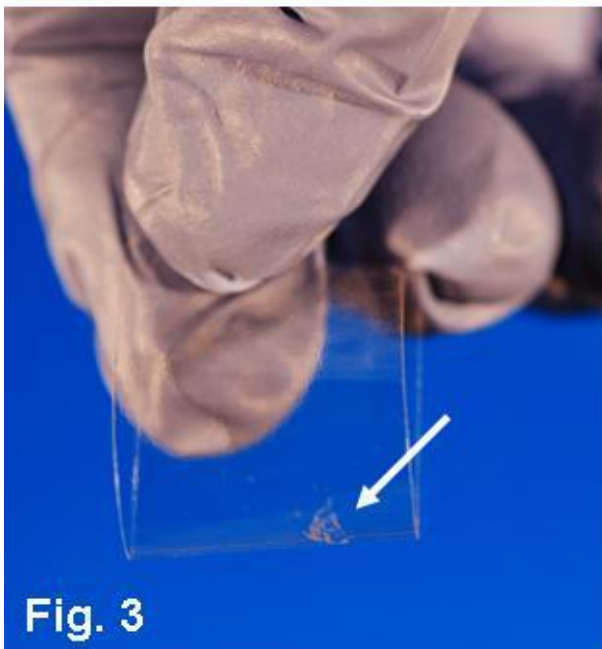
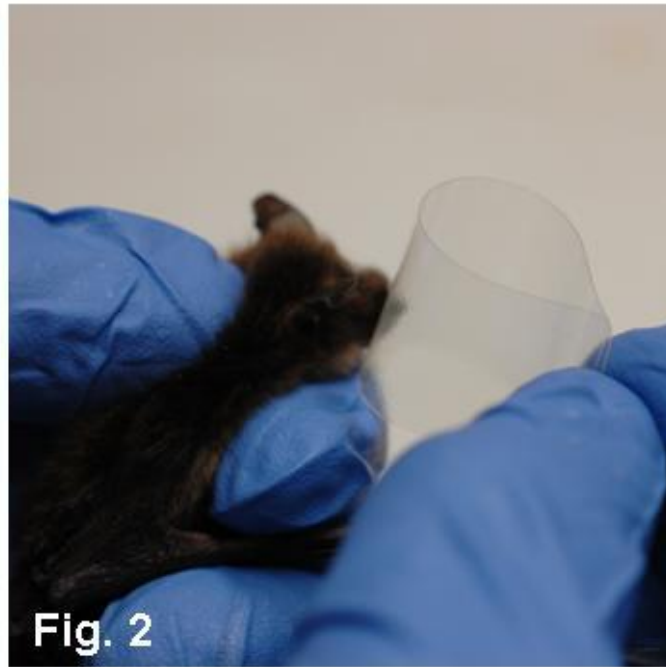
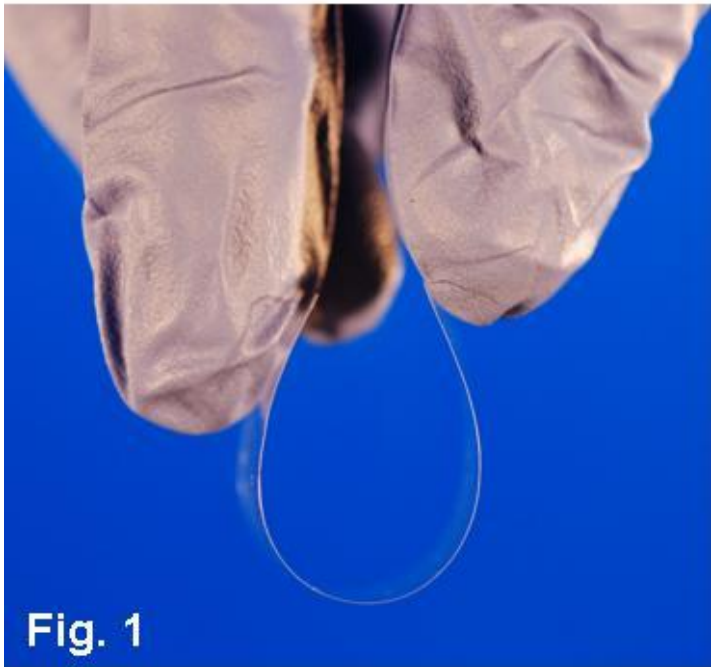
### Procedure:

