USGS National Wildlife Health Center

Bat White-Nose Syndrome (WNS)/*Pd* Surveillance Submission Guidelines Winter 2025/2026 (November – May)

The following sample submission guidelines are for use when evaluating unusual bat morbidity or mortality during Winter 2025/2026 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/*Pd* 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 sample type received. **The primary objective of this document is to identify range expansion of *Pseudogymnoascus destructans* (*Pd*) while opportunistically identifying new species of bats affected by WNS.** National guidance on active surveillance for the early detection of Pd (aka "Pd Surveillance 3.0") to assist partners with site selection and prioritization are also outlined in this document. Instructions for sample collection at designated long-term monitoring sites for disease progression among western bat species (aka "Pd surveillance 4.0) are provided elsewhere. 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.

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Winter 2025/2026 NWHC Bat Submission Quick Reference Chart

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

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

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

Samples to submit (5–8 bats)
- Photos AND
- Fresh, intact carcasses
- MAX. of 3 euthanized non-T/E bats per site

Bats with signs suggestive of WNS (NOV-MAY) Pg. 6

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

Samples to submit (1–5 bats)
- 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 A – Pg. 9)

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

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

Samples to submit (5–8 bats)
- Photos AND
- Fresh, intact carcasses
- MAX. of 3 euthanized non-T/E bats per site

Bats with signs suggestive of WNS (NOV-MAY) Pg. 6-7

Priority Samples
- Any species in a county of unconfirmed WNS/Pd status
- Species not previously confirmed with WNS in a WNS+ county

Samples to submit (1–5 bats)
- 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 (3.0): (Appendix A: Map B – Pg. 10)

ENDEMIC AREA (DEC-MAY)

Priority Samples
- Limited to any species with clinical signs of WNS from an ecosection of unknown WNS/*Pd* status

Samples to submit
- Requires prior arrangement with NWHC

INTERMEDIATE & AT-RISK AREAS Bats with no signs of WNS (DEC-MAY) Pg. 8

Priority Samples
- Any species at site considered "Inconclusive for *Pd*" (if desired) in ecosection of unconfirmed Pd presence
- Species with confirmed susceptibility to WNS at a hibernaculum within an ecosection of unknown WNS/*Pd* status
- Species of unknown susceptibility co-roosting with susceptible species at a hibernaculum within an ecosection of unknown Pd status
- Banded bats originating from *Pd*-contaminated areas detected in an ecosection of unknown Pd status
- Spring trapping of *Myotis* & others on landscape or environmental sampling in ecosections of unknown Pd status where overwintering sites are unknown or inaccessible

Samples to submit
- 25–50 samples per site (minimum colony size = 15 bats)
- Skin swabs ± guano from individual bats (using NWHC kits)
- Environmental swabs/guano at bat roosts (supplemental)

*https://www.usgs.gov/centers/nwhc/science/diagnostic-case-submission-guidelines

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, early return to summer roost)
- 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 late spring/early summer

Species with confirmed WNS in North America:

- Big brown bat (*Eptesicus fuscus*)
- Cave bat (*Myotis velifer*)
- Eastern small-footed bat (*Myotis leibii*)
- Fringed bat (*Myotis thysanodes*)
- Gray bat (*Myotis grisescens*) *endangered
- Indiana bat (*Myotis sodalis*) *endangered
- Little brown bat (*Myotis lucifugus*)
- Long-legged bat (*Myotis volans*)
- Northern long-eared bat (*Myotis septentrionalis*) *endangered
- Tricolored bat (*Perimyotis subflavus*) *proposed endangered
- Western long-eared bat (*Myotis evotis*)
- Yuma bat (*Myotis yumanensis*)

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

- California bat (*Myotis californicus*)
- Canyon bat (*Parastrellus hesperus*)
- Eastern red bat (*Lasiurus borealis*)
- Mexican free-tailed bat (*Tadarida brasiliensis*)
- Pallid bat *(Antrozous pallidus)*
- Rafinesque's big-eared bat (*Corynorhinus rafinesquii*)
- Silver-haired bat (*Lasionycteris noctivagans*)
- Townsend's big-eared bat (*Corynorhinus townsendii*)
  - Ozark big-eared bat (*Corynorhinus townsendii ingens*) *endangered
  - Virginia big-eared bat (*Corynorhinus townsendii virginianus*) *endangered
- Western red bat (*Lasiurus frantzii*)
- 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.

   COVID-19 Guidance: Additional biosecurity recommendations for bat-related activities due to circulating SARS-CoV-2 variants are available.
   https://www.iucnbsg.org/bsg-publications.html;
   https://www.fishwildlife.org/application/files/1915/9230/2350/Covid-19_Guidance_for_Bats_4-13-2020a11.docx

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 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 skin swabs or wing biopsy samples are collected from bats with suspicious clinical signs, we request close-up images of individual live bats prior to sampling. E-mail photos to NWHC-epi@usgs.gov 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. **You MUST have the proper permits or authorization for specimen collection and record the method of euthanasia on the datasheet/submission form**. For guidance on acceptable methods of euthanasia in bats for WNS evaluation, see Appendix G or visit https://www.usgs.gov/media/videos/approved-euthanasia-methods-bats-microchiroptera.

