

Texas Parks and Wildlife Department
White-Nose Syndrome
Action Plan
February 2017



Texas Parks and Wildlife Department
Wildlife Diversity Program

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PURPOSE

This plan describes the management actions Texas Parks and Wildlife Department (TPWD) will take in order to minimize the spread and impact of white-nose syndrome (WNS) on bats in Texas. The primary goal is for Texas to maintain healthy, diverse, and abundant bat populations. This is a dynamic document and subject to change in response to new information regarding both the *Pseudogeyomyces destructans* (*Pd*) fungus and WNS. The guidelines in this document are intended to provide guidance to TPWD and its partners. As new information becomes available, it may be necessary for a response to vary from a recommendation in this document.

WHITE-NOSE SYNDROME SUMMARY AND STATUS

WNS is a fungal disease caused by the non-native *Pseudogymnoascus destructans* (*Pd*).^{1,2} WNS is named for the accumulation of white fungal material around the nose, ears and wings of infected individuals and, as of January 2012, is responsible for the deaths of at least 5.7 million hibernating bats³ in 26 US states and five Canadian provinces (Figure 1).

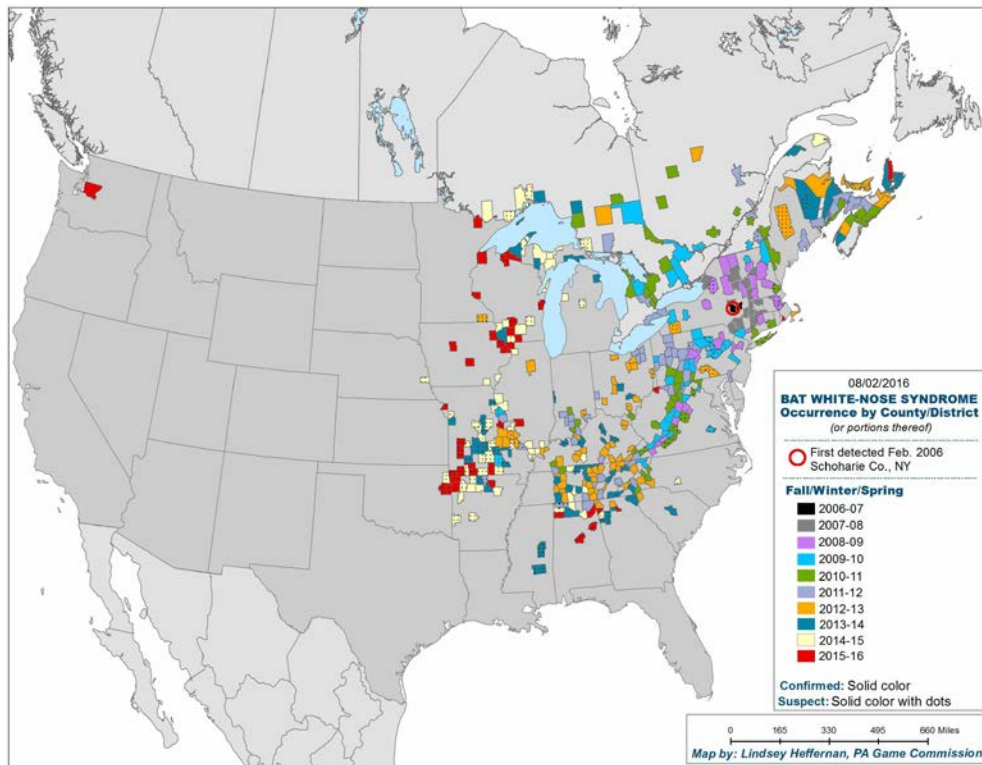


Figure 1. Spread of WNS as of August 2, 2016 (<https://www.whitenosesyndrome.org/resources/map>).

Bats affected with WNS can generally be recognized by abnormal winter behavior and a visible accumulation of white fungus on the nasal area. Hibernating bats will arouse more frequently⁴ than energy reserves allow, resulting in a cascade of physiological effects⁵, ultimately causing bats to emerge

from roosts to forage for food and often starve. Bats affected by WNS will also cluster at the entrances of hibernacula.⁶ Although infected bats usually exhibit visible physical signs of the disease (Figure 2), many bats that lack visible fungal growth have tested positive for *Pd*. In addition, bats that carry the spores on their bodies may not become infected but may distribute the spores to roosts.^{7,8} Confirmation of the presence of *Pd* or WNS requires histological and/or genetic verification through the National Wildlife Health Center, the Southeast Cooperative Wildlife Disease Study, or the Canadian Cooperative Wildlife Health Centre.

Potential Signs of WNS in Bats

- Excessive or unexplained mortality at/near hibernaculum
- Visible fungus on flight membranes, muzzle, or ears of live or fresh dead bats
- Abnormal behaviors including daytime activity or population shift to entrance of the hibernaculum
- Moderate to severe wing damage in non-torpid bats
- Thin body condition
- Yellow-orange fluorescent pattern of non-haired skin under UVA light

Note: not all signs must be present; however, detection confidence improves with more signs observed

FOR SUSPECTED WNS OBSERVATIONS
Contact Jonah Evans (830) 331-8739

Figure 2. WNS Field Signs in Winter/Spring.⁹

Where WNS has been detected, large-scale and often-complete colony mortality has been observed.^{10,11,12} Bats have very low reproductive rates with females often giving birth to one pup a year, making population recovery very slow and difficult. This destructive disease has spread rapidly and poses a considerable threat to hibernating bats throughout North America. The magnitude of the threat from this disease is not fully understood but it appears to be far-reaching; bats in large colonies as well as those roosting singly in any roost type are susceptible.¹³

Pd is acknowledged to be the cause of WNS,¹⁴ although the triggers for infection such as hibernacula microclimate, body condition prior to or during hibernation (e.g. malnutrition, dehydration, or suppressed immune system during hibernation), or other environmental vector-enhancing issues, are not yet widely understood.¹⁵ The disease is primarily transmitted bat-to-bat, but accidental spread of the disease by humans appears possible.¹⁶ White-nose syndrome has not been shown to affect any species other than bats.

Seven bat species have been documented with WNS in the U.S. and Canada; two of these are known to occur in Texas (Table 1). Four additional species that occur in Texas have been documented with *Pd* but

shown no signs of WNS. There is concern that WNS may infect additional bat species in different climates and ecoregions as *Pd* spreads. Species with the most heavily impacted populations are the little brown myotis (*Myotis lucifugus*), tri-colored bats (*Perimyotis subflavus*), the federally threatened northern long-eared bat (*Myotis septentrionalis*), and the federally endangered Indiana bat (*Myotis sodalis*). Populations of the big brown bat (*Eptesicus fuscus*) and the eastern small-footed myotis (*M. leibii*) have also been impacted.

Table 1. North American bat species with documented WNS or *Pd*.

Scientific Name	Common Name	U.S. Status	WNS Confirmed
<i>Corynorhinus rafinesquii</i>	Rafinesque’s big-eared bat	Not Listed	No
<i>Corynorhinus townsendii virginianus</i>	Virginia big-eared bat	Endangered	No
<i>Eptesicus fuscus</i>	big brown bat	Not Listed	Yes
<i>Lasionycteris noctivagans</i>	silver-haired bat	Not Listed	No
<i>Lasiurus borealis</i>	eastern red bat	Not Listed	No
<i>Myotis austroriparius</i>	southeastern bat	Not Listed	No
<i>Myotis grisescens</i>	gray bat	Endangered	Yes
<i>Myotis leibii</i>	eastern small-footed bat	Not Listed	Yes
<i>Myotis lucifugus</i>	little brown myotis	Under Review	Yes
<i>Myotis septentrionalis</i>	northern long-eared myotis	Threatened	Yes
<i>Myotis sodalis</i>	Indiana myotis	Endangered	Yes
<i>Perimyotis subflavus</i>	tri-colored bat	Not Listed	Yes

Note: Species in bold text are known to occur in Texas.

Some species in regions where WNS or *Pd* have been detected do not appear to be diseased and it is unclear whether they will contract the disease.^{17,18} WNS has been confirmed in bat species that hibernate in caves, trees, crevices, talus slopes, bat boxes, and underground structures; several of these species occur in Texas and may warrant additional surveillance (Table 2). The detection of *Pd* in bat houses, mines, culverts and bunkers demonstrate the ability for the fungus to persist in roosts apart from caves.^{19,20,21} Currently, it is unknown whether bats that contract the disease from an infected roost and harbor viable *Pd* spores could transmit the fungus to other types of roosts (such as crevices, cliffs, human-created structures [e.g. bridges, buildings], hollow trees, under loose tree bark, in leaf litter, and similar locations). Additionally, species such as the tri-colored bat, which can occupy different roost types (e.g. caves in winter, trees in spring/summer), may become a vector for fungal spread to non-cave habitats.

Table 2. Texas bat species and known winter roost types.²²

Scientific Name	Common Name	Federal Status	State Status	State Rank	Roost Type
<i>Antrozous pallidus</i>	Pallid bat	-	-	S5	crevice/cave/artificial
<i>Choeronycteris mexicana</i>	Mexican long-tongued bat	-	-	-	cave/mine
<i>Corynorhinus rafinesquii</i>	Rafinesque's big-eared bat	-	-	S3	cave/tree
<i>Corynorhinus townsendii</i>	Townsend's big-eared bat	-	-	S3	cave/tree/artificial
<i>Diphylla ecaudata</i>	Hairy-legged vampire bat	-	-	-	cave/mine
<i>Eptesicus fuscus</i>	Big brown bat	-	-	S5	tree/artificial
<i>Euderma maculatum</i>	Spotted bat	-	-	S2	crevice
<i>Eumops perotis</i>	Western mastiff bat	-	-	S3	cave/crevice
<i>Lasionycteris noctivigans</i>	Silver-haired bat	-	-	-	tree
<i>Lasiurus blossevillii</i>	Western red bat	-	-	-	tree
<i>Lasiurus borealis</i>	Eastern red bat	-	-	-	tree
<i>Lasiurus cinereus</i>	Hoary bat	-	-	-	tree
<i>Lasiurus ega</i>	Southern yellow bat	-	T	S1	palm
<i>Lasiurus intermedius</i>	Northern yellow bat	-	-	-	moss/palm
<i>Lasiurus seminolus</i>	Seminole bat	-	-	-	tree
<i>Lasiurus xanthinus</i>	Western yellow bat	-	-	S1	palm/yucca
<i>Leptonycteris nivalis</i>	Mexican long-nosed bat	E	E	S1	cave
<i>Mormoops megalophylla</i>	Ghost-faced bat	-	-	S2	cave/crevice
<i>Myotis austroriparius</i>	Southeastern myotis	-	-	S3	cave/tree/artificial
<i>Myotis californicus</i>	California myotis	-	-	S4	artificial
<i>Myotis ciliolabrum</i>	Western small-footed bat	-	-	S3	crevice
<i>Myotis septentrionalis</i>	Northern long-eared bat	T	-	-	cave
<i>Myotis thysanodes</i>	Fringed myotis	-	-	S3	cave/crevice/artificial
<i>Myotis velifer</i>	Cave myotis	-	-	S4	cave/mine
<i>Myotis volans</i>	Long-legged myotis	-	-	S4	caves
<i>Myotis yumanensis</i>	Yuma myotis	-	-	S4	cave/crevice/artificial
<i>Nycticeius humeralis</i>	Evening bat	-	-	-	tree/vegetation

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<i>Nyctinomops femorosaccus</i>	Pocketed free-tailed bat	NL	NL	S3	cave/crevice
<i>Nyctinomops macrotis</i>	Big free-tailed bat	NL	NL	S3	crevice
<i>Parastrellus hesperus</i>	Western parastrelle	NL	NL	S5	crevice/artificial
<i>Perimyotis subflavus</i>	Tri-colored bat	NL	NL	S5	cave/crevice/artificial
<i>Tadarida brasiliensis</i>	Brazilian free-tailed bat	NL	NL	S5	cave/mine/artificial
<p>Bold type - obligatory hibernators (other species likely utilize short-term torpor) NL= not listed, T= threatened, E= endangered Artificial roost = bridge, mine, culvert, building, bat house</p>					

The current bat and WNS research and conservation community believes that any bat species dependent on winter hibernation is potentially at risk for WNS because *Pd* grows optimally at temperatures ranging from 5° to 14°C (40° to 55° F), and at humidity levels of 90% or greater.²³

GOALS AND ACTIONS

PURPOSE: This plan describes the management action(s) TPWD will take in order to minimize the spread and impact of WNS on bats in Texas.

Goals of the TPWD WNS Plan:

Before WNS Detection in Texas:

- 1) Determine Texas bat population parameters pre-WNS exposure
- 2) Detect WNS/*Pd* as early as possible
- 3) Slow the spread of WNS into Texas

After WNS Detection in Texas:

- 1) Coordinate initial response actions
- 2) Prevent spread of WNS into new areas
- 3) Monitor the spread and impact of WNS in Texas
- 4) Consider application of new disease management options as they become available

BEFORE WNS DETECTION IN TEXAS

As of 2016, WNS and *Pd* have not been detected in Texas. We recommend *Pd* and WNS diagnosis be performed by the U.S. Geological Survey (USGS) National Wildlife Health Center (NWHC) in Madison, Wisconsin, the Southeastern Cooperative Wildlife Disease Study (SCWDS) at the University of Georgia, or the Canadian Cooperative Wildlife Health Centre (submission forms and instructions in Appendix 1).

GOAL 1 — DETERMINE TEXAS BAT POPULATION PARAMETERS PRE-WNS EXPOSURE

Rationale: Gathering pre-WNS exposure population information on potentially susceptible bat species will support TPWD in: identifying areas in need of regular WNS monitoring, predicting the spread of WNS, documenting the impacts of WNS, setting future recovery targets, and effectively prioritizing potential treatment and recovery actions.

Actions:

- 1) Determine winter and summer abundance and distribution of Texas bats.
 - a. Monitor bats at winter roosts/hibernacula statewide (focusing on vulnerable and high priority areas) in order to obtain data on pre-WNS populations and distributions.
 - i. Count hibernating bats at roost sites.
 - ii. Test bats for WNS/*Pd*.
 - iii. Test hibernacula surfaces for WNS/*Pd*.
 - iv. Measure hibernacula environmental conditions to determine vulnerability to WNS/*Pd*.

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- b. Implement the North American Bat Monitoring (NABat) project to monitor summer bat activity. This will provide broad scale bat population trend and distribution information that can be compared regionally and nationally.
 - c. Work with conservation partners to gather existing bat data for entry into the TXNDD.
- 2) Identify key hibernacula that harbor WNS susceptible (and potentially susceptible) species and environmental conditions favorable to *Pd*.
 - 3) Use models that predict the spread of WNS across Texas to identify the most vulnerable and highest priority winter roosts.

GOAL 2 — DETECT WNS/*Pd* AS EARLY AS POSSIBLE

Rationale: Early detection of WNS/*Pd* in Texas is important for coordinating timely and effective response actions.