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. Muzzle samples yield the clearest results and are the preferred 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 grossly visible areas of fungal growth on the muzzles of bats. When possible, avoid collecting samples from wing membranes as analyses of unfurred skin have not been reliable in detection of *P.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).

## APPENDIX C - Fungal tape-lift protocol for bats – con't

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(s) on the tape with a permanent marker containing the material sampled from the bat.
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 clean Ziploc bag to store or mail to the lab.
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 [NWHC-epi@usgs.gov](mailto:NWHC-epi@usgs.gov). Contact the NWHC Field Epidemiologist if you have any additional questions (608-270-2480).

**APPENDIX C. Illustrations – Fungal tape-lift protocol for bats**  
**-Photographs by D. Berndt and D. Johnson, USGS – NWHC**



## APPENDIX D - Instructions for Taking a Wing Tissue Biopsy

Updated by Pat Ormsbee (NFS) and Jan Zinck 5/14/09 (original: Shonene Scott, Portland State University 5/2003)

Modified by Anne Ballmann (USGS-NWHC)12/27/13; 3/1/2015

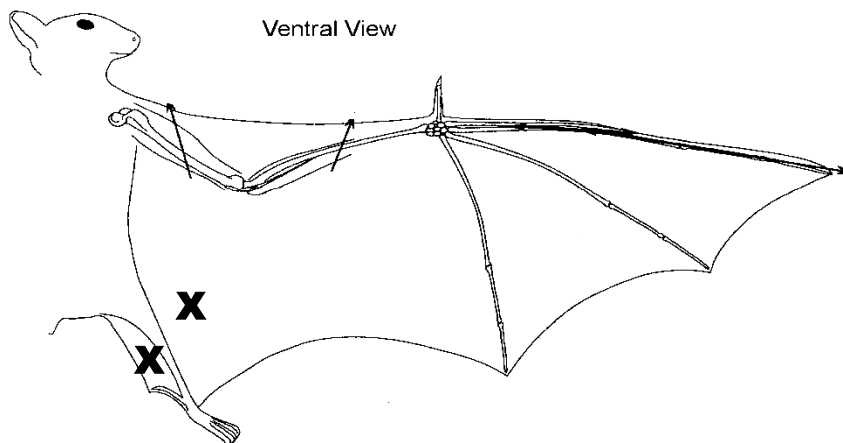
**NOTE:** If punch biopsies are the only sample type to be submitted to the lab in a particular case, it is highly recommended that 2 biopsies per bat be collected (from different wings). Additional population genetic sampling should not be attempted in these individuals to reduce the number of holes in the wings. **This technique may NOT confirm White-nose Syndrome (WNS) on bats and should not be used as the sole sampling methodology in areas where WNS has not been previously confirmed in the bat population.**

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 bat, 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:

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

3. Have a fresh cardboard square, a labeled tube, a new tissue punch, and a sterilized forceps ready for each bat. Do not touch (contaminate) the end of the punch, the forceps, or the inside of the tube lid with fingers or environmental debris.
4. 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.
5. 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 F).** If possible, locate an affected area near the body wall within the lower half of the wing membrane or uropatagium. These locations have been demonstrated to have faster healing rates and are less disruptive to flight aerodynamics (Faure PA et al. 2009. J Mammalogy 90(5): 1148-56.) 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 bat flight membranes.



## APPENDIX D - Instructions for Taking a Wing Tissue Biopsy -con't

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. For fungal PCR analysis, place tissue into an empty sterile vial (no storage media) for storage. For histopathological evaluation, place tissue into a storage vial containing 10% buffered neutral formalin (1 part tissue to 10 parts formalin).
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, unfixed samples should be frozen until submitted for fungal PCR analysis. Formalin-fixed samples should be held at room temperature (not frozen).
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, rinse in alcohol and flame sterilize. Allow forceps to cool before contacting bat tissue. 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 contaminants, 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 unfixed samples cannot be shipped overnight, freeze them and ship as soon as possible.

Send an electronic copy of the completed hibernaculum/bat datasheets(Appendix B.2) to the NWHC-epi@usgs.gov. Shipping address and examples of appropriate shipping materials are in Appendix G. 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.*

- 3-5 mm biopsy punches Fisher Scientific Catalog # NC9515874 (\$106.73/pack of 50)
- 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)
- 10% buffered neutral formalin (if histopathological analysis is desired)
- Sterile rinse water
- 2ml 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 for Non-lethal Swab Sampling of Bat Skin for Detection of *Pseudogymnoascus destructans* (Pd)**

**Prepared by:** USGS – National Wildlife Health Center (October 2013)

**Purpose:** The following procedure is designed to detect the presence of Pd while minimizing harm to the sampled bat. **This technique will NOT confirm White-nose Syndrome (WNS) on bats and should not be used as the sole sampling methodology in areas where WNS has not been previously confirmed in the bat population.**

### **Materials**

#### **Provided by NWHC:**

- Sterile and individually wrapped Puritan Pur-Wrap polyester-tipped swabs with plastic shafts (27)
- Sterile, nuclease-free, pre-labeled 2 ml microcentrifuge tubes, each containing 150 µl of sterile nuclease free water (25)
- 2 Ziplock bags (1 quart-size) for vial storage & trash
- Datasheet
- 1 Ziplock bag (1 gallon-size) for clean outer storage & packaging of sample vials and datasheet (do not carry bag inside hibernaculum)
- Insulated shipper box with 2 ice packs (for return shipment only; do not carry inside hibernaculum)

#### **Needed:**

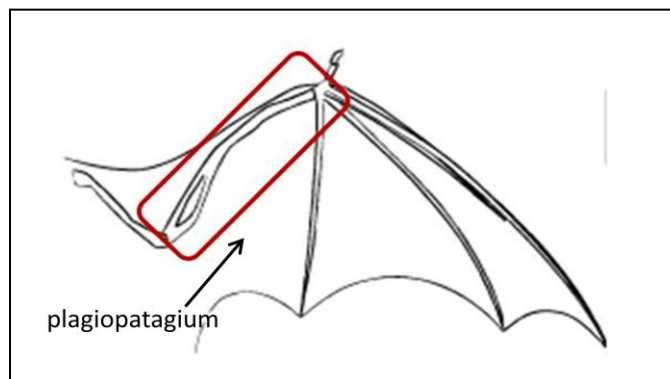
- Disposable exam gloves
- Pencil or indelible ink pen
- Clipboard
- Decontamination supplies
- Cooler with ice for sample transport

### **Bat Swab Collection Protocol:**

1. Persons collecting swab sample from bats or handling sample tubes should wear disposable exam gloves. It is not necessary to change gloves between each bat/sample tube provided the persons performing these tasks do not directly contact individual bats or inside rim of sample vial lid.
2. Identify a bat to be sampled.
3. Remove a pre-labeled sample tube from the Ziplock bag and record the requested individual bat information on the provided data sheet. Remember to include the unique Swab Vial # from the selected sample tube.
4. Tap sample tube to ensure all liquid is pooled at the bottom of the tube.
5. Remove a swab from its packaging without touching the polyester tip.
6. Dip the tip of the swab into the sample tube containing sterile water to moisten the tip (most water will be absorbed by the swab).
7. Bats may be sampled without removing them from their roosting location. If direct handling of the bat is required for other work, hold bat face down with one wing pulled slightly away from the body at the elbow.
8. Sample one of the bat's forearms and adjacent wing tissue between the elbow and wrist (see diagram) by gently ROLLING the swab across the surface of skin (three passes back & forth). Rolling the swab as it is moved along the skin prevents abrading the delicate wing skin.
9. Roll the same swab across the muzzle of the same bat 3 times.
10. Place the swab tip into the same sample tube used to moisten the swab, and break off the protruding plastic shaft of the swab without touching the rim of the tube or inside of lid with your fingers. Close the lid of tube tightly.



11. Place sample tube containing swab into the second Ziplock bag (1 quart-size) maintained at ambient cave temperature.
12. Dispose of swab handles, paper wrappers and contaminated exam gloves as necessary into third Ziplock bag (1 quart-size).
13. Repeat the above process for each bat sampled.
14. Upon exiting the hibernaculum but prior to leaving the area, place the datasheet inside of the emptied Ziplock bag (1 quart-size). Decontaminate the outer surfaces of all Ziplock bags taken inside the hibernaculum following current USFWS Decontamination Guidelines. Place the Ziplock bags containing all sample tubes and datasheet inside the clean Ziplock bag (1 gallon-size) for storage and shipment. Ensure all excess air is removed from the bags.
15. Following removal of collected samples from the hibernaculum, store them on ice for transport to an office refrigerator or freezer.



### **Sample Storage:**

Hold swab samples chilled (4°C) if they are to be shipped within 2 days following collection; otherwise freeze samples at -20°C until they are shipped. If you are sampling multiple sites, samples can be stored frozen to facilitate batch shipping at your convenience. Avoid multiple freeze-thaw cycles.

### **Sample Shipment:**

Package bagged samples between frozen ice-packs for shipment by overnight courier to the USGS – National Wildlife Health Center. Ensure that ice-packs are frozen solid prior to sealing the package for shipment. Ship early in the week to avoid weekend deliveries (DO NOT ship on Fridays or the day before a holiday). Notify Anne Ballmann (608-270-2445; [aballmann@usgs.gov](mailto:aballmann@usgs.gov)) with the courier service and package tracking number of the return shipment.

*Ship samples to:* USGS – National Wildlife Health Center  
Necropsy Loading Dock  
Diagnostic Microbiology  
6006 Schroeder Road  
Madison, WI 53711  
608-270-2400 (emergency contact number)

## APPENDIX F—Longwave ultraviolet (UVA) fluorescence screening of bat wings

Authors: Anne Ballmann, Carol Meteyer (modified from G. Turner & J. Gumbs 2011)

Date: 5/7/2012, revised 12/27/13; 3/1/15

**Purpose:** To examine bat wings with little to no visible fungal growth for evidence of yellow-orange fluorescence areas suggestive of an infection by *Pseudogymnoascus destructans*. **This is a screening technique with unknown specificity outside the WNS endemic area. It will NOT confirm White-nose Syndrome (WNS) on bats and should not be used as the sole sampling methodology in areas where WNS has not been previously confirmed.**

### 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 and visible light filter (LED Wholesaler #7202UV385; Polman Minerals) or 368 nm wavelength 9 V UV box (Contact Greg Turner [grturner@pa.gov] for more details on UV box system)
- Disposable exam gloves
- Digital camera
- Permanent marker
- PPE: UVA blocking safety glasses, SPF15+ sunblock on exposed human skin

*Additional equipment for non-lethal wing biopsy 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 NWHC-epi@usgs.gov.

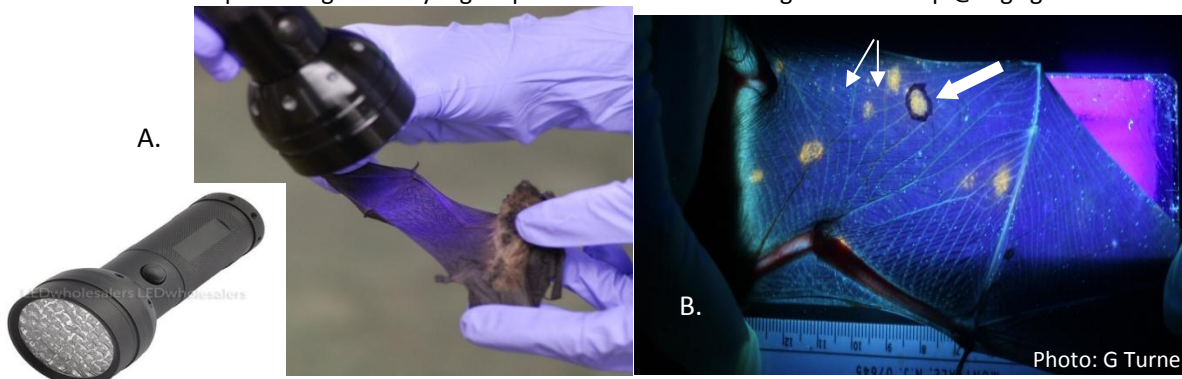


Figure 1.A) UV flashlight examination of ventral bat wing to be conducted in total darkness. B) Digital photo of backlit extended wing held over 368 nm UV light box. Arrows identify yellow-orange fluorescent areas of various diameters associated with suspect *G. destructans* infection.

## USGS – National Wildlife Health Center INSTRUCTIONS FOR COLLECTION AND SHIPMENT OF SPECIMENS



### Contact the NWHC Field Epidemiology Team

Email: [NWHC-epi@usgs.gov](mailto:NWHC-epi@usgs.gov), Phone: 608-270-2480, Fax: 608-270-2415

For Hawaii/Pacific Islands shipping instructions, contact Thierry Work.

Email: [thierry\\_work@usgs.gov](mailto:thierry_work@usgs.gov), Phone: 808-792-9520

The following instructions should be used for collecting and shipping wildlife carcasses, carcass parts, and samples extracted from animals to the USGS National Wildlife Health Center (NWHC) in Madison, Wisconsin, to ensure adequate and well preserved specimens.

- Complete the “Wildlife Mortality Reporting and Diagnostic Services Request Form” and email/fax it to the NWHC epidemiology team to initiate discussion of the case you would like to submit and get shipping approval. **Packages will not be opened if form does not arrive first.**
- For most cases, NWHC prefers to receive fresh chilled specimens if they can be sent within 24-36 hours of collection or death, as freezing/thawing impedes isolation of some pathogens and causes tissue damage. As a general guideline: if you cannot call or ship within 24-36 hours, immediately freeze the animal(s) and keep frozen during shipment.
- Specimens should be shipped 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, prior arrangements must be made. Email/fax shipment tracking number to NWHC.
- 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. Use rubber, vinyl, or nitrile gloves when picking up sick or dead animals. If you do not have gloves, invert a plastic bag over your hand and use it as a glove to scoop specimen directly in to the 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. Record on carcass tags and “Wildlife Mortality Reporting and Diagnostic Services Request Form” which animals were euthanized.
- Collect specimens that are representative of all species and geographic areas involved.
- Suitable specimens should have intact body, eyes, and body cavity; have no maggots; and have no foul odors. 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 specimens or samples from animals that are too large to ship. Other specimens might also require unique collection and shipping instructions (e.g., amphibians, bats, snakes); contact NWHC.
- Immediately attach a leg tag to each animal with the following information in pencil or waterproof ink:
  - Date collected
  - Location (specific site, town, county, state)
  - Collector (name/address/phone)
  - Species
  - Found dead or euthanized
  - Your reference #
- Place each animal in a separate plastic bag, close, and seal the bag. Cover zipper bag closure with strapping or duct tape after sealing zipper. Twist non-zipper bags closed, fold over on itself, and secure with package strapping or duct tape.



- Place 1<sup>st</sup> bag inside a 2<sup>nd</sup> bag, close and seal. More than one individually bagged animal can be placed in the 2<sup>nd</sup> bag. This prevents cross-contamination of individual specimens and leaking shipping containers.
- Tag the outside of 2<sup>nd</sup> bag and list 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, 3<sup>rd</sup> layer of bags).
- Place absorbent material in the 3<sup>rd</sup> plastic bag to absorb any liquids that might leak during shipping.  
**See appendix for examples of bags and absorbent materials.**
- Pack individually bagged animal(s) contained within the 2<sup>nd</sup> sealed bag into the 3<sup>rd</sup> 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 ship using dry ice.**
- Seal the 3<sup>rd</sup> bag with methods described for 1<sup>st</sup> bag.
- Place the completed “Wildlife Mortality Reporting and Diagnostic Services Request Form” and return shipping label (if you want the cooler returned) in a Ziploc bag and tape to the inside lid of the cooler. NWHC cannot pay for shipping.
- 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 information below to the outside of each cooler in a resealable pouch. Also attach “to” and “from” addresses and phone numbers directly to the cooler.

To Address:

**Necropsy Loading Dock**  
**National Wildlife Health Center**  
**6006 Schroeder Road**  
**Madison, WI 53711**  
**608-270-2480**

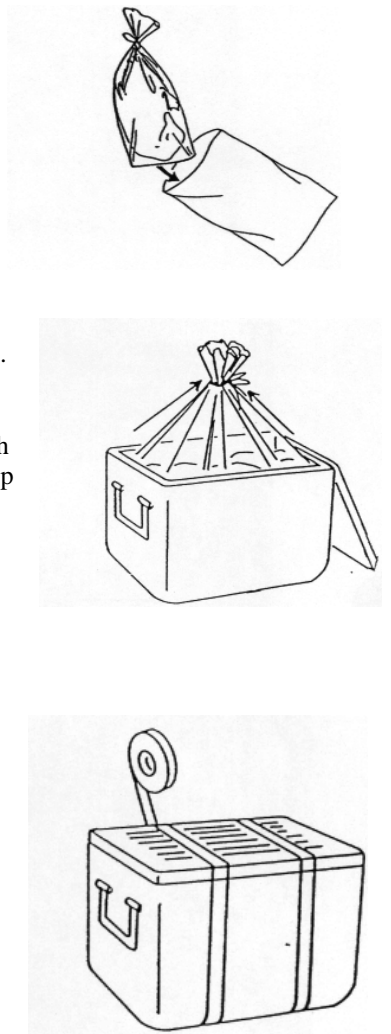
From Address/Emergency Contact:

**Your Agency’s Address**  
**Your Phone Number**

Supplementary Labels:

**Keep Cold**

- Mark the cooler with the appropriate information:  
 (See last page of these instructions for printable marking labels – do not shrink size of labels)
  - Carcasses of animals that died of unknown causes:  
**UN3373 and BIOLOGICAL SUBSTANCE, CATEGORY B.**
  - Blood and tissue samples from dead or sick animals:  
**UN3373 and BIOLOGICAL SUBSTANCE, CATEGORY B.**
  - Blood and tissue samples from apparently healthy animals (hunter-killed, live captured):  
**EXEMPT ANIMAL SPECIMENS.**
- Note the shipment tracking number in case packages are delayed.
- These instructions cover 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
- Hefty Freezer – 1 gal
- Hefty Jumbo – 2.5 gal

- Ziploc Freezer – 1 gallon
- Ziploc Big Bag – 20 gallon
- Glad Freezer – 1 qt, 2 qt, 1 gal

Third layer for cooler liner:

- Hefty Cinch Sak (1.1 mil) – 33 and 39 gal
- Hefty Lawn and Leaf (1.1 mil) – 33 and 39 gal
- House brand large trash (1.1 mil) – 30 gal

- Glad Force Flex (1.05 mil) – 25 gal
- Hefty Ultra Flex (1.3 mil) – 30 gal
- House Lawn - Leaf (1.2 mil) – 39 gal

Absorbent material:

- Super absorbent packet or pads for water
- Paper towels
- Do not use packing peanuts or shredded paper.

- Cellulose wadding
- Cotton batting or cotton balls



**BIOLOGICAL SUBSTANCES, CATEGORY B**

---

**EXEMPT ANIMAL  
SPECIMENS**