   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 fresh carcasses (intact body, no evidence of scavenging, fur does not pull out easily) representing each affected species. If fresh carcasses are unavailable, intact desiccated carcasses free of excessive fungal overgrowth 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 H. If no agency reference # exists, use the following format: state code, MMDDYY, collector's initials, ### *(i.e.*: WI010120AB###). 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 H for NWHC shipping instructions.**

5. **Non-lethal Sampling Techniques:** Non-lethal sampling techniques serve as adjunct or alternative means to evaluate the presence of *P. destructans* among bats with clinical signs suggestive of WNS at a location. The maximum number of bat carcasses per site 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 early Pd detection in new areas (Pd Surveillance 3.0), the target sample size is 25 bats (minimum 15) at sites where the bat population lacks clinical signs of WNS. Supplemental environmental samples are required whenever fewer than 25 bats per site are sampled. *Note: Bats from WNS-confirmed counties with visible evidence of WNS (white material on muzzle and/or wing membranes) are considered suspect positive for WNS. Disturbance of these bats may compromise survival and further sampling is not advised unless there is a specific need.* ***Non-lethal sampling techniques may have a reduced reliability to confirm WNS or 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 2025/2026, active *Pd* surveillance kits provided by NWHC will be allocated using a hybrid approach for early Pd detection (Pd surveillance 3.0) in ecosections of negative or unknown Pd status and long-term monitoring of WNS progression at select sites in western states (Pd surveillance 4.0).** 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 replaced 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. *NOTE: Absence of fluorescence does NOT equate with absence of infectious Pd on bats.*

- **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 qPCR technique.

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), and ship chilled (≤36 hrs since collection) or frozen (>36 hrs) to NWHC for evaluation according to packaging and shipping instructions in Appendix H. 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: Map 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 at the time of submission.** Take field photos and submit up to 5 fresh, intact carcasses or up to 3 bats (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 unless specifically requested by NWHC. 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: Map A)-

Note: It is recommended that 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 qPCR are recommended for submission. Sites designated for Pd surveillance 4.0 (long-term monitoring) are sampled in accordance to the project specific protocol (provided separately).

Priority samples to submit for laboratory diagnostics:

1. Any bat species with suspicious clinical signs (e.g., visible fungus, wing damage) or aberrant behavior in a county of unconfirmed WNS status

2. Species not previously confirmed with WNS in a county where WNS has been confirmed

Site prioritization recommendations:

Hibernacula located in counties of suspect or unknown disease status. Once WNS is confirmed in a county, surveillance should be limited to hibernacula of critical biological or management significance during the winter period and/or targeted towards confirmation of disease in new species. If WNS is first confirmed on a bat found on the above-ground landscape, effort should be made to identify the environmental reservoir.

At sites NOT designated for Pd surveillance 4.0 long-term monitoring, 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 bat species with unknown WNS susceptibility ONLY that appear affected from a WNS 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. You must have the appropriate permit or management authority for lethal sampling. If conducting surveillance activity under the NWHC IACUC protocol, you are required to follow our bat euthanasia protocol. For guidance on acceptable methods of euthanasia in bats for WNS evaluation, visit https://www.usgs.gov/media/videos/approved-euthanasia-methods-bats-microchiroptera or see Appendix G.

    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 by wing punch biopsies may be reduced as compared to whole carcass evaluation. Thus, negative results do not rule out the possibility of a bat being positive for WNS.*

       Submit photos and specimens to NWHC (Appendix H). 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 (3.0)

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 Pd surveillance 3.0 to assist partners with determining a level of participation that suits their resources and objectives. Priority ecosections (https://www.fs.usda.gov/rds/archive/Catalog/RDS-2016-0003) for early detection of Pd expansion are those of unknown Pd status. Continuation of targeted, passive surveillance of bats with suspicious clinical signs/behaviors is encouraged throughout all WNS Management Areas in accordance to guidance described in the previous section. **Partners that wish to participate in active Pd surveillance should contact Anne Ballmann (aballmann@usgs.gov) to receive sampling kits and detailed protocols.**

Site prioritization recommendations: (1-highest priority; 3-lowest priority)

1. Sites considered "Inconclusive for *Pd*" in an ecosection within the Intermediate and At-Risk WNS Management Areas (if desired), with emphasis on those located in ecosections of unknown WNS/*Pd* status.
2. Hibernacula or summer congregation areas located within an ecosection of unknown Pd status. Sites receiving experimental treatments are excluded. *Note- Coordination of surveillance efforts with adjacent states sharing overlapping priority ecosections is encouraged to maximize resources.*
3. Limit the number of sites selected within an ecosection where *Pd* is already known to occur to only those of critical biological or management significance.

Sites known to contain 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.

Priority skin swab samples to submit for laboratory diagnostics:

1. Species with confirmed susceptibility to WNS at hibernaculum of unknown or inconclusive WNS/*Pd* status
2. Species of unknown susceptibility to WNS co-roosting with species of confirmed susceptibility at hibernaculum of unknown or inconclusive WNS/*Pd* status
3. Bats banded within *Pd*-contaminated areas detected in a county or ecosection of unknown *Pd* status
4. Spring trapping or opportunistic sampling of *Myotis* spp. & others on landscape where overwintering sites are unknown or inaccessible and *Pd* status of area is unknown. Fresh guano from trapped individuals can be collected opportunistically or obtained from roost environments to supplement individual skin swabs.

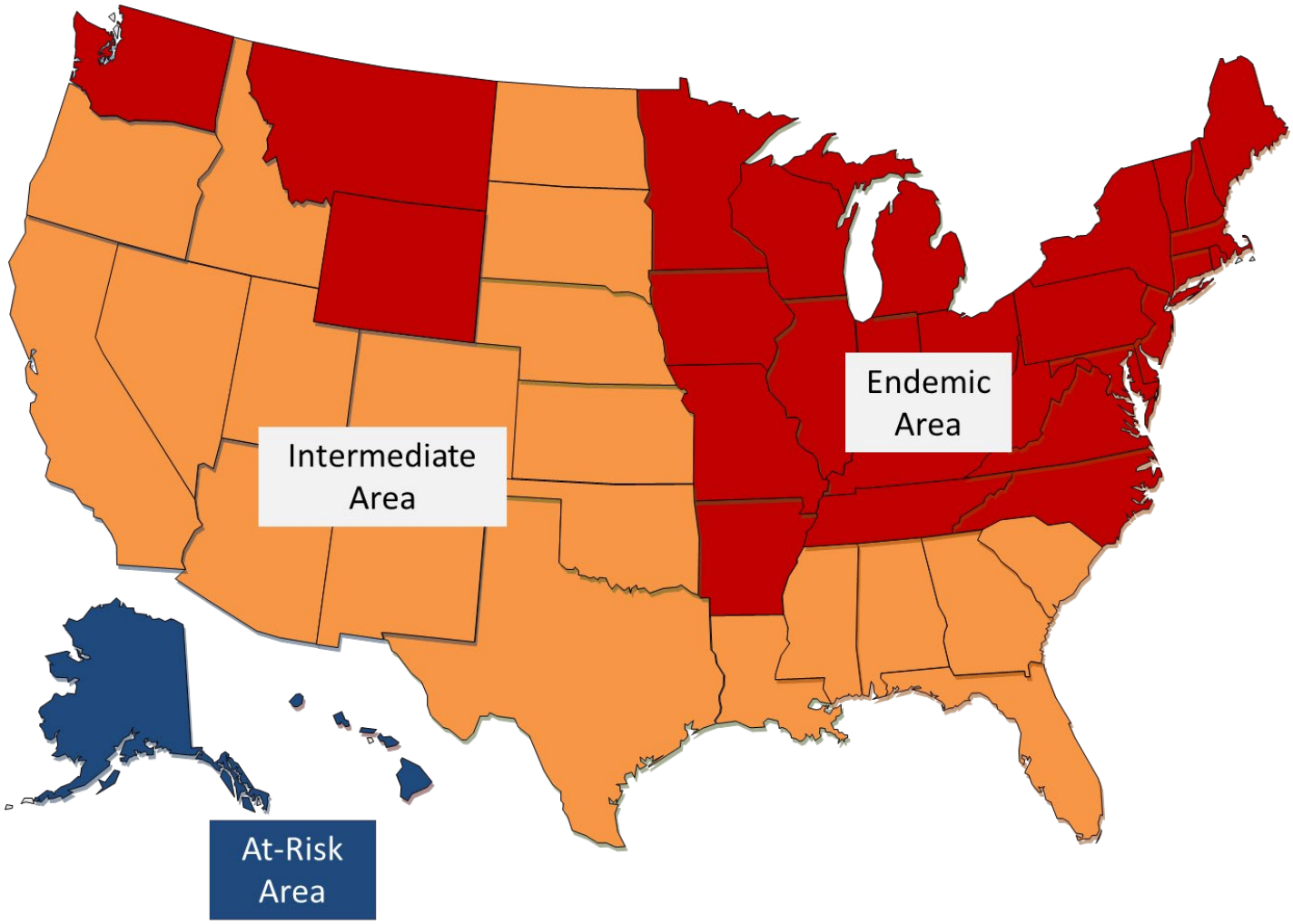
Skin swab samples from a total of 25 bats (minimum sample size = 15 bats) per site are requested using kit materials provided by NWHC. A single kit may be split among neighboring roosts if bats are known to move between sites. Collect swabs from individual bats roosting within arms' reach and from representative roosting areas throughout the hibernaculum. Environmental swabs should supplement skin swab samples (5x substitution factor) whenever fewer than 25 bats per site are sampled. Environmental sampling exclusively at a site requires a larger sample size (n=50) 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 or to nominate sites for *Pd* surveillance 4.0 long-term monitoring.

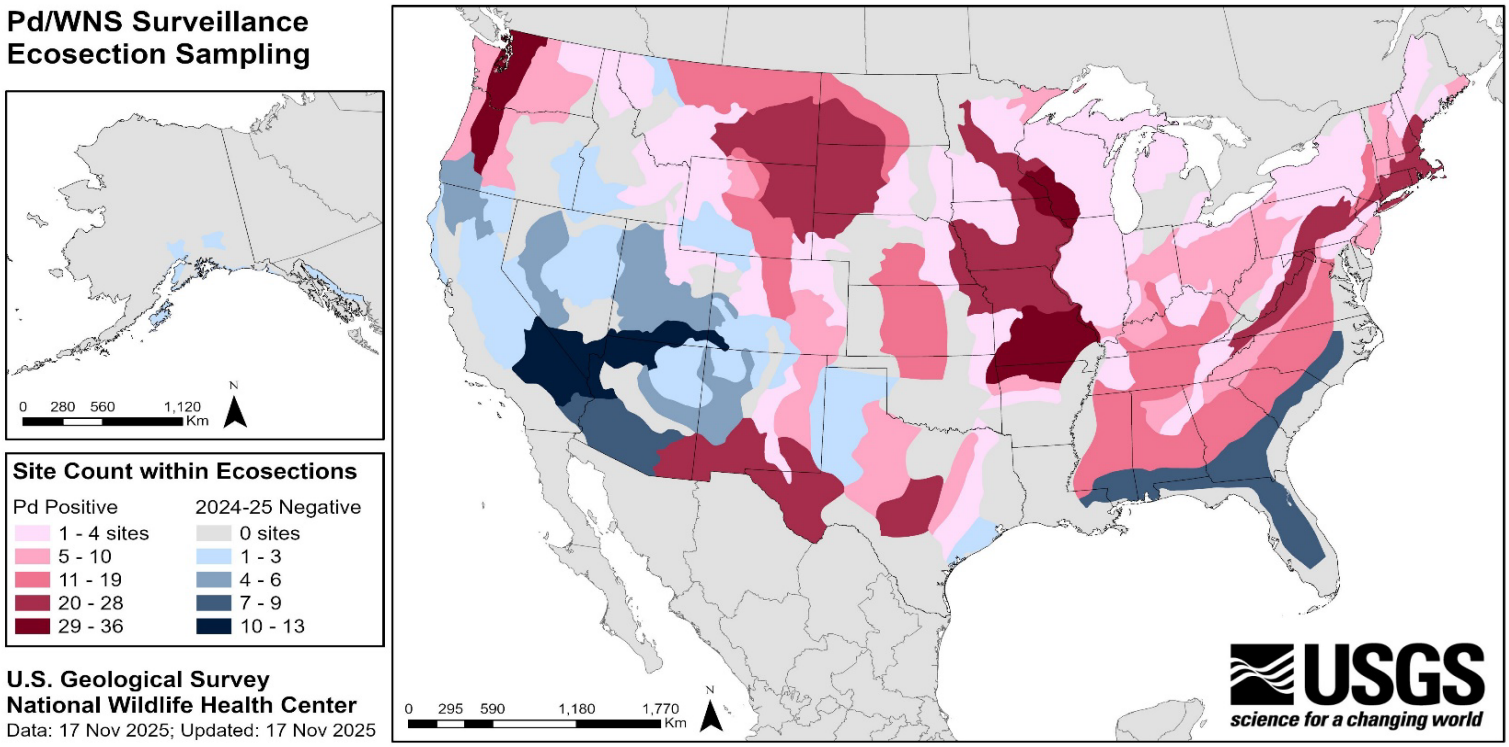
APPENDIX A

MAP A: WNS Management Areas within the United States based on WNS Distribution (as of Nov 2025)



APPENDIX A (con't)

MAP B: Pd status of ecosections in the US based on cumlative surveillance data 2008-2025 (as of Nov 2025)



APPENDIX B

USGS NWHC Wildlife Mortality Reporting and Diagnostic Services Request

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

Access the form: Wildlife Mortality Reporting and Diagnostic Services Request Worksheet (amazonaws.com)

Please complete this form for each unique location when submitting bat carcass(es) obtained through passive surveillance efforts. If submitting bats collected from multiple locations and dates for WNS screening only, contact nwhc-epi@usgs.gov to request a "Rabies Lab/Rehab Datasheet" to include with your submission request.

APPENDIX C

USGS NWHC **Site Information Datasheet**

OMB# 1028-0147
Expires: 10/31/2027

Investigator(s): _____ **Date:** _____

Phone/Email: _____

Sampling Authority: (check one) **(live bats only)**	□ Agency Management Authority	□ Investigator's IACUC Protocol Protocol #: Exp. Date:	□ NWHC IACUC Protocol

State:	**County:**	**Site Name:**
Latitude:	**Longitude:**	Datum:

Site Ownership: (check one)
□ Private □ Public □ Tribal
□ Other (specify): _____

Site Access: (check one) □ N/A-on landscape
Open- □ all year, □ seasonal/restricted
Gated- □ all year, □ seasonal, □ breech

Winter Site Use: (check one) □ Yes □ No □ Unknown □ N/A-on landscape

Summer Site Use: (check one) □ No □ Unknown □ N/A-on landscape
Yes- □ Maternity Roost, □ Night Roost, □ Day Roost (non-maternity)

Site Classification: (check one) □ N/A- on landscape
Cave- □ undeveloped, □ recreational, □ show
Mine- □ active, □ inactive, □ show
□ Tunnel/culvert □ Building/bunker □ Bridge
□ Well/cistern □ Bat box □ Rock crevice/talus
□ Other (specify):
_____

Sampling Method(s): □ **Roosting/Hand-capture** □ **Trap** (type:_____) □ **Env Swabs Only** □ **Pooled Guano**

Population Information: (check if applicable) □ No bats present □ No population info available

Survey Date[1]	**Survey Method**[1] **R**oost/Visual count **T**rap count **E**xit count *Other (specify in Notes)* **circle one per line**	**Site Count** **F**ull **P**artial **N**ot **A**pplicable	**Species**[1] 4-letter code include "?" if unsure	**# Live**[2]	**# Dead**[2]	**Species ID Method** **M**orphology only **A**coustics **G**enetics **circle any that apply**	**Notes**
	R T E	F P N/A				M A G	
	R T E	F P N/A				M A G	
	R T E	F P N/A				M A G	
	R T E	F P N/A				M A G	
	R T E	F P N/A				M A G	
	R T E	F P N/A				M A G	
	R T E	F P N/A				M A G	

[1]Separate popn information by method and date for each species, [2]Indicate if number is an estimated count

Pd was first detected at site: (e.g. Winter 2020-21, Summer 2021)	□ N/A	Season _____ Year_____

WNS Clinical Signs Present at Site: (check all that apply) (check if applicable) □ No clinical signs observed

- □ Visible fungus on bats (**note # affected[2] per species;** *Ex: MYLU-3*)
- □ UV positive bats (pale orange fluorescent areas on hairless skin)
- □ Moderate to severe wing damage (WDI ≥ 2)
- □ Increased mortality/significant reduction in population count
- □ Unusual roosting near entrance of hibernaculum
- □ Increased day flight in winter/early return to summer roost

Species with clinical sign:

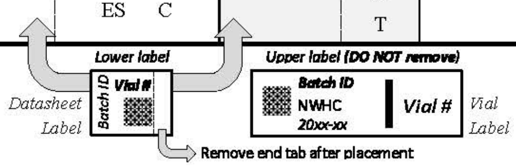
Comments:

EMAIL A SCANNED COPY OF ALL DATASHEETS AT TIME OF SHIPMENT

v20251222

USGS NWHC – Individual Specimen Collection Datasheet (pg. ___ of ___) Site Name: ______________________ Date: __________

Vial #[1] *See example label below.* **Datasheet Label** from vial.	Sample Type **B**at Skin Swab, **G**uano, **T**issue, **E**nv **S**wab, **C**arcass circle ONE	Additional Sample from Same Bat[1] *If applicable,* Add Datasheet Label Vial #[1]	Bat Skin Swab, Guano, Tissue circle ONE	Species 4-letter code Include "?" if unsure	Status **L**ive, **D**ead, **E**uth* *specify method in comments	On-Site Location[2] **O**utside, **E**ntrance, **I**nside Roost Pattern[3] **S**olitary, **C**luster *Hibernacula Only*	Visible Fungus **Y**es, **N**o UV Status[4]	Sex **M**ale, **F**emale WDI[5] *if bat handled*	Age Class **A**dult, **J**uv, **U**nkn Repro. Status[6] *Trap Surveys Only*	Body Wt. (0.01 g) Forearm Length (0.1 mm) *Trap Surveys Only*	Band No. *if applicable* Recapture **Y**es, **N**o	Comments: - Agency's Ref. ID. - Additional Vial # for samples from same bat (indicate sample type). -Protocol deviations, etc. -Method of euthanasia. - Env swab: specify as ceiling, wall, trap, etc.
	B G T ES C		B G T		L D E	O E I S C	Y N + --	M F 0 1 2 3	A J U PG LA PL SC NR U		 Y N	
	B G T ES C		B G T		L D E	O E I S C	Y N + --	M F 0 1 2 3	A J U PG LA PL SC NR U		 Y N	
	B G T ES C		B G T		L D E	O E I S C	Y N + --	M F 0 1 2 3	A J U PG LA PL SC NR U		 Y N	
	B G T ES C		B G T		L D E	O E I S C	Y N + --	M F 0 1 2 3	A J U PG LA PL SC NR U		 Y N	
	B G T ES C		B G T		L D E	O E I S C	Y N + --	M F 0 1 2 3	A J U PG LA PL SC NR U		 Y N	
	B G T ES C		B G T		L D E	O E I S C	Y N + --	M F 0 1 2 3	A J U PG LA PL SC NR U		 Y N	
	B G T ES C		B G T		L D E	O E I S C	Y N + --	M F 0 1 2 3	A J U PG LA PL SC NR U		 Y N	
	B G T ES C		B G T		L D E	O E I S C	Y N + --	M F 0 1 2 3	A J U PG LA PL SC NR U		 Y N	
	B G T ES C		B G T		L D E	O E I S C	Y N + --	M F 0 1 2 3	A J U PG LA PL SC NR U		 Y N	
	B G T ES C		B G T		L D E	O E I S C	Y N + --	M F 0 1 2 3	A J U PG LA PL SC NR U		 Y N	



(1) If no vial # label exists: create a unique ID *Ex: AZ020126AB001* (state, MMDDYY, collector, ###).
(2) Entrance: area impacted by daylight (twilight zone), **Inside:** beyond twilight zone. **(3) Cluster:** ≥2 bats in direct contact.
(4) UV Status: pale orange fluorescence suggestive of WNS; note other colors in comments.
(5) WDI: Wing Damage Index (see Table 2; Reichard & Kunz 2009).

(6) PG: Pregnant, **LA:** Lactating
PL: Post-lactating, **SC:** Scrotal
NR: Non-repro, **U:** Unknown

v20251222

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 December 2025)
https://www.usgs.gov/media/videos/collecting-a-skin-swab-white-nose-syndrome-surveillance

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:

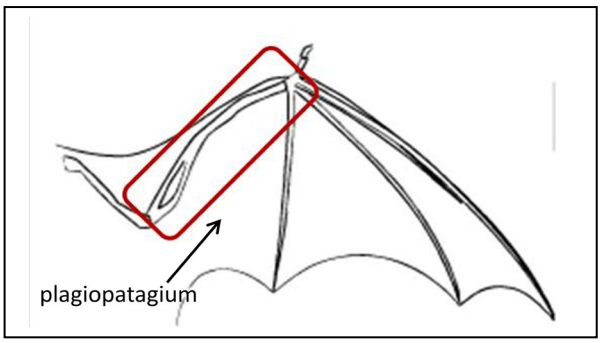
- Sterile, individually wrapped polyester-tipped swabs with plastic shafts
- Sterile, pre-labeled 1.5-ml microcentrifuge tubes, each containing 150 µl of nuclease-free water
- Plastic bags for vial storage (1 quart-size) & "TRASH" (1 gallon-size)
- Datasheets on waterproof paper that can be decontaminated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

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 Skin Swab Collection Protocol:

1. Persons collecting swab samples from bats or handling sample vials should wear disposable exam gloves. It is not necessary to change gloves between each bat/sample vial 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 individual bat information on the Individual Specimen Datasheet. Remove a pre-labeled sample vial from the "SWAB VIALS" bag. **Place the Datasheet Label from the sample tube on the datasheet. Remove and dispose of end tab to allow label to lie flat.**
4. Tap sample vial 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 vial 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 necessary, 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 (**five** 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 **5 times**.



10. After collecting the sample, transfer swab to the same sample vial used to moisten it. Break off the shaft **near the applicator tip**. Avoid touching the vial rim or inside of lid with your fingers. Screw closed the vial lid tightly.
11. Place swab sample vials into the "SAMPLES" bag and follow instructions for sample handling & storage.
12. Dispose of swab handles, wrappers, end tabs of Datasheet Labels, 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 a 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 frozen 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. **Email NWHC (nwhc-epi@usgs.gov) with scanned copies of completed datasheets prior to returning samples.** NWHC can provide a pre-paid FedEx label for priority overnight return shipment upon request; include package dimensions and weight (if using a non-NWHC provided shipper box) in the email. Ship early in the week (Mon-Wed). Do NOT ship on Fridays or the day before a federal holiday. NWHC cannot receive weekend deliveries. Remember to disinfect datasheets prior to scanning and shipment.

Ship samples to:

Necropsy Loading Dock
USGS National Wildlife Health Center
Attn: WNS/Pd Surv Project
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): 11/17/2022
https://www.usgs.gov/media/videos/collecting-a-bat-skin-biopsy-white-nose-syndrome-surveillance

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 is not recommended 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 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").

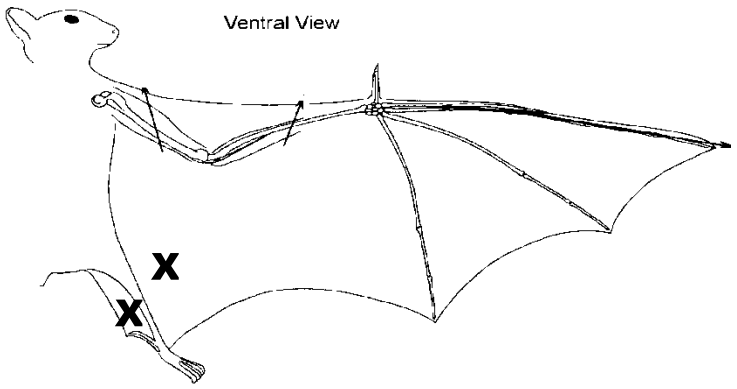
   State, Date (MMDDYY), Collector initials, sequential number ### (ex: WI061609AB001)

3. Have a fresh cardboard square, a labeled tube, a new tissue punch, and 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 with 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 locate 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 ice packs. 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 H. 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 # NC0002980
- □ 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/ice packs

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): 11/6/2019

https://www.usgs.gov/media/videos/uv-screening-bat-white-nose-syndrome-surveillance

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 9V 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.

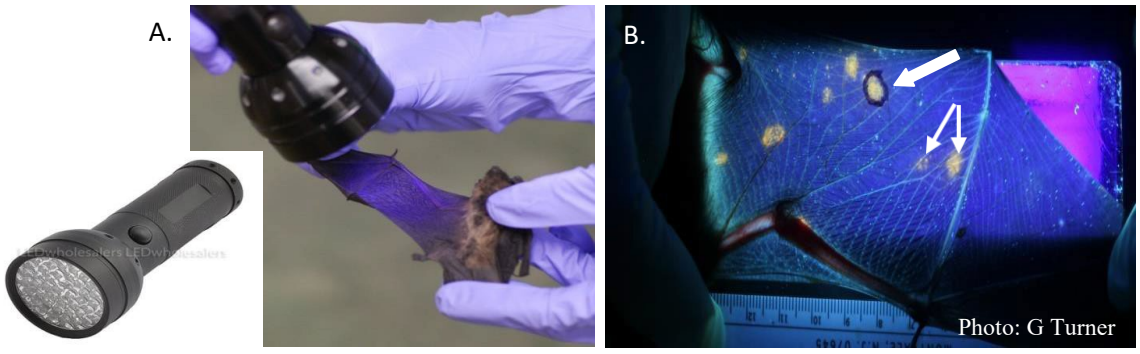


Figure 1. A) UV flashlight examination of ventral bat wing to be conducted in total darkness. B) Digital photo of backlit extended wing held over 368 nm UV light box. Arrows identify yellow-orange fluorescent areas of various diameters associated with suspect *P. destructans* infection.

APPENDIX G - Acceptable Euthanasia Techniques in Bats for WNS testing

Note: Ideally, bats with clinical signs of WNS that will be lethally collected for diagnostic evaluation should be anesthetized (i.e.: isoflurane, CO2) prior to euthanasia whenever it is safe and effective to do so. Alternatively, an injectable barbiturate overdose may be administered (IV, IP). Bats in torpor have greatly reduced respiratory and heart rates and may remain unresponsive to stimuli such that pre-anesthesia is not necessary if manual euthanasia is administered immediately upon handling. Inhalant anesthetics (such as isoflurane) are reportedly ineffective in cold environments such as that found in many hibernacula as it may not sufficiently vaporize to be inhaled. Humans are also susceptible to the effects of inhalant anesthetics and should protect the bottle from breakage and only open the bottle in well-ventilated areas. If not possible or more stressful for the animal to transport to a well-ventilated area at least 20° C/68° F to administer inhalant anesthesia and the bat is moribund or in torpor, then cervical dislocation or decapitation without anesthesia are acceptable methods of euthanasia. Decapitation is recommended as a secondary method to confirm death following cervical dislocation.

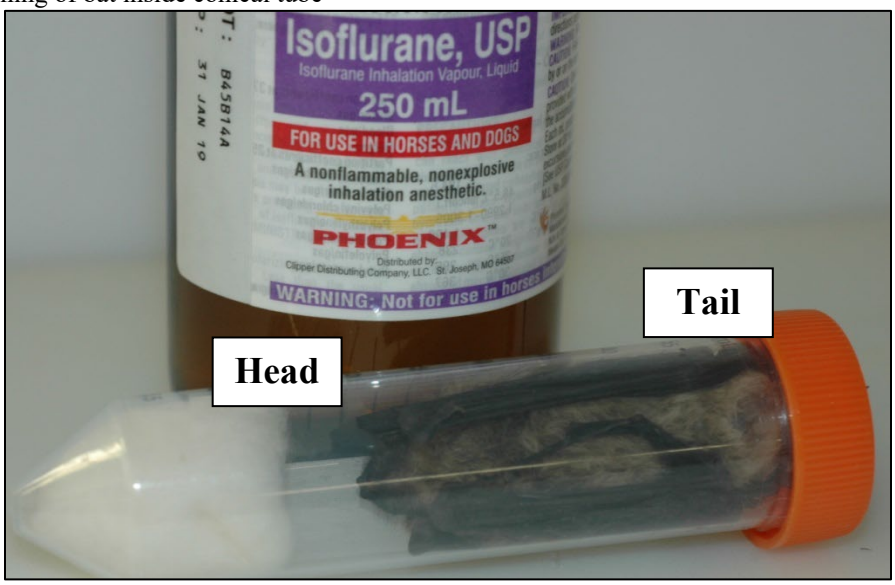
Materials Needed:

- 50 ml plastic conical tube with screw cap
- Cotton balls
- Isoflurane
- Disposable exam gloves
- Well-ventilated preparation area (Do NOT carry the bottle of isoflurane into the hibernaculum)

Inhalant – Isoflurane

- Place dry cotton ball in bottom of 50 ml conical tube.
- In a well-ventilated area, add sufficient volume (approx. 2 ml) of isoflurane into the tube to saturate the cotton ball but not result in free-standing liquid. Secure lid tightly on tube. Prepare 3 tubes in advance (up to 24 hours) of capture activity and ensure cotton ball remains saturated with anesthetic.
- Remove lid, carefully place the bat inside the conical tube headfirst and secure lid tightly. Prevent the bat from coming into direct contact with the saturated cotton ball (Fig. 1). Allow a minimum of 10-15 minutes for the anesthetic overdose to result in unconsciousness and cessation of breathing. It may take longer for bats that are in torpor. You should pinch the toe of the bat to confirm that the bat is unconscious, as breathing may be difficult to see.
- Perform euthanasia or confirm death by quickly removing the animal from the conical tube in a well-ventilated area and performing cervical dislocation (see below). Return carcass to tube for shipment to the lab.

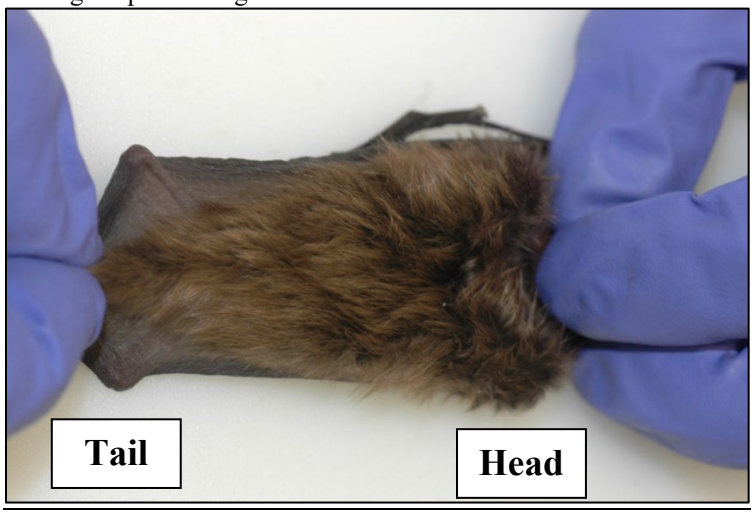
Figure 1. Proper positioning of bat inside conical tube



Physical – Cervical Dislocation (can be used alone in a moribund/unconscious bat or in conjunction with isoflurane)

- With gloved hands, hold the animal with the thumb and middle finger on either side of the base of the skull. Using the index finger, apply pressure dorsally to the first cervical vertebra where it connects at the base of the skull against a firm surface (cave wall, clipboard, thigh). Refer to Fig. 2.
- With the other hand, grasp the base of the tail and quickly pull backward so that pressure from the index finger causes separation of the cervical vertebrae. You may hear/feel a pop as separation occurs.
- Observe animal for lack of responsiveness and cessation of breathing. Confirm by cervical separation by palpation of the neck. Place carcass inside a clean, sealed bag or return to the 50 ml conical tube for shipment.

Figure 2. Proper hand positioning for performing cervical dislocation in a bat.



Physical – Decapitation (can be used alone in a moribund/unconscious bat or in conjunction with isoflurane)

- With gloved hands, hold the bat around the chest, with the wings tucked around the body.
- Using a large, sharp pair of scissors, quickly cut across the neck at the base of the skull, ensuring that the cut goes all the way through the spinal cord.
- If using this method for a bat in torpor, it must be done immediately upon handling. Do not move the bat and allow it to become conscious before decapitating.
- Movement of the jaw may occur after decapitation and does not indicate consciousness if the spinal cord has been severed.

Physical – Thoracic Compression (only for use in an anesthetized bat)

NOTE: This method of euthanasia often compromises lung and heart tissues for histopathological evaluation and should be avoided if complete necropsy evaluation is desired.

- With gloved hands, hold the animal in dorsal recumbency in one hand (bat's back in contact with the palm) and use the thumb and index finger of the other hand to apply firm, steady pressure to the sides of the bat's chest at the level of the armpits for 3-5 minutes. Take care to avoid including the wings in the compression or fracturing ribs with overly aggressive pressure.
- Death is confirmed by lack of palpable heartbeat or chest movement.
- Place carcass inside a clean, sealed bag with appropriate labels for shipment.

References

1. AVMA Guidelines for the Euthanasia of Animals: 2020 edition. https://www.avma.org/kb/policies/documents/euthanasia.pdf
2. Simmons, N and Voss, R. 2009. Ch. 42: Collection, Preparation, and Fixation of Bat Specimens and Tissues. In Kunz T, S Parson, eds. Ecological and behavioral methods for the study of bats. Baltimore, MD: Johns Hopkins Univ. Press, Pp. 315-325.
3. www.michigan.gov/documents/emergingdiseases/Humane_Euthanasia_of_Bats-Final_244979_7.pdf
4. Approved euthanasia methods for bats (Microchiroptera) training video. 2019. https://www.usgs.gov/media/videos/approved-euthanasia-methods-bats-microchiroptera

APPENDIX H

USGS – National Wildlife Health Center

INSTRUCTIONS FOR COLLECTION AND SHIPMENT OF SPECIMENS

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, and samples extracted from animals to the National Wildlife Health Center (NWHC) in Madison, Wisconsin to ensure adequate and well-preserved specimens.

☐ First, you must report the mortality and request our service prior to shipping. Please go to: Report Mortality Events and Submit Specimens https://www.usgs.gov/centers/nwhc/science/report-mortality-events-and-submit-specimens.

☐ 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 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, prior arrangements must be made. Email/fax history and tracking number to NWHC. NWHC does not have weekend delivery.

☐ 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, 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. Record on carcass tags and "Wildlife Mortality Reporting and Diagnostic Services Request Form" which animals were euthanized.

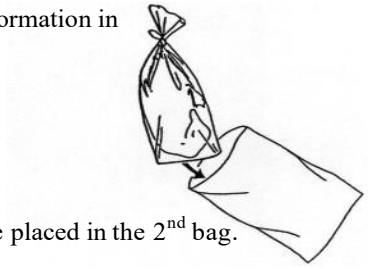
☐ Collect specimens that are representative of all species affected and geographic areas involved.

☐ Suitable specimens should have intact body cavity and eyes; 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).

☐ 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 containers.

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

☐ Use a hard-sided cooler in good condition for shipment. Close the drain plug of cooler and tape over inside. Line cooler with a thick bag (1 mil thickness, 3rd layer of bags).

☐ Place absorbent material in the 3rd plastic bag to absorb any liquids that might leak during shipping. See appendix for examples of bags and absorbent materials.

☐ Pack the individually bagged animal(s) that are contained within the 2nd sealed bag into the 3rd bag with enough FROZEN BLUE ICE PACKS or similar coolant to keep carcasses cold. Use enough coolant to keep samples chilled if there is a delay in delivery.

- Blue ice (unfrozen) can be obtained at hardware, sporting goods, or grocery stores.
- Wet ice can be used if frozen in a sealed plastic container (i.e., soda or water bottle).
- DO NOT USE DRY ICE.

☐ Seal the 3rd bag with methods described for 1st bag.

☐ Place the completed specimen history and return shipping label in a ziplock bag and tape to the inside lid of the cooler (if you want 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:

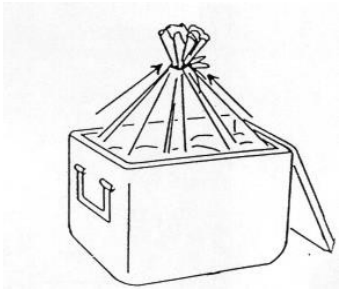
Necropsy Loading Dock
National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711
608-270-2480

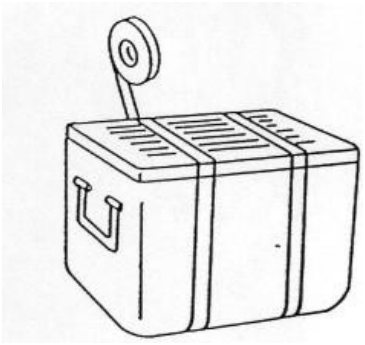
From Address/Emergency Contact:

Your Agency Address & Phone Number

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