Actions:

- 1) Identify high priority and highly susceptible hibernacula.
- 2) Work with universities and other conservation partners to regularly test high priority hibernacula for WNS/*Pd*.
- 3) Test bats for WNS through spring emergence mist netting, when possible.
- 4) Send suspected WNS/*Pd* positive samples to the National Wildlife Health Center (or other authorized lab) for testing.
- 5) Distribute survey, sampling, and decontamination protocols to Texas bat researchers.

GOAL 3 — SLOW SPREAD OF WNS INTO TEXAS

Rationale: Human-assisted spread of WNS/*Pd* could potentially introduce the disease into TX. There are several simple actions that can help reduce this risk.

Actions:

- 1) Encourage or require WNS Decontamination Protocols (Appendix 2). Distribute decontamination protocols to cavers, bat biologists, landowners, and land managers. Any equipment that has contacted bats or has been inside caves or mines or other potential hibernacula in confirmed WNS-affected states should not be used in Texas.
- 2) Require bat researchers to visually evaluate all bats handled for signs of WNS. Any bats suspected of having WNS will be handled in accordance with the NWHC Bat Submission Protocols (Appendix 1) and TPWD will be contacted as soon as possible.²⁴
- 3) Restrict access to highly vulnerable and important hibernacula on state lands as needed. Encourage landowners and managers to restrict access to hibernacula on private lands.
- 4) Take steps to limit the import of bats into Texas from WNS/*Pd* positive states.
- 5) Assist with and contribute to research into WNS/*Pd* treatments.

AFTER DETECTION OF WNS

GOAL 1 — COORDINATE IMMEDIATE RESPONSE ACTIONS

Rationale: Promptly notifying the appropriate stakeholders and conservation partners when WNS/*Pd* is detected will enable a smooth and consistent response. TPWD may be able to help slow the spread of the disease through notifying the public about the risks of human-assisted spread and key preventative measures.

Actions:

- 1) Notification:
 - a. Notify the TPWD Executive Director and leadership within 24 hours.
 - b. Notify the property owner within 48 hours.
 - c. Notify cooperating agencies/partners involved with WNS within 48 hours.
 - d. Direct all media communications through the TPWD Communications Division.
 - i. Develop a press release. Make sure to specify if the detection is confirmed or suspected. Coordinate the press release with the National Communications and Outreach Working Group and USFWS (Catherine Hibbard; 413-253-8569; Catherine.Hibbard@fws.gov).
 - e. Notify the Texas bat research community through the Google group (<https://groups.google.com/d/forum/texas-bat-working-group>) or email (texas-bat-working-group@googlegroups.com) and through other social media.
 - f. Consider avenues to notify other stakeholder groups such as: universities, cavers, TPWD biologists, land managers, rehabilitators, pest control businesses, etc.
- 2) Response Actions
 - a. Survey accessible caves within a 10-mile radius for presence of WNS/*Pd* as soon as possible.
 - b. Conduct annual surveillance within a 50-mile radius of the infected site.
 - c. Continue winter WNS/*Pd* monitoring in hibernacula across Texas and summer NABat monitoring.
 - d. Encourage landowners of infected sites to limit access and require WNS decontamination protocols.
 - e. Consider possible field-testing of experimental control methods to stop or slow disease spread.
 - f. Maintain records of confirmed and suspected WNS/*Pd* detections in Texas and distribute to conservation partners.

GOAL 2 — MONITOR THE SPREAD AND IMPACT OF WNS IN TEXAS

Rationale: Monitoring the spread of WNS/*Pd* and its impact on Texas species will provide information needed to effectively respond to the disease.

Actions:

- 1) Conduct annual surveys for WNS/*Pd* at susceptible sites.
- 2) Conduct bat surveys to estimate population levels.
- 3) Monitor naïve species of Texas bats for signs of WNS/*Pd*.

GOAL 3 — PREVENT SPREAD OF WNS INTO NEW AREAS

Rationale: It may be possible to reduce the impact of WNS by implementing actions to slow its spread.

Actions:

- 1) Restrict access to caves on state lands within 50 miles²⁵ of WNS/*Pd* positive sites.
- 2) Contact private landowners within 10 miles of WNS/*Pd* positive sites with properties that contain known caves to recommend restricting access to hibernacula.
- 3) Increase surveillance for WNS at caves within 50 miles²⁶ of the infected site.
- 4) Disseminate WNS decontamination protocols to any groups that may enter caves.
- 5) Require rehabilitators to contact TPWD before releasing any rehabilitated bats to reduce the chances of accidental spread of WNS/*Pd*.
- 6) Continue to prohibit scientific research permit holders from bringing any equipment into Texas that has been used in WNS-affected states and to and to follow the USFWS decontamination protocol for all bat-related work conducted in Texas.
- 7) Update this policy as needed to stay in line with USFWS protocols.

GOAL 4 — ADMINISTER WNS/*PD* TREATMENTS WHEN OPTIONS BECOME AVAILABLE

Rationale: Disease treatments could prove key to preventing the further spread of *Pd* and reducing the impact of WNS. However, the potential impact of any treatment on cave ecosystems must be carefully weighed. Research and testing is underway at several universities to find an effective treatment for WNS/*Pd*. Treatment of caves with chemicals is not advised due to the potential impacts on the cave ecosystem and associated species. As treatments become available TPWD will evaluate whether or not such treatments will be used in Texas in the event WNS is detected.

Actions:

- 1) Evaluate WNS/*Pd* control measures for use in TX. Coordinate with USFWS and other key partners to carefully weigh any adverse impacts to bats and their associated cave ecosystems before proceeding with any treatment.
- 2) Through a carefully considered and coordinated effort, TPWD will evaluate the costs/benefits of new treatments for WNS/*Pd* as they become available.
- 3) Conduct treatment trials if an effective treatment with minimal risks to cave environments is identified.
- 4) Conduct widespread treatment application if feasible and treatment trials are effective.

APPENDIX 1. NWHC SUBMISSION GUIDELINES



**Bat White-Nose Syndrome (WNS)/*Pd* Surveillance Submission Guidelines
Winter 2016/2017 (November – May)**

The following sample submission guidelines are for use when evaluating unusual bat morbidity or mortality during Winter 2016/2017 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* (formerly *Geomyces*) *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.

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Winter 2016/2017 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
<p><u>Priority Samples</u></p> <ul style="list-style-type: none"> Any species Any county ≥ 5 dead/sick bats at one location For other situations- consult with NWHC <p><u>Samples to submit</u> (5-8 bats)</p> <ul style="list-style-type: none"> Photos AND Fresh, intact carcasses MAX. of 3 euthanized non-T/E bats per site 	<p><u>Priority Samples</u></p> <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 <p><u>Samples to submit</u> (1-5 bats)</p> <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
<p><u>Priority Samples</u></p> <ul style="list-style-type: none"> Any species Any county ≥ 5 dead/sick bats at one location For other situations- consult with NWHC <p><u>Samples to submit</u> (5-8 bats)</p> <ul style="list-style-type: none"> Photos AND Fresh, intact carcasses MAX. of 3 euthanized non-T/E bats per site 	<p><u>Priority Samples</u></p> <ul style="list-style-type: none"> Species with confirmed susceptibility to WNS at a suspect positive hibernaculum Any hibernating bat species in a county of unconfirmed status <p><u>Samples to submit</u> (1-5 bats)</p> <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
<p><u>Priority Samples</u></p> <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 <p><u>Samples to submit</u></p> <ul style="list-style-type: none"> Requires prior arrangement with NWHC 	<p><u>Priority Samples</u></p> <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 <p><u>Samples to submit</u></p> <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 approx. decreasing frequency of occurrence)

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

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

- Eastern red bat (*Lasiurus borealis*)
- Rafinesque's big-eared bat (*Corynorhinus rafinesquii*)
- Silver-haired bat (*Lasionycteris noctivagans*)
- Southeastern myotis (*Myotis austroriparius*)
- Virginia big-eared bat (*Corynorhinus townsendii virginianus*)

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 protocols for containment and decontamination of field gear and personnel described in "National White-Nose Syndrome Decontamination Protocol Version 04.12.2016" (www.whitenosesyndrome.org/sites/default/files/resource/national_wns_decon_protocol_04.12.2016.pdf).

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 or not 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 much of the visible fungus to disappear before specimens arrive at the lab. Please take good quality field photographs of representative affected bats, particularly in regions where WNS has yet to be identified, to be included with all bat submissions. Digital photos can be e-mailed to 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, 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 the 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 suspect bats at a particular location. The maximum number of individuals (in any sample combination of carcasses, wing biopsies) per site that will be accepted for WNS/*Pd* diagnostic evaluation is 10 per season unless prior arrangements have been made with the lab. Not all submitted samples may be tested; this will be at the discretion of the lab. For 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 2016/2017, 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. 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; known susceptible species in an unconfirmed county within the WNS endemic area with physical evidence (visible fungus, wing damage). This non-lethal sampling is the preferred, more sensitive method to fungal tape lifts for diagnostic evaluation when fungus is present on both flight membranes and muzzle as PCR and/or histopathology may be performed.

To reduce the risk of cross-contamination among bats, all equipment (i.e.: gloves, tissue punches, biopsy boards, and forceps) should be cleaned or changed between each sampled bat. Collect wing biopsies only on live bats with visible fungal growth or characteristic UV fluorescence ([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 bat 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 requires 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. 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

UNUSUAL BAT MORTALITY/BEHAVIOR NOT ASSOCIATED WITH WNS

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.

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

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.

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

Priority samples to submit for laboratory diagnostics:

1. Bat species not previously confirmed with WNS with suspicious lesions (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 3–5 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 3–5 clinically affected bats at sites of unknown *Pd* status within a WNS confirmed county if laboratory confirmation of *Pd* is desired.

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

Note: It is recommended that 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) 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 hibernating bat species with suspicious lesions (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 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 3–5 fresh carcasses of new bat species with unknown WNS susceptibility that appear affected from a confirmed county. If county is of suspect or unknown WNS status, submit 3–5 carcasses of any affected species (see pg. 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](http://www.michigan.gov/documents/emergingdiseases/Humane_Euthanasia_of_Bats-Final_244979_7.pdf).
 2. Perform paired skin swab and UV-guided wing punch biopsy on 3–5 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/*Pd* detection in wing punch biopsies may be reduced as compared to whole carcass evaluation. Thus, negative results do not rule out the possibility of an animal being infected.*

Submit photos 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 3–5 visibly affected live bats using kit materials provided 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.

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 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. 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 tri-colored bats are encouraged as *Pd* has been detected more commonly on these species. Please consult the National Surveillance Implementation Plan for prioritization guidance.

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

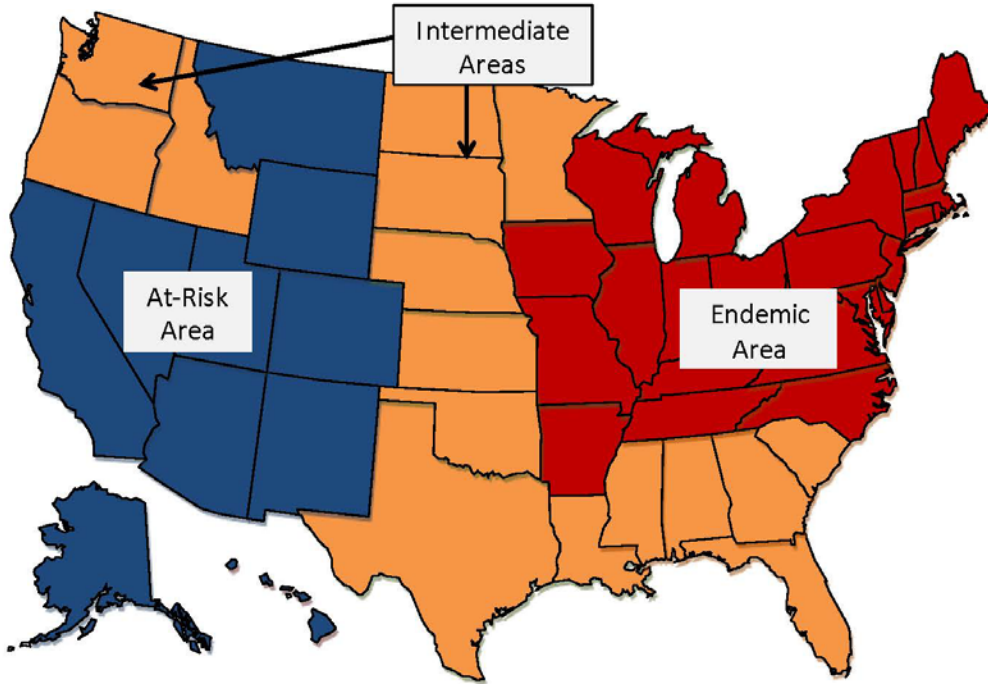
Skin swab samples from a total of 25 bats (minimum sample size = 15 bats) per site are requested using kit materials provided by NWHC. Collect swabs from bats roosting within arms' reach and from representative roosting areas throughout the hibernaculum. This may include bats roosting individually or in separate clusters. Environmental samples may supplement skin swab samples to achieve the target sample size. Environmental sampling exclusively at a site requires a larger sample size (n=30) and can result in delayed detection of *Pd* into 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 2016)



APPENDIX B

USGS NWHC Wildlife Mortality Reporting and Diagnostic Services Request

www.nwhc.usgs.gov/services/Wildlife_Mortality_Reporting_and_Diagnostic_Services_Request_Form_120415_saveable.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).

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 Open- <input type="checkbox"/> all year, <input type="checkbox"/> seasonal/restricted Gated- <input type="checkbox"/> all year, <input type="checkbox"/> seasonal, <input type="checkbox"/> breach	Site Classification: (check one) <input type="checkbox"/> N/A <i>Cave</i> - <input type="checkbox"/> undeveloped, <input type="checkbox"/> recreational, <input type="checkbox"/> show <i>Mine</i> - <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"/> Building/bunker <input type="checkbox"/> Other (specify): _____
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		

Survey Type: (check one) Full; Partial; Trap (specify): _____

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

Location ¹ <u>Trap, Outside,</u> <u>Entrance, Inside</u> circle one per line	Bat species 4-letter code	# live ²	# dead ²	# moribund ²	# with fungus visible ²	Distribution of affected bats Solitary, Clustered ³	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	
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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 zone
²Indicate if number is an estimate count; ³Cluster: ≥2 bats in direct contact

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: _____ _____ _____ _____
--	--

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

APPENDIX C: North American Bat Species Codes

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

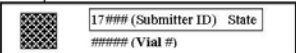
TPWD White-nose Syndrome Action Plan: February 2017

APPENDIX C- USGS NWHC Individual Specimen Collection Datasheet (pg. 1 of 2)

Submitter: _____

Site ID: _____ State: _____ County: _____ Date: _____

Vial #* <small>17 - - - - Submitter ID (from vials) see example label below</small>	Sample Type(s) Whole Carcass Wing Tissue Bat Swab Soil Enviro Swab Guano <small>circle all that apply</small>	Species 4-letter code <small>Include wings if unsure</small>	On-site Location ¹ <u>Trap</u> <u>Outside</u> <u>Entrance</u> <u>Inside</u> <small>circle one</small>	Status <u>Live</u> <u>Dead</u> <u>Euth</u>	Roost Pattern <u>Solitary</u> <u>Cluster</u> ² <small>Non-trap surveys only</small>	Visible Fungus <u>Muzzle</u> <u>Ear</u> <u>Wing</u> <u>Tail</u> <small>circle all that apply</small>	UV	Sex <u>Male</u> <u>Female</u>	Wing Damage Index <small>Reichard et al 2009</small> 0 1 2 3	Age Class <u>Adult</u> <u>Juvenile</u> <u>Unknown</u>	Rep. Status ³ <small>See descriptions for abbrev. below</small> PG LA PL SC NR	Weight (0.01 g)	Comments: Band no / Agency's Ref. ID/or (format: WI100113AB###) Deviations from protocol, photo file ID, etc. Enviro swabs: specify as ceiling, wall, trap type, etc.
												Forearm Length (0.1 mm)	
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		
	CTBSE G		TOEI	LDE	SC	MEWT	+ --	MF	0 1 2 3	AJU	PG LA PL SC NR		

¹Entrance: area impacted by daylight (twilight zone), **Inside:** beyond twilight zone
 Example vial label:  ²Cluster: ≥2 bats in direct contact

If individual bat is handled

Trap surveys only

³PG: pregnant; LA: lactating; PL: post-lactating;
 SC: scrotal; NR: non-reproductive

*Label additional sample tubes from a single bat with the same Vial #; specify sample type

TPWD White-nose Syndrome Action Plan: February 2017

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

Submitter: _____

Site ID: _____

State: _____

County: _____

Date: _____

Vial #* <small>17 --- Submitter ID (from vials) see example label below</small>	Sample Type(s) Whole Carcass Wing Tissue Bat Swab Soil Enviro Swab Guano <small>circle all that apply</small>	Species 4-letter code <small>Include wsp if unsure</small>	On-site Location ¹ Trap Outside Entrance Inside <small>circle one</small>	Status Live Dead Euth	Roost Pattern Solitary Cluster ² <small>Non-trap surveys only</small>	Visible Fungus Muzzle Ear Wing Tail <small>circle all that apply</small>	UV	Sex Male Female	Wing Damage Index <small>Reichard et al 2009</small>	Age Class Adult Juvenile Unknown	Rep. Status ³ <small>See descriptions for abbrev. below</small>	Weight (0.01 g)	Comments: Band no / Agency's Ref. ID/or (format: WI100113AB###) Deviations from protocol, photo file ID, etc. Enviro swabs: specify as ceiling, wall, trap type, etc.
												Forearm Length (0.1 mm)	
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		
	C T B S E G		T O E I	L D E	S C	M E W T	+ --	M F	0 1 2 3	A J U	PG LA FL SC NR		

¹Entrance: area impacted by daylight (twilight zone), Inside: beyond twilight zone
 Example vial label: _____
²Cluster: ≥2 bats in direct contact

If individual bat is handled Trap surveys only



*Label additional sample tubes from a single bat with the same Vial #; specify sample type

³PG: pregnant; LA: lactating; PL: post-lactating;
 SC: scrotal; NR: non-reproductive

APPENDIX D - Instructions for Taking a Wing Tissue Biopsy

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

Modified by Anne Ballmann (USGS-NWHC) 12/27/13; 3/1/2015, 12/18/16

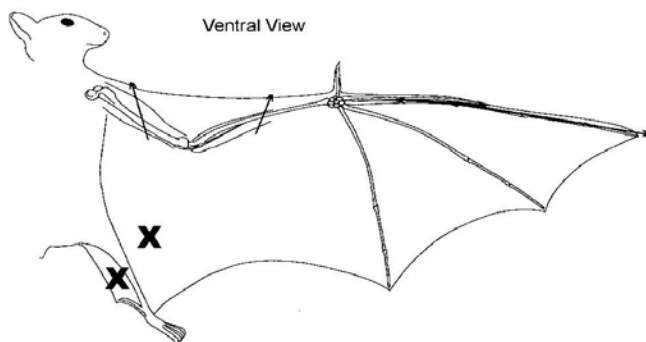
NOTE: If punch biopsies are the only sample type to be submitted to the lab in a particular case, it is highly recommended that 2 biopsies per bat be collected (from different wings). Additional population genetic sampling should not be attempted in these individuals to reduce the number of holes in the wings. Alternatively, a skin swab of 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. In order to do this, use a small clean piece of sturdy cardboard that can be discarded after each animal, a new tissue punch for each bat, sterilized forceps, and disposable gloves.
2. Label a sterile vial using a black ultra-fine Sharpie permanent marker to indicate "Biopsy". Use the following naming convention to uniquely identify the bat if a skin swab vial number for the same individual doesn't already exist:

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

3. Have a fresh cardboard square, a labeled tube, a new tissue punch, and a sterilized forceps ready for each bat. Do not touch (contaminate) the end of the punch, the forceps, or the inside of the tube lid with fingers or environmental debris.
4. Identify 2 representative lesions to biopsy on the affected wings/tail of the bat. Place the bat on the cardboard on its back and extend one wing membrane (Avoid sampling from bats with large wing tears). For people inexperienced in this technique, it works best when one person holds the bat and another person collects the biopsy.
5. When collecting wing tissue biopsies, avoid bones and major blood vessels. (Figure 1). ***Long-wave UV light can optimize biopsy placement and allows for additional histopathological evaluation (target areas exhibit faint yellow-orange fluorescent spotting-See APPENDIX F). If possible, locate an affected area near the body wall*** within the lower half of the wing membrane or uropatagium. These locations have been demonstrated to have faster healing rates and are less disruptive to flight aerodynamics (Faure PA et al. 2009. J Mammalogy 90(5): 1148-56.) Press the punch firmly through the membrane and twist the punch slightly to ensure 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 D - Instructions for Taking a Wing Tissue Biopsy -con't

6. Carefully lift the bat off the biopsy board and look for the tissue sample. It should either be on the board or inside the tip of the punch. Be careful on windy days since the wind can blow the tissue off of the board. A new 25 ga needle or sterile forceps can be used to pick up the tissue and transfer each biopsy to separate storage vials. For fungal PCR analysis, place tissue into an empty sterile vial (no storage media). For histopathological evaluation, place tissue into a separate storage vial containing 10% buffered neutral formalin (1 part tissue to 10 parts formalin).
7. Release the bat only after tissue samples have been placed into the tubes, the tubes have been closed, and any bleeding has stopped. The number of biopsies is limited to 2 per bat to prevent compromising flight.
8. While in the field, sample tubes should be stored on ice. Subsequently, unfixed samples should be frozen until submitted for fungal PCR analysis. Formalin-fixed samples should be held at room temperature (not frozen).
9. Dispose of the used biopsy punch after each animal. DO NOT reuse the same biopsy punch on multiple bats. The punches are very sharp. Be careful to not cut yourself. Change into new gloves before handling each bat.
10. Before reusing forceps while in the field, rinse in alcohol and flame sterilize. Allow forceps to cool before contacting bat tissue. Upon returning to the office, perform a more thorough cleaning and disinfection of nondisposable biopsy equipment with detergent washing followed by soaking in a 10% bleach solution for 10 min with a thorough clean water rinse. Once dry, forceps can be placed into a clean hard surface container (not plastic bags), free of contaminants, marked for cleaned forceps, and with handles all pointing in the same direction.
11. Ship wing tissues to NWHC. Ensure that all 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 overnight, freeze them and ship as soon as possible.

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

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

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

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

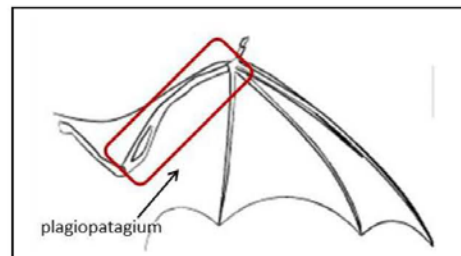
- Sterile, individually wrapped polyester-tipped swabs with plastic shafts (27)
- Sterile, pre-labeled 1.5 ml microcentrifuge tubes, each containing 150 µl of nuclease-free water (25)
- 2 plastic bags (1 quart-size) for vial storage & “TRASH” (1 gallon-size)
- Datasheets
- Plastic bag (1 gallon-size) for “CLEAN” outer storage & packaging of sample vials and datasheet (**do not carry bag inside hibernaculum**)
- Insulated shipper box with 2 ice packs (for return shipment only; do not carry inside hibernaculum)

Needed:

- Disposable exam gloves
- Pencil or indelible ink pen
- Clipboard
- Decontamination supplies
- Cooler with ice for sample storage & transport in the field

Bat Swab Collection Protocol:

1. Persons collecting swab sample from bats or handling sample tubes should wear disposable exam gloves. It is not necessary to change gloves between each bat/sample tube provided the persons performing these tasks do not directly contact individual bats or inside rim of sample vial lid.
2. Identify a bat to be sampled.
3. Record the requested individual bat information on the Individual Specimen Datasheet. Remove a pre-labeled sample tube from the “SWAB VIALS” bag. **Remember to include the unique Vial # from the selected sample tube.**
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.
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 as close to the applicator tip as possible. Avoid touching the rim of the tube or inside of lid with your fingers. Close the tube lid tightly.
 11. Place swab sample tubes into the "SAMPLES" bag and maintain at ambient temperature while underground.
 12. Dispose of swab handles, wrappers, and contaminated exam gloves as necessary into "TRASH".
 13. Repeat the above process for each bat sampled.
 14. Upon exiting the hibernaculum but prior to leaving the area, place the datasheet inside of the emptied plastic bag (1 quart-size). Decontaminate the outer surfaces of all bags taken inside the hibernaculum following current USFWS Decontamination Guidelines. Place the bags containing all sample tubes and datasheet inside the "CLEAN" bag for storage and shipment. Ensure all excess air is removed from the bags.
 15. Following removal of collected samples from the hibernaculum, store them on ice for transport to an office refrigerator or freezer.

Sample Storage:

Hold swab samples chilled (4°C) if they are to be shipped within 2 days following collection. If you are sampling multiple sites, samples can be stored frozen at -20°C (preferably not a frost-free freezer 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 standard 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. Ship early in the week (Mon-Wed) to avoid weekend deliveries (DO NOT ship on Fridays or the day before a holiday). Notify Anne Ballmann (608-270-2445; aballmann@usgs.gov) with the courier service and package tracking number of the return shipment.

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

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

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

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

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

Equipment:

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

- 380-385 nm wavelength UV 51 bulb LED flashlight and visible light filter (LED Wholesaler #7202UV385; Polman Minerals) or 368 nm wavelength 9 V UV box (Contact Greg Turner [grturner@pa.gov] for more details on UV box system)
- Disposable exam gloves
- Digital camera
- Permanent marker
- PPE: UVA blocking safety glasses, SPF15+ sunblock on exposed human skin

Additional equipment for non-lethal wing biopsy collection-

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

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

1. In complete darkness, shine the UV flashlight facing down approximately 3-5 inches (7.5-12.5 cm) above the extended ventral surface of the flight membranes (Fig. 1A). If using a UV box, place the bat on its back and extend the wing and corresponding foot over the UV light source to transilluminate the wing surface. Disinfect surface of UV box between bats. Avoid shining the light into the unprotected eyes of the bat or people or exposing bat skin to UV light for more than 3 minutes.
2. Examine wing membrane for circular areas of yellow-orange fluorescence (Fig. 1B). Fluorescence will be faint when viewed with the naked eye using a hand-held UV flashlight. Visualization is greatly enhanced by examining a digital photograph of the UV-illuminated wing surface when using the UV box. Photography does not improve visualization with the UV flashlight.
3. If the bat is to be euthanized, use a permanent marker to circle representative areas of fluorescence on the wing membrane to target sampling in the laboratory. Place marks outside of the fluorescent border.
4. If live-sampling techniques are used, collect paired wing punch biopsies (3-5 mm diameter, See [Appendix 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 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.

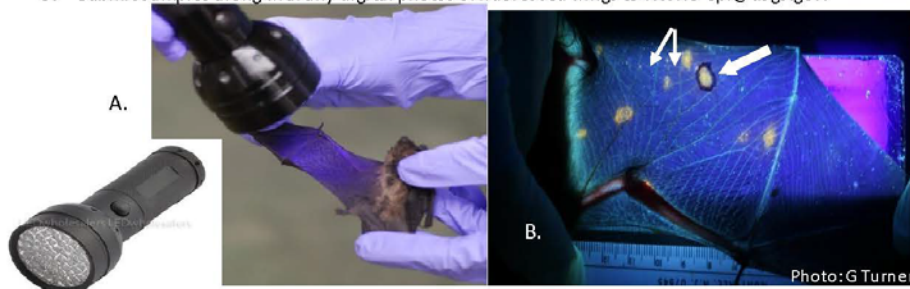


Figure 1. A) UV flashlight examination of ventral bat wing to be conducted in total darkness. B) Digital photo of bat's extended wing held over 368 nm UV light box. Arrows identify yellow-orange fluorescent areas of various diameters associated with suspect *G. destructans* infection.

APPENDIX 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 FIT to get shipping approval and discuss shipping arrangements. Typically, ship specimens by 1-day (overnight) service, Monday through Wednesday, to guarantee arrival at NWHC before the weekend. If specimens are fresh and need to be shipped on Thursday or Friday, special arrangements can be made.
- Email/fax history and tracking number to FIT. Packages will not be opened if history does not arrive first!
- Use rubber, vinyl, or nitrile gloves when picking up sick or dead animals. If you do not have gloves, insert your hand into a plastic bag.
- More than one disease may be affecting the population simultaneously. When possible, collect both sick and dead animals. Note behavior of sick animals before euthanizing.
- Collect specimens that are representative of all species affected and geographic areas.
- Collect the freshest dead specimens. Decomposed or scavenged carcasses are usually of limited diagnostic value. If you plan to collect animals in the field, take along a cooler containing ice to immediately chill carcasses.
- Collect animals under the assumption that an infectious disease or toxin is involved and other animals may be at risk. Protect yourself as some diseases and toxins are hazardous to humans.
- Place each animal in a plastic bag, close, and seal the bag. Twist non-zipper bags closed, fold over on itself, and secure with package strapping or duct tape. Label the outside of this bag with the following information in waterproof ink:
 - Date collected
 - Location (specific site, town, county, state)
 - Collector (name/address/phone)
 - Species
 - Found dead or euthanized
 - Your reference #
- Place 1st bag inside a 2nd bag, close and seal. More than one individually bagged animal can be placed in the 2nd bag. This prevents cross-contamination of individual specimens and leaking shipping containers.
- Tag the outside of 2nd bag and number of animals and type, date collected, location, and name of collector. Reminder order: TAG, BAG, BAG, TAG.
- Use a hard-sided cooler in good condition for shipment. Close the drain plug of cooler and tape over inside. Line cooler with a thick bag (1 mil thickness, 3rd layer of bags).

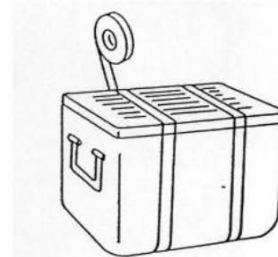
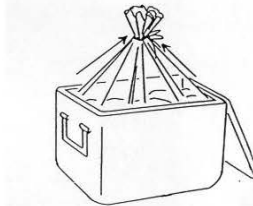


- Place absorbent material in the 3rd plastic bag to absorb any liquids that might leak during shipping.
See appendix for examples of bags and absorbent materials.
- Pack the individually bagged animal(s) that are contained within the 2nd sealed bag into the 3rd bag with enough FROZEN BLUE ICE PACKS or similar coolant to keep carcasses cold. Use enough coolant to keep samples chilled if there is a delay in delivery.
 - o Blue ice (unfrozen) can be obtained at hardware, sporting goods, or grocery stores.
 - o Wet ice can be used if frozen in a sealed plastic container (i.e., soda or water bottle).
 - o 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 FIT emergency
608-270-2400

Supplementary Labels:
Keep Cold



- Mark the cooler with the appropriate information: (See Pg. 3 for printable marking labels)
 - o Carcasses of animals that died of unknown causes:
BIOLOGICAL SUBSTANCE, CATEGORY B and UN 3373.
 - o Blood and tissue samples from apparently healthy animals (hunter-killed, live captured):
EXEMPT ANIMAL SPECIMENS.
 - o 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**

APPENDIX 2. WNS DECONTAMINATION PROTOCOLS

National White-Nose Syndrome Decontamination Protocol - Version 04.12.2016

I. INTRODUCTION

The fungus *Pseudogymnoascus destructans* (*Pd* – formerly identified as *Geomyces destructans*) is the cause of white-nose syndrome (WNS), a disease that has resulted in unprecedented mortality of hibernating bats throughout eastern North America. Since first documented in New York in 2006, WNS continues to threaten hibernating populations of bats across the continent, having spread rapidly through the Northeast, mid-Atlantic, Midwest, and Southeast states, as well as eastern Canada.

Best available science indicates that *Pd* arrived in North America from a foreign source. Once *Pd* has been detected, either on bats or in the hibernaculum environments, the county of occurrence is considered contaminated indefinitely due to the long-term persistence of the fungus. Because of the devastating effects of WNS in North America, recommendations detailed in this document were developed to minimize the risk of human-assisted transmission. All persons who come into contact with bats, their environments, and/or associated materials for any reason (*e.g.*, research, recreation, etc.) are advised to take precautions to avoid additional, inadvertent transport of *Pd* to uncontaminated bats or habitats.

Observations of live or dead bats (multiple individuals at a single location) should be reported to local USFWS Field Office or State agency wildlife office <http://www.whitenosesyndrome.org/partners>. **Do not handle bats unless you are properly trained, vaccinated, and, where necessary, authorized in writing to do so by the appropriate government agency.**

II. PURPOSE:

The purpose of this document is to provide recommendations based on the best available scientific information known to effectively clean and treat (herein referred to as decontaminate, or similar derivation thereof) clothing, footwear, and/or gear (herein collectively referred to as equipment) that may have been exposed to *Pd*. When activities involve contact with bats, their environments, and/or associated materials the following decontamination procedures are designed to reduce the risk of human-assisted transmission of the fungus to other bats and/or habitats.

For the protection of bats and their habitats: 1) comply with all current cave and mine closures, advisories, and regulations on federal, state, tribal, and private lands; 2) follow relevant recommendations found in this document; and 3) **do not transport any equipment into or out of the United States of America (USA)** that has been in contact with bats or their environments.

Local, state, federal, or other management agencies may have additional requirements or clarifications for equipment used on lands under their jurisdictions¹ or work involving public trust resources. Always follow all state and/or federal permit conditions. Contact the respective agency representatives for supplemental documents or additional information.

III. PRODUCT USE:

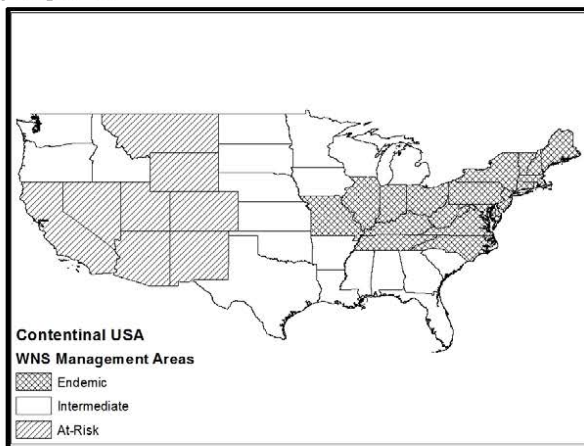
Ensuring the safety of individuals using any of the applications and/or products identified in this document must be the first priority. Safety data sheets (SDS) for chemicals and user's manuals for equipment developed by product manufacturers provide critical information on the physical properties, reactivity, potential health hazards, storage, disposal, and appropriate first aid procedures for handling, application, and disposing of each product in a safe manner. Familiarization with the SDS for chemical products, and manufacturer's product care and use standards, will help to ensure appropriate use of these materials and safeguard human health. Read

product labels in advance of intended field use. Ensure availability of adequate emergency eye-wash supplies or facilities at intended site of use. Always store cleaning products out of the reach of children or pets.

It is a violation of federal law to use, store, or dispose of a regulated product in any manner not prescribed on the approved product label and associated SDS. Products, or their contaminated rinse water, must be managed and disposed of in accordance with local environmental requirements and, where applicable, product label, to avoid contamination of groundwater, drinking water, or non-municipal water features such as streams, rivers, lakes, or other bodies of water. Follow all local, state and federal laws. Requirements for product disposal may vary by state. Note: Quaternary ammonium wastewaters should not be drained through septic systems because of the potential for system upset and subsequent leakage into groundwater.

IV. TRIP PLANNING/ORGANIZATION:

1.) Identify the appropriate WNS Management Area (Figure 1) in which the equipment has been used and will be used in the future. Users of new or site-dedicated equipment (that has been and will be used in only one site) may skip to #3.



“Site” is loosely defined in this document as the location of a discrete bat roost (cave, barn, talus slope, etc.) or as a specific field location for mist netting or other trapping. Since conditions vary considerably, delineating sites will be at the discretion of the appropriate local regulatory or land management agency.

Figure 1. WNS Management Areas by state.

2.) Once the appropriate Management Areas have been determined using Figure 1, use Figure 2 to determine appropriate uses for A. Subterranean Equipment or B. Terrestrial Equipment. **“Subterranean equipment” includes any equipment that has ever been exposed to a cave/mine environment. “Terrestrial equipment” includes any equipment that has not previously been exposed to a cave/mine environment.** Regardless of the equipment designation, equipment should only be reused at similarly classified or progressively more contaminated locations². In addition, given uncertainties in the distribution of *Pd* in the Pacific Northwest (i.e., ID, OR, & WA), subterranean and terrestrial equipment should not be transferred between the PNW and eastern USA (endemic/intermediate).

3.) Contact local state/federal regulatory or land management agencies for additional requirements, exemptions, or addendums on lands under its jurisdiction that supplement guidance provided in Figure 2A and 2B.

4.) Choose equipment that can be most effectively decontaminated [e.g., rubber or synthetic rather than leather boots], otherwise commit use of equipment to a specific location (herein referred to as equipment dedication). Equipment should always be inspected for defects prior to use. Replace all defective or degraded equipment with new equipment. Brand new equipment can be used at any location where access is permitted, as long as it has not been stored or come in contact with contaminated equipment.

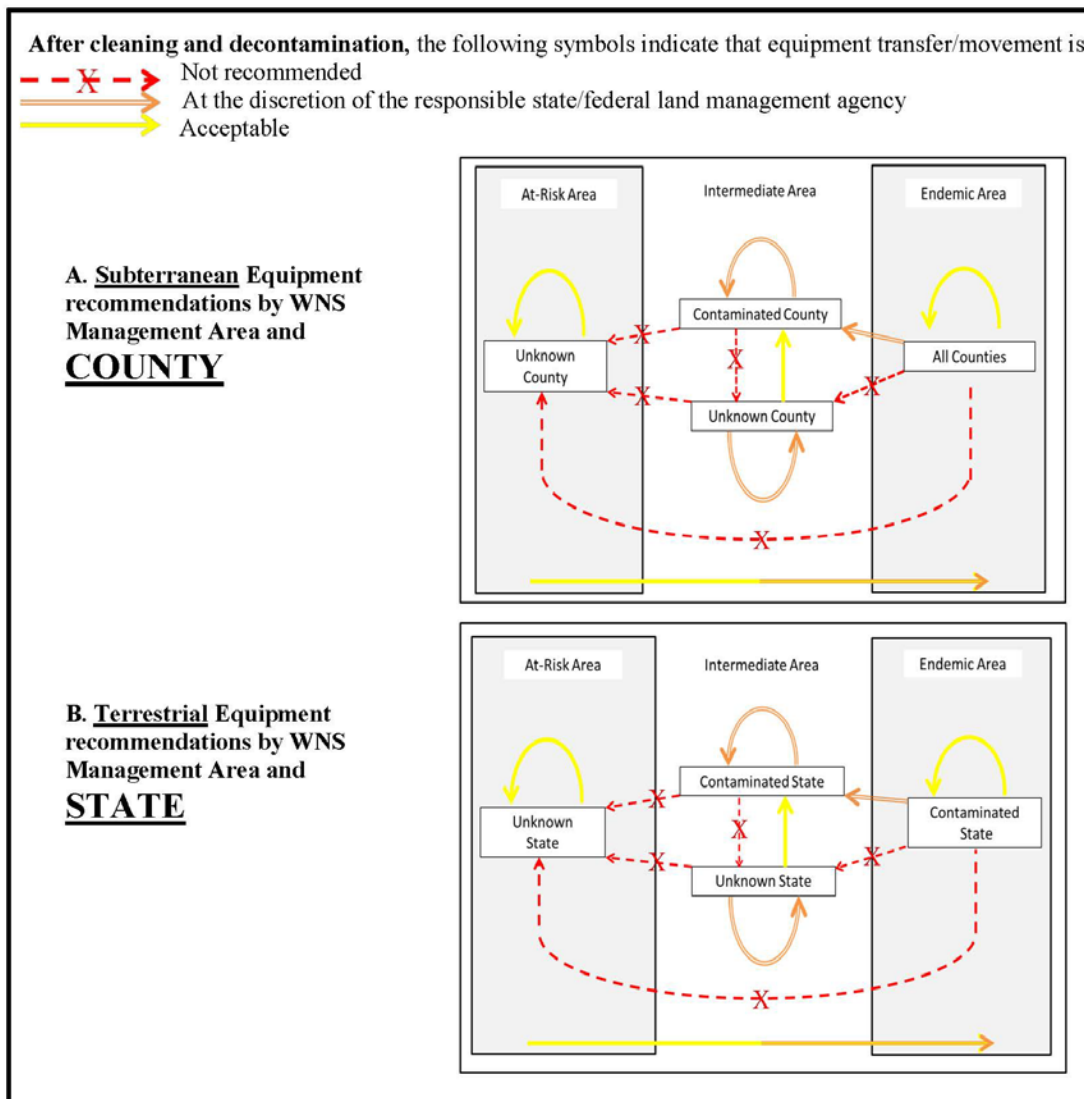


Figure 2. Movement recommendations for decontaminated (A) Subterranean and (B) Terrestrial equipment.

5.) Prepare a strategy (*i.e.*, Outline how/where all equipment and waste materials will be contained, stored, treated and/or discarded after returning to the vehicle/base area) that allows daily decontamination of equipment and, where applicable, between individual sites visited on the same day, **unless** otherwise directed by local state/federal or land management agency instructions. Confirmed *Pd* contaminated sites or those with a high index of suspicion for contamination should be visited **only after** those sites of unknown *Pd*/WNS status² have been visited, to further reduce the risk of inadvertent transmission.

V. PROCEDURES FOR DECONTAMINATION:

1.) On site:

- a.) Thoroughly remove sediment/dirt from equipment immediately upon exiting from the site.

b.) Contain all exposed and potentially contaminated equipment in sealed bags/containers for treatment away from the location. Decontaminate the outside hard, non-porous surfaces of containers and bags prior to moving them to a secondary location (e.g., vehicles, labs, or storage). Store all exposed and decontaminated equipment separately from unexposed equipment.

c.) Clean hands, forearms, and exposed skin using hand/body soaps/shampoos and, when feasible, change into clean clothing and footwear prior to entering a vehicle.

2.) Off site:

a.) *REMOVE* dirt and debris from the outside of vehicles (especially wheels/undercarriage) prior to additional site visits, especially when traversing WNS Management areas or scenarios categorized as “Not Recommended” (Figure 2).

b.) *CLEAN* submersible and non-submersible equipment according to manufacturer’s specifications. Sediments and debris significantly reduce the effectiveness of treatments. Laboratory trials^{3&4} demonstrate that the use of conventional cleansers like Woolite® detergent or Dawn® dish soap aided in the removal of sediments and debris prior to treatment, contributing to the effectiveness of decontamination.

c.) *TREAT* submersible or non-submersible equipment only in a safe manner according to the equipment and product labels using the most appropriate application or product listed in Table 1. For equipment that cannot safely be treated in accordance with both the manufacturer’s recommendations and product labeled instructions, dedicate to individual sites as determined appropriate in Section IV.

i. Submersible Equipment (i.e., equipment that can safely withstand submersion in water or other specified product for the recommended amount of time without compromising the integrity of the item):

Treatment of submersible equipment must be done in accordance with manufacturer’s recommendations for your equipment. The preferred treatment for all submersible equipment is submersion in hot water that maintains a temperature of at least 55°C (131°F) for a minimum of 20 minutes. Ensure that all equipment surfaces remain in direct contact (i.e., avoid all trapped air) with the hot water treatment for the duration of the treatment period. Consider that although many commercial and home washing machines with sanitize (or allergen) cycles may be capable of submerging gear in the recommended hot water application for the required time, it is incumbent on the user to be sure that machines to be used attain and sustain the needed temperatures throughout the process. If heat may compromise the safety and/or integrity of the otherwise submersible equipment, consider equipment dedication or other products listed in Table 1. When considering other products found in Table 1, recognize that the applicability and effect of such products on the safety and integrity of equipment remains untested. Be aware the use of preferred applications and products in Table 1 should be done with extreme caution and proper personal protective gear due to the risk of personal injury.

ii. Non-submersible Equipment (i.e., equipment that may be damaged by liquid submersion):

Treat all non-submersible equipment using the most appropriate application or product in Table 1 that complies with the equipment manufacturer’s recommendations and product label instructions, where applicable. The listed applications or products may not be appropriate or safe for non-submersible equipment. Dedication of equipment should always be considered the preferred application in these circumstances.

d.) *RINSE* equipment, as appropriate, thoroughly in clean water, particularly items that may contact humans, bats, or sensitive environments. Allow all equipment to completely dry prior to the next use.

e.) DECONTAMINATE the equipment bins, sinks, countertops and other laboratory, office, or home areas with the most appropriate applