



Bat White-Nose Syndrome (WNS)/*Pd* Surveillance Submission Guidelines Winter 2018/2019 (November – May)

The following sample submission guidelines are for use when evaluating unusual bat morbidity or mortality during Winter 2018/2019 identified through either passive surveillance efforts (*i.e.*: public reporting, rabies lab submissions) or active surveillance efforts (*i.e.*: hibernacula surveys, spring trapping). They are meant to assist with prioritizing appropriate field samples for laboratory submission based on presence/absence of WNS clinical signs, geographic location, and prior knowledge of WNS status at a site. 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 *Pd*-contaminated sites with greater emphasis on suspect WNS bats found at or beyond the current disease boundaries, and 3) the type of sample received. This document also provides information on the National *Pd* Surveillance Project to assist partners with determining a level of participation that fits their capabilities and interests. **The primary objectives of this surveillance design are to identify range expansion of *Pseudogymnoascus destructans* (*Pd*) and new species of bats affected by WNS.** 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) with any questions, suggestions, or concerns.

TABLE OF CONTENTS	PAGE
Winter 2018/2019 NWHC Bat Submission Quick Reference Chart	2
WNS Clinical Signs and Affected Species	3
 Specimen and Data Collection	 3-5
Biosecurity, Survey Site Data Collection, Field Photographs, Carcass Collection, and Non-lethal Sampling Techniques	
 Submission Guidance	
Unusual bat mortality/behavior <u>not associated with WNS</u> (all areas)	6
Bats with <u>clinical signs suggestive of WNS</u>	
<input type="checkbox"/> Within the WNS Endemic Area (See Map in Appendix A)	6
<input type="checkbox"/> Outside of the WNS Endemic Area (See Map in Appendix A)	6-7
<i>Pd</i> surveillance <u>in absence of clinical signs of WNS</u>	
<input type="checkbox"/> Overview of the NWHC National <i>Pd</i> Surveillance Project	8
 Appendix A: Map of Current WNS Management Areas within the U.S. (Nov 2018)	 9
Appendix B: NWHC Wildlife Mortality Reporting and Diagnostic Services Request (<i>passive sampling</i>)	9
Appendix C: Site Information and Individual Specimen Datasheets (<i>active sampling</i>)	10-13
Appendix D: Protocol for Non-lethal Swab Sampling of Bat Skin for Detection of <i>Pd</i>	14-15
Appendix E: Wing Punch Biopsy Instructions	16-17
Appendix F: Longwave Ultraviolet (UVA) Fluorescence Screening of Bat Wing	18
Appendix G: USGS National Wildlife Health Center Packaging and Shipping Instructions	19-21

Winter 2018/2019 NWHC Bat Submission Quick Reference Chart

Within the WNS Endemic Area: (Appendix A Map – Pg. 9)

Unusual bat mortality/behavior not associated with WNS (NOV-MAY) Pg. 6	Bats with signs suggestive of WNS (NOV-MAY) Pg. 6
<u>Priority Samples</u> <ul style="list-style-type: none"> Any species Any county ≥ 5 dead/sick bats at one location For other situations- consult with NWHC <u>Samples to submit</u> (5–8 bats) <ul style="list-style-type: none"> Photos AND Fresh, intact carcasses MAX. of 3 euthanized non-T/E bats per site 	<u>Priority Samples</u> <ul style="list-style-type: none"> Species not previously confirmed with WNS from any county Any species at/near a hibernaculum of suspect or unknown status in an unconfirmed county <u>Samples to submit</u> (1–5 bats) <ul style="list-style-type: none"> Photos AND fresh, intact carcass OR UV-guided wing biopsies Skin swab only if WNS confirmation is NOT required Euthanasia of sick bats is not advised except for species not previously confirmed with WNS (MAX. of 3 euthanized non-T/E bats per site)

Outside of the WNS Endemic Area: (Appendix A Map – Pg. 9)

Unusual bat mortality/behavior not associated with WNS (NOV-MAY) Pg. 6	Bats with signs suggestive of WNS (NOV-MAY) Pg. 6-7
<u>Priority Samples</u> <ul style="list-style-type: none"> Any species Any county ≥ 5 dead/sick bats at one location For other situations- consult with NWHC <u>Samples to submit</u> (5–8 bats) <ul style="list-style-type: none"> Photos AND Fresh, intact carcasses MAX. of 3 euthanized non-T/E bats per site 	<u>Priority Samples</u> <ul style="list-style-type: none"> Species with confirmed susceptibility to WNS at a suspect positive hibernaculum Any bat species in a county of unconfirmed status <u>Samples to submit</u> (1–5 bats) <ul style="list-style-type: none"> Photos AND fresh, intact carcass of any species OR UV-guided wing biopsies from T/E species or banded bats Skin swabs from biopsied bats, supplement with other affected species MAX. of 3 euthanized non-T/E bats per site

NWHC National *Pd* Surveillance Project:

ENDEMIC AREA (DEC-MAY)	INTERMEDIATE & AT-RISK AREAS Bats with no signs of WNS (DEC-MAY) Pg. 8
<u>Priority Samples</u> <ul style="list-style-type: none"> Any species with clinical signs from a hibernaculum or county of unknown WNS/<i>Pd</i> status Other research priorities identified in conjunction with the WNS Coordination Team/WNS Steering Committee <u>Samples to submit</u> <ul style="list-style-type: none"> Requires prior arrangement with NWHC 	<u>Priority Samples</u> <ul style="list-style-type: none"> Species with confirmed susceptibility to WNS at hibernaculum of unknown WNS/<i>Pd</i> status Species of unknown susceptibility co-roosting with susceptible species at a hibernaculum of unknown status Banded bats originating from contaminated areas detected in a county of unknown status Spring trapping or opportunistic submissions of <i>Myotis</i> spp. & others on landscape where overwintering sites are unknown or inaccessible and <i>Pd</i> status of area is unknown <u>Samples to submit</u> <ul style="list-style-type: none"> 25–30 samples per site Skin swabs ± guano from individual bats (using NWHC kits) Environmental substrates associated with roosting bats (supplemental) Requires prior arrangement with NWHC

WNS CLINICAL SIGNS & AFFECTED SPECIES

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) or areas of yellow-orange fluorescence on hairless skin of bats examined under long-wave UV light through May

WNS has been confirmed in the following North American bat species:

(listed in approximately decreasing frequency of occurrence)

- Little brown bat (*Myotis lucifugus*)
- Tricolored bat (*Perimyotis subflavus*)
- Northern long-eared bat (*Myotis septentrionalis*)
- Indiana bat (*Myotis sodalis*)
- Eastern small-footed bat (*Myotis leibii*)
- Big brown bat (*Eptesicus fuscus*)
- Gray bat (*Myotis grisescens*)
- Southeastern myotis (*Myotis austroriparius*)
- Yuma bat (*Myotis yumanensis*)
- Cave bat (*Myotis velifer*)
- Long-legged bat (*Myotis volans*)

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

- Eastern red bat (*Lasiurus borealis*)
- Rafinesque's big-eared bat (*Corynorhinus rafinesquii*)
- Silver-haired bat (*Lasionycteris noctivagans*)
- Virginia big-eared bat (*Corynorhinus townsendii virginianus*)
- Ozark big-eared bat (*Corynorhinus townsendii ingens*)
- Townsend's big-eared bat (*Corynorhinus townsendii townsendii*)
- Mexican free-tailed bat (*Tadarida brasiliensis*)
- Western small-footed bat (*Myotis ciliolabrum*)

SPECIMEN AND DATA COLLECTION

1. **Biosecurity:** A site contaminated with *P. destructans* retains this designation indefinitely regardless of the presence of affected bats. Prior to leaving each survey site, follow the most current **guidelines for containment and decontamination of field gear and personnel** described in the National White-Nose Syndrome Decontamination Protocol (<https://www.whitenosesyndrome.org/static-page/decontamination-information>).

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.

2. **Survey Site Data Collection:** Fill out the Site Information Datasheet ([Appendix C](#)) whenever hibernacula or roost sites are surveyed, regardless of what state or county you are in and whether you submit specimens to the lab. These data will increase our understanding of the epidemiology of WNS, and records of negative data (*i.e.*: no fungus or abnormal behaviors observed) are important in this effort.
3. **Field Photographs:** Handling bats may cause 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 NWHC-epi@usgs.gov for further submission consultation.

When non-lethal swabs or biopsy 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) with the Site Information/Individual Specimen Collection Datasheets ([Appendix C](#)) including the date photos were taken, site name, and the photographer's name.

4. **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) or visit 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, desiccated carcasses are preferable to wet, slimy carcasses and may be accepted upon consultation with NWHC. If carcasses are being submitted for diagnostic evaluation, keep individual carcasses chilled in separate bags with ID labels according to instructions in [Appendix G](#). If no agency reference # exists, use the following format: state code, MMDDYY, collector's initials, ### (*i.e.*: WI100113AB###). 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 C](#)). **Please contact NWHC-epi@usgs.gov prior to submitting samples. See [Appendix G](#) for NWHC shipping instructions.**

5. **Non-lethal Sampling Techniques:** Non-lethal sampling techniques serve as adjunct or alternative means to evaluate for the presence of *P. destructans* among bats with clinical signs suggestive of WNS at a location. The maximum number of bat carcasses 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 participants in the NWHC National *Pd* Surveillance Project, the target sample size is 25 bats (minimum 15) at sites where the bat population lacks clinical signs of WNS. *Note: Bats from WNS-confirmed counties with visual evidence of WNS (white material on muzzle and/or 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.*

- **Bat skin swab:** see [Appendix D](#) for detailed instructions

Advised application- known susceptible species observed in a hibernaculum of unknown *Pd* status or on the landscape 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 within hibernacula can be sampled using this technique without removing them from roost locations to minimize disturbance. **For Winter 2018/2019, bat swab sampling kits provided by NWHC are available for approx. 5 -10 sites per state within the Intermediate Area and the At-Risk Area for *Pd* surveillance ([Appendix A](#)).** Kits are also available on a limited scale within the WNS Endemic Area. Contact Anne Ballmann (608-270-2445, aballmann@usgs.gov) for details.

- **Wing punch biopsy:** see [Appendix E](#) for detailed instructions

Advised application- any threatened/endangered bat species **with visible fungus or characteristic fluorescence on wing membranes under UVA light**; any known susceptible species in an unconfirmed county within the WNS Endemic Area with physical evidence (visible fungus, wing damage) suggestive of WNS.

To reduce the risk of cross-contamination among bats, all equipment (i.e.: gloves, biopsy punch, cutting surface, and forceps) should be disinfected or changed between each sampled bat. Collect wing biopsies only on live bats with visible fungal growth or characteristic UV fluorescence ([Appendix F](#)) 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 and be accompanied by a skin swab ([Appendix D](#)) from the same bat. E-mail Site Information/Individual Specimen datasheet ([Appendix C](#)) to Anne Ballmann (NWHC-epi@usgs.gov) and overnight ship samples to the NWHC.

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

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

This technique involves handling individual bats to examine extended wings and thus results in hibernation disturbance as well as unknown safety risks to bats. Alternatively, it may be performed to a limited extent on forearms and ears while the bat is roosting in-situ. 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. Also, absence of fluorescence does NOT equate with absence of infectious *Pd* on the bat.

- **Fungal tape-lift**

Earlier versions of this document included fungal tape-lifts as a method for detecting *Pd* on bats. This methodology has been replaced by the skin swab which is analyzed by a highly sensitive and efficient PCR technique.

SUBMISSION GUIDANCE

Before entering hibernacula of any threatened or endangered 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. A copy of your federal permit must accompany samples obtained from endangered species that are sent to the lab.

UNUSUAL BAT MORTALITY/BEHAVIOR NOT ASSOCIATED WITH WNS

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 (see Pg. 4, Carcass Collection), 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 NWHC Wildlife Mortality Reporting and Diagnostic Services Request Form ([Appendix B](#)).

BATS WITH CLINICAL SIGNS SUGGESTIVE OF WNS

- **Sites within the WNS Endemic Area** (see [Appendix A](#))-

Priority samples to submit for laboratory diagnostics:

1. Bat species not previously confirmed with WNS observed with suspicious clinical signs (e.g., visible fungus, wing damage) or aberrant behavior from any county
2. Any bat species with suspicious signs at/near a hibernaculum of suspect or unknown WNS 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 Endemic 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 1 - 5 bats (fresh dead or euthanized) with physical or concurrent behavioral evidence suggestive of WNS along with completed Site Information/Individual Specimen datasheets ([Appendix C](#)). If bats aren't associated with a hibernaculum, submit with [Appendix B](#) form. Once WNS is confirmed in the county, only bat species of unknown susceptibility will typically be accepted for WNS diagnostic evaluation from that county. Bat skin swabs ([Appendix D](#)), however, may be submitted from up to 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 any previously identified *Pd*-contaminated hibernacula outside the WNS Endemic Area be surveyed mid- to late-winter for the development of WNS in the bat population. Specimen types that allow histopathological evaluation (whole carcasses, wing biopsy + skin swab) in conjunction with PCR are recommended for submission.

Priority samples to submit for laboratory diagnostics:

1. Species with confirmed susceptibility to WNS at a suspect positive hibernaculum
2. Any bat species with suspicious clinical signs (e.g., visible fungus, wing damage) or aberrant behavior in a county of unconfirmed status

Site prioritization:

To be determined by the wildlife management agency. Please consult the National WNS Surveillance Implementation Plan or Overview of the NWHC National *Pd* Surveillance Project (pg. 8) for site prioritization guidance.

The following sample collection descriptions apply to bats with clinical signs suggestive of WNS regardless of the area where they are detected. Consult the NWHC Bat Submission Quick Reference Chart (pg. 2) 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 the appropriate submission form (Appendix B-passive surveillance OR Appendix C-active surveillance) and e-mail to NWHC-epi@usgs.gov (608-270-2415 fax). Submit 1–5 fresh carcasses of new bat species with unknown WNS susceptibility that appear affected from a confirmed county. If the county is of suspect or unknown WNS status, submit up to 5 carcasses total of any affected species (see pg. 3 for list of WNS susceptible species).
- **If live bats have behavioral or physical evidence 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 or visit www.michigan.gov/documents/emergingdiseases/Humane_Euthanasia_of_Bats-Final_244979_7.pdf.
 2. Collect a paired skin swab and UV-guided wing punch biopsy on up to 3 individuals (See Appendices D&E) 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 C). *NOTE: The diagnostic reliability for WNS confirmation 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 positive for WNS.*

Submit photos and specimens to NWHC (Appendix G). Include completed Site Information/ Individual Specimen datasheets (Appendix C).
 - **WNS confirmation is NOT required**, follow the method below:
 1. Collect a skin swab from 1–5 visibly affected live bats using kit materials provided by NWHC (See Appendix D for detailed instructions). Photograph the bat prior to swabbing and record associated geographic, demographic, and physical data on the Site Information/Individual Specimen datasheets (Appendix C).

OVERVIEW OF NWHC NATIONAL *Pd* SURVEILLANCE PROJECT

Before entering hibernacula of threatened or endangered 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. A copy of your federal permit must accompany samples obtained from endangered species that are sent to the lab.

This section gives an overview of the National *Pd* Surveillance Project to assist partners with determining a level of participation that fits their capabilities and interests. **Partners that wish to participate should contact Anne Ballmann (aballmann@usgs.gov; 608-270-2445) to receive complete protocols and sampling kits prior to sampling.**

Priority skin 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 within *Pd*-contaminated areas detected in a county of unknown *Pd* status
4. Spring trapping or opportunistic submissions of *Myotis* spp. & others on landscape where overwintering sites are unknown or inaccessible and *Pd* status of area is unknown.

Site prioritization recommendations:

To be determined by the wildlife management agency and should not include hibernacula participating in similar projects for early detection of *Pd* in hibernating bat populations unless requested specifically by NWHC. Broad spatial distribution of selected hibernacula within the state is desirable for a surveillance program. Hibernacula known to contain winter populations of *Myotis* spp. (particularly little brown bats and/or northern long-eared bats) or tricolored bats are encouraged as *Pd* has been detected more commonly on these species. Please consult the National Surveillance Implementation Plan (<https://www.whitenosesyndrome.org/working-group/surveillance-and-diagnostics>) for prioritization guidance.

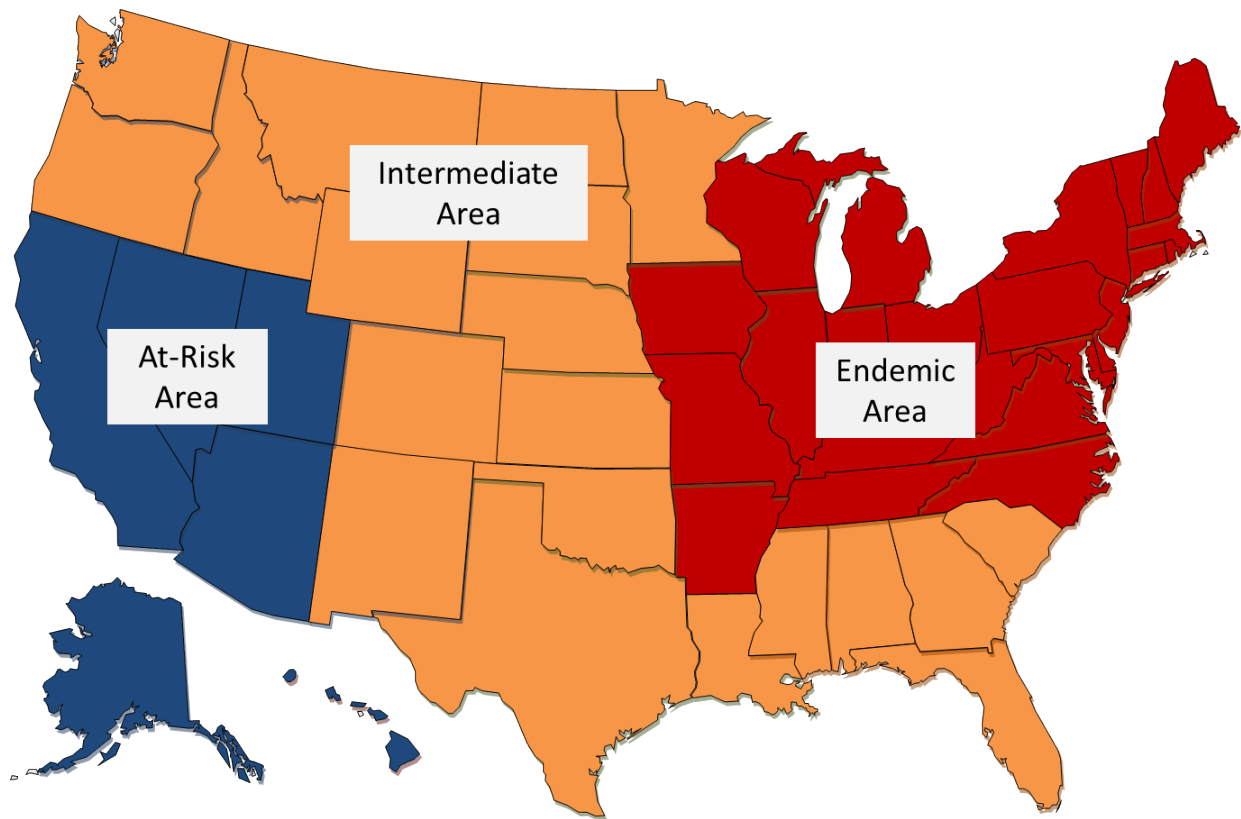
Skin swab samples from a total of 25 bats (minimum sample size = 15 bats) per site are requested using kit materials provided by NWHC. Collect swabs from bats roosting within arms' reach and from representative roosting areas throughout the hibernaculum. This may include bats roosting individually or in separate clusters. Environmental samples may supplement skin swab samples to achieve a total of 25 samples per site. Environmental sampling exclusively at a site requires a larger sample size (n=30) and can result in delayed detection of *Pd* in new areas. Complete the Site Information/Individual Specimen datasheets ([Appendix C](#)) 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. 6-7).

Contact Anne Ballmann (aballmann@usgs.gov; 608-270-2445) to discuss alternative strategies for *Pd* surveillance in bats not associated with winter hibernacula in more detail.

APPENDIX A

MAP A: WNS Management Areas within the United States based on WNS Distribution (as of November 2018)



APPENDIX B

USGS NWHC Wildlife Mortality Reporting and Diagnostic Services Request

Instructions available at: www.usgs.gov/nwhc/submit

Access the form: https://prd-wret.s3-us-west-2.amazonaws.com/assets/palladium/production/s3fs-public/atoms/files/Wildlife_Mortality_Reporting_and_Diagnostic_Services_Request_Form_Sep2018.pdf

Please complete this form for each unique location when submitting bat carcass(es) obtained through passive surveillance efforts (i.e.: public reports, rabies laboratory or rehabilitation facility submissions). Minimum information requested from rabies lab submissions include: State, County where bat was collected, Date of collection, Species, Reference lab case number.

APPENDIX C

Site Information Datasheet

Investigator Name(s): _____ Date: _____

Phone /e-mail: _____

State:	County:	Site Name:	
Latitude:	Longitude:	Datum:	Nearest Pd+ Site: (name)
Site Ownership: (check one) <input type="checkbox"/> Private <input type="checkbox"/> Public <input type="checkbox"/> Military		Site Access: (check one) <input type="checkbox"/> N/A-landscape Open- <input type="checkbox"/> all year, <input type="checkbox"/> seasonal/restricted Gated- <input type="checkbox"/> all year, <input type="checkbox"/> seasonal, <input type="checkbox"/> breech	
Site Use (at time of survey): (check one) <input type="checkbox"/> Hibernaculum <input type="checkbox"/> Day roost <input type="checkbox"/> Night roost <input type="checkbox"/> N/A-landscape		Site Classification: (check one) <input type="checkbox"/> N/A-landscape Cave- <input type="checkbox"/> undeveloped, <input type="checkbox"/> recreational, <input type="checkbox"/> show Mine- <input type="checkbox"/> active, <input type="checkbox"/> inactive, <input type="checkbox"/> show <input type="checkbox"/> Tunnel/culvert <input type="checkbox"/> Well/cistern <input type="checkbox"/> Bldg/bunker <input type="checkbox"/> Bat box <input type="checkbox"/> Bridge <input type="checkbox"/> Rock crevice/talus <input type="checkbox"/> Other (specify): _____	

Population Summary Information:				Survey Type: (check one) <input type="checkbox"/> Full <input type="checkbox"/> Partial <input type="checkbox"/> Trap (circle one): harp mist (check if applicable) <input type="checkbox"/> No bats present <input type="checkbox"/> No population info available		
Location ¹ Trap, Outside, Entrance, Inside circle one per line	Bat species 4-letter code Include "?" if unsure	# live ²	# dead ²	# with fungus visible ²	Distribution of affected bats ³ Solitary, Clustered	Notes Ex: band #s observed, photo file IDs, uncertainty of species ID
T O E I					S C N/A	
T O E I					S C N/A	
T O E I					S C N/A	
T O E I					S C N/A	
T O E I					S C N/A	
T O E I					S C N/A	
T O E I					S C N/A	
T O E I					S C N/A	
T O E I					S C N/A	
T O E I					S C N/A	

¹Separate popn information by location for each species, **Entrance:** area impacted by daylight (twilight zone), **Inside:** beyond twilight zone²Indicate if number is an estimate count; ³Cluster: ≥ 2 bats in direct contact, N/A: not applicable

Other WNS Clinical Signs Present at Site: (check all that apply) <input type="checkbox"/> UV positive bats <input type="checkbox"/> Moderate to severe wing damage (WDI ≥ 2) <input type="checkbox"/> Increased mortality/significant reduction in population count <input type="checkbox"/> Unusual roosting near entrance of hibernaculum <input type="checkbox"/> Increased day flight at entrance, # of bats flying in 5 min: _____	List species with each clinical sign: _____ _____ _____ _____
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Comments:

Please attach a map of the hibernaculum with marked locations of sampled bats/environment within the site.
 Complete the Individual Specimen Collection Datasheet(s). **For trap surveys**, include copies of any additional datasheets.

EMAIL A SCANNED COPY OF ALL DATASHEETS AT TIME OF SHIPMENT

Version 2019-20 (1)

APPENDIX C- USGS NWHC Individual Specimen Collection Datasheet (pg. 1 of 3)

Site Name: _____

Date: _____

Vial # ¹ <i>See example label below.</i> Replace written vial # with Spot Label prior to shipment Include submitter ID if no Spot Label	Sample Type Whole <u>C</u> arcass Wing <u>T</u> issue <u>B</u> at Swab <u>S</u> oil <u>E</u> nviro Swab <u>G</u> uano circle ONE	Additional Sample from <u>Same Bat</u> if applicable Wing <u>T</u> issue <u>B</u> at Swab <u>G</u> uano Vial # ¹ circle ONE	Species 4-letter code Include "???" if unsure	On-site Location ² Trap <u>O</u> utside <u>E</u> ntrance <u>I</u> nside circle ONE	Status <u>L</u> ive <u>D</u> ead <u>E</u> uth	Roost Pattern <u>S</u> olitary <u>C</u> luster ³ <i>Non- trap surveys only</i>	Visible Fungus <u>M</u> uzzle <u>E</u> ar <u>W</u> ing <u>T</u> ail circle all that apply	UV	Wing Damage Index <small>Reichard et al. 2009</small>	Age Class	Body Wt. <small>(0.01 g)</small>	Band No. <i>if applicable</i>	Comments: -Agency's Ref. ID -Additional Vial # for bat – identify sample type -Deviations from protocol, photo file ID, etc. Enviro swabs: Specify as ceiling, wall, trap type, etc.
									Sex	Repro. Status ⁴	Forearm Length <small>(0.1 mm)</small>	Recapture	
									Male Female				
	CTBSEG		T B G	T O E I	L D E	S C	MEWT	+-	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+-	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+-	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+-	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+-	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+-	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+-	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+-	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+-	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+-	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	

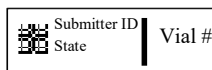
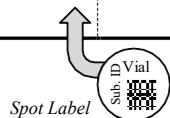
¹If no vial # label exists: create a unique ID Ex: *WT010118AB001* (state, MMDDYY, collector, ###)

²Entrance: area impacted by daylight (twilight zone), Inside: beyond twilight zone;

³Cluster: ≥2 bats in direct contact

⁴PG: pregnant, LA: lactating, PL: post-lactating, SC: scrotal, NR: non-reproductive

Version 2018-19 (1)



Vial Label

APPENDIX C- USGS NWHC Individual Specimen Collection Datasheet (pg. 2 of 3)

Site Name: _____

Date: _____

Vial # ¹ <i>See example label below.</i> Replace written vial # with Spot Label prior to shipment Include submitter ID if no Spot Label	Sample Type Whole <u>C</u> arcass Wing <u>T</u> issue <u>B</u> at Swab <u>S</u> oil <u>E</u> nviro Swab <u>G</u> uano circle ONE	Additional Sample from <u>Same Bat</u> if applicable Wing <u>T</u> issue <u>B</u> at Swab <u>G</u> uano Vial # ¹ circle ONE	Species 4-letter code Include "?" if unsure	On-site Location ² Trap <u>O</u> utside <u>E</u> ntrance <u>I</u> nside circle ONE	Status <u>L</u> ive <u>D</u> ead <u>E</u> uth	Roost Pattern <u>S</u> olitary <u>C</u> luster ³ <i>Non- trap surveys only</i>	Visible Fungus <u>M</u> uzzle <u>E</u> ar <u>W</u> ing <u>T</u> ail circle all that apply	UV	Wing Damage Index <small>Reichard et al. 2009</small>	Age Class	Body Wt. <small>(0.02 g)</small>	Band No. <i>if applicable</i>	Comments: -Agency's Ref. ID -Additional Vial # for bat – identify sample type -Deviations from protocol, photo file ID, etc. Enviro swabs: Specify as ceiling, wall, trap type, etc.
									Sex <u>M</u> ale <u>F</u> emale	Repro. Status ⁴	Forearm Length <small>(0.1 mm)</small>	Recapture	
	CTBSEG		T B G	T O E I	L D E	S C	MEWT	+-	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	

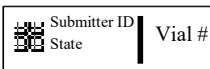
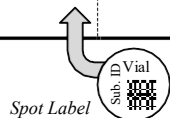
¹If no vial # label exists: create a unique ID Ex: W1010118AB001 (state, MMDDYY, collector, ###)

²Entrance: area impacted by daylight (twilight zone), Inside: beyond twilight zone;

³Cluster: ≥2 bats in direct contact

⁴PG: pregnant, LA: lactating, PL: post-lactating, SC: scrotal, NR: non-reproductive

Version 2018-19 (1)



Vial Label

APPENDIX C- USGS NWHC Individual Specimen Collection Datasheet (pg. 3 of 3)

Site Name: _____

Date: _____

Vial # ¹ <i>See example label below.</i> Replace written vial # with Spot Label prior to shipment Include submitter ID if no Spot Label	Sample Type Whole <u>C</u> arcass Wing <u>T</u> issue <u>B</u> at Swab <u>S</u> oil <u>E</u> nviro Swab <u>G</u> uano circle ONE	Additional Sample from <u>Same Bat</u> if applicable Wing <u>T</u> issue <u>B</u> at Swab <u>G</u> uano Vial # ¹ circle ONE	Species 4-letter code Include "???" if unsure	On-site Location ² Trap <u>O</u> utside <u>E</u> ntrance <u>I</u> nside circle ONE	Status <u>L</u> ive <u>D</u> ead <u>E</u> uth	Roost Pattern <u>S</u> olitary <u>C</u> luster ³ <i>Non- trap surveys only</i>	Visible Fungus <u>M</u> uzzle <u>E</u> ar <u>W</u> ing <u>T</u> ail circle all that apply	UV	Wing Damage Index <small>Reichard et al. 2009</small>	Age Class	Body Wt. <small>(0.03 g)</small>	Band No. <i>if applicable</i>	Comments: -Agency's Ref. ID -Additional Vial # for bat – identify sample type -Deviations from protocol, photo file ID, etc. Enviro swabs: Specify as ceiling, wall, trap type, etc.
									Sex <u>M</u> ale <u>F</u> emale	Repro. Status ⁴	Forearm Length <small>(0.1 mm)</small>	Recapture	
	CTBSEG		T B G	T O E I	L D E	S C	MEWT	+ -	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+ -	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+ -	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+ -	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+ -	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+ -	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+ -	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+ -	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+ -	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	
	C T B S E G		T B G	T O E I	L D E	S C	MEWT	+ -	0 1 2 3 MF	A J U PG LA PL SC NR		Y N	

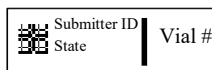
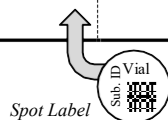
¹If no vial # label exists: create a unique ID Ex: *WT010118AB001* (state, MMDDYY, collector, ###)

²Entrance: area impacted by daylight (twilight zone), Inside: beyond twilight zone;

³Cluster: ≥2 bats in direct contact

⁴PG: pregnant, LA: lactating, PL: post-lactating, SC: scrotal, NR: non-reproductive

Version 2018-19 (1)



Vial Label

APPENDIX D - Protocol for Non-lethal Swab Sampling of Bat Skin for Detection of *Pseudogymnoascus destructans* (Pd)

Prepared by: USGS – National Wildlife Health Center (October 2013; updated 11/6/2018)

Purpose: The following procedure is designed to detect the presence of Pd while minimizing disturbance 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:

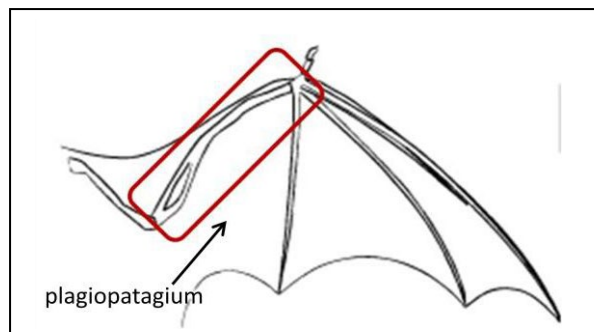
- Sterile, individually wrapped polyester-tipped swabs with plastic shafts (27)
- Sterile, pre-labeled 1.5-ml microcentrifuge tubes, each containing 150 µl of nuclease-free water (25)
- Plastic bags for vial storage (1 quart-size) & “TRASH” (1 gallon-size)
- Datasheets on waterproof paper that can be decontaminated appropriately
- Plastic bag (1 gallon-size) for “CLEAN” outer storage & packaging of sample vials and datasheet (**do not carry this bag inside hibernaculum**)
- Spot Labels containing barcodes for datasheets
- Insulated shipper box with 2 ice packs (for return shipment only; **do not carry inside hibernaculum**)
- Pre-paid return FedEx shipping label & airbill pouch

Needed:

- Disposable exam gloves
- Ultra-violet UVA (368-385nm) light source (optional)
- Pencil or indelible ink pen
- Plastic clipboard
- Decontamination supplies
- Cooler with ice packs for sample on-site storage & transport from site

Bat Swab Collection Protocol:

1. Persons collecting swab samples 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 the environmental substrate.
2. Identify a bat to be sampled.
3. Record the requested individual bat information on the Individual Specimen Datasheet. Remove a pre-labeled sample tube from the “SWAB VIALS” bag. **Remember to record the unique Vial # from the selected sample tube. Spot Labels with barcodes should replace written vial #s on datasheet prior to shipment to the lab.**
4. Tap sample tube to ensure all liquid is pooled at the bottom.
5. Remove a swab from its packaging **without touching the polyester tip**.
6. Dip the tip of the swab into the sample tube to moisten (most water will be absorbed by 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 and maximizes contact with the swab surface.
9. Roll the same swab across the muzzle of the same bat 3 times.
 10. After collecting the sample, transfer swab to the same sample tube used to moisten it. Break off the shaft **near the applicator tip**. Avoid touching the rim of the tube or inside of lid with your fingers. Screw closed the tube lid tightly.
 11. Place swab sample tubes into the “SAMPLES” bag and follow instructions for sample handling and storage.
 12. Dispose of swab handles, wrappers, and contaminated exam gloves as necessary into “TRASH”.
 13. Repeat the above process for each bat sampled.

Sample Handling and Storage:

- Samples collected inside the hibernaculum can be maintained at ambient temperature while underground. Whenever above-ground, hold collected samples on frozen ice packs for transport to an office refrigerator or freezer.
- Prior to leaving the site, spray datasheets with a non-alcohol-based disinfectant and place inside the emptied “SWAB VIALS” bag. Decontaminate the outer surfaces of all bags carried on-site following current USFWS Decontamination Guidelines (<https://www.whitenosesyndrome.org/static-page/decontamination-information>).
- Place samples bag and datasheet bag inside the “CLEAN” bag (1 gallon-size) for storage and shipment-**this bag should not be carried on-site**. Remove all excess air from bags.
- Hold all samples chilled (4°C) if they are to be shipped within 2 days after collection. If you are sampling multiple sites, samples can be stored frozen at -20°C (preferably not a frost-free unit that undergoes periodic freeze-thaw cycles) to facilitate batch shipping at your convenience. However, **frozen samples MUST be received by the lab no later than 4 weeks after collection**. If only a frost-free freezer is available, package samples between ice packs within the freezer to protect them from temperature fluctuations. Longer-term storage at -80°C is possible. 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. A prepaid FedEx priority overnight return shipment label is included with each shipper provided. Ship early in the week (Mon-Wed). DO NOT ship on Fridays or the day before a federal holiday. NWHC cannot receive weekend deliveries. **Email NWHC (nwhc-epi@usgs.gov) when you are ready to return samples**. Include the package tracking number and scanned copies of the completed datasheets in the email. Remember to replace written Vial #s with barcode Spot Labels onto dry, disinfected datasheets prior to scanning and 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 E - Instructions for Taking a Wing Tissue Biopsy

Modified by Pat Ormsbee (NFS) and Jan Zinck 5/14/2009 (original: Shonene Scott, Portland State University 5/2003)

Updated by Anne Ballmann (USGS-NWHC): 11/6/2018

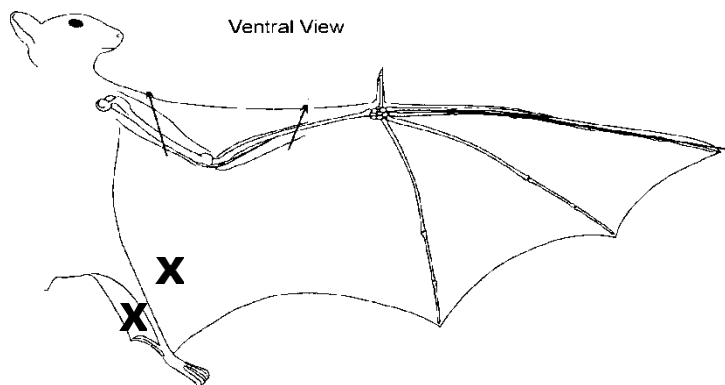
NOTE: If punch biopsies are the only sample type to be submitted to the lab in a particular case, it is highly recommended that 2 biopsies per bat be collected (from different wings). Additional population genetic sampling should not be attempted in these individuals to reduce the number of holes in the wings. Alternatively, a skin swab can be substituted for one of the biopsy samples and should be collected first. **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. Use a small clean piece of sturdy cardboard for a flat cutting surface that can be discarded after each animal, a new biopsy punch for each bat, sterilized forceps, and disposable gloves.
2. Label each sterile vial using a black ultra-fine Sharpie permanent marker with the unique bat ID number using the format shown below. Indicate the sample type on the vial (“**T**issue” or “**B**at swab”).

State, Date (MMDDYY), Collector initials, sequential number ### (ex: WI061609AB001)

3. Have a fresh cardboard square, a labeled tube, a new tissue punch, and some 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. When collecting wing tissue biopsies, avoid sampling from bats with large wing tears or in areas over bones and major blood vessels (Figure 1). Identify up to 2 representative lesions to biopsy on the affected wings/tail of the bat. ***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).
5. Place the bat on the cardboard on its back and extend one wing membrane. For people inexperienced in this technique, it works best when one person holds the bat and another person collects the biopsy. Position the biopsy punch perpendicular to the skin, press the punch firmly through the membrane and twist the punch slightly to ensure complete penetration. 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 E - Instructions for Taking a Wing Tissue Biopsy -con't

6. Carefully lift the bat off the cardboard and look for the tissue sample. It should either be on the cardboard or inside the tip of the punch. A new 25 ga needle or the plastic shaft of a sterile swab 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) if a skin swab sample is not available. For histopathological evaluation, place tissue into a storage vial containing 10% buffered neutral formalin (1part tissue to 10 parts formalin). If formalin is unavailable, place biopsy in an empty sterile vial.
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 is limited to 2 per bat to prevent compromised 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. Ship wing tissues to NWHC. Ensure that all vials are labeled and lids are secured in place to prevent cross-contamination of samples. Wrap lid of vials 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 within 2 days of collection, freeze them (-20°C) and ship no later than 1 week after collection. **NOTE: There are additional packaging and labelling requirements for shipment of specimens stored in formalin.** Contact NWHC for more details.

Send an electronic copy of the completed datasheets ([Appendix C](#)) 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 sterile biopsy punches Fisher Scientific Catalog # NC9515874 (\$106.73/pack of 50)
- ☐ 25-gauge needles **OR** sterile plastic-shafted swabs
- ☐ Sharps collection container
- ☐ 10% buffered neutral formalin (if histopathological analysis is desired)
- ☐ 2ml sterile plastic vials with caps
- ☐ Fine point permanent marker
- ☐ Vial labels
- ☐ Disposable exam gloves
- ☐ Stiff cutting surface (cardboard square)
- ☐ Parafilm sealant
- ☐ Ziploc bags and cooler with blue ice

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

Authors: Anne Ballmann, Carol Meteyer (modified from G. Turner & J. Gumbs 2011), 5/7/2012

Updated by Anne Ballmann (USGS-NWHC): 11/6/2018

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 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 taken with a camera tripod setup.
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 E](#)) 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 sterile vial for PCR and keep chilled in the field. Alternatively, a combined wing/muzzle swab (Appendix D) can be substituted for the 2nd wing biopsy. Label vials with the unique bat ID number.
5. Submit samples along with any digital photos of fluoresced wings to NWHC-epi@usgs.gov.

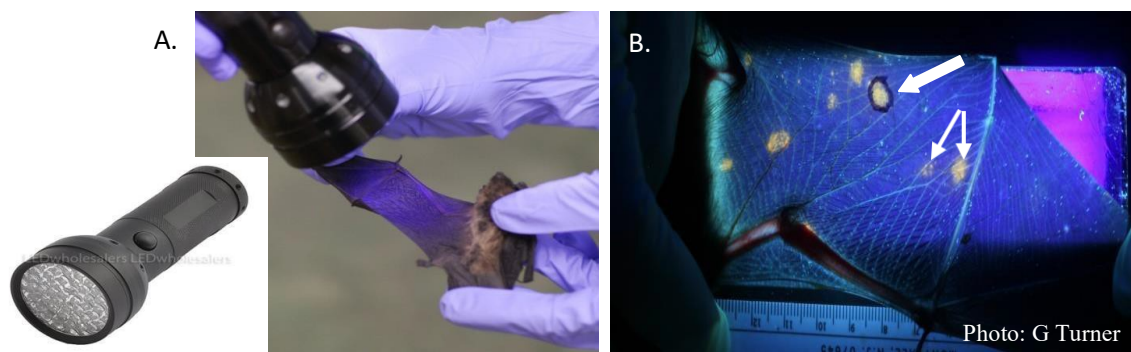


Figure 1. A) UV flashlight examination of ventral bat wing to be conducted in total darkness. B) Digital photo of backlit extended wing held over 368 nm UV light box. Arrows identify yellow-orange fluorescent areas of various diameters associated with suspect *P. destructans* infection.

APPENDIX G

USGS – National Wildlife Health Center

INSTRUCTIONS FOR COLLECTION AND SHIPMENT OF AVIAN AND MAMMALIAN CARCASSES

Contact the NWHC Field Epidemiology Team before shipping.

Alaska, continental US, or Puerto Rico: NWHC-epi@usgs.gov, 608-270-2480

Hawaii/Pacific Islands: thierry_work@usgs.gov, 808-792-9520



The following instructions should be used for collecting and shipping wildlife carcasses, carcass parts 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 NWHC to get shipping approval and discuss shipping arrangements. Typically, ship specimens by 1-day priority 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 NWHC. 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.
- ☐ 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 agency's internal 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 contents.
- ☐ 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 the 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:

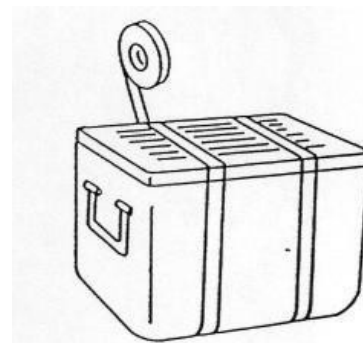
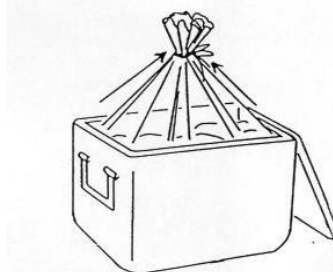
**National Wildlife Health Center
Necropsy Loading Dock
6006 Schroeder Road
Madison, WI 53711**

Emergency Contact:

NWHC FET emergency 608-270-2400

Supplementary Labels:

Keep Cold
- ☐ Mark the cooler with the appropriate information: (See last page 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
Hefty Freezer – 1 gal
Hefty Jumbo – 2.5 gal

Ziplock Freezer – 1 gallon
Ziplock 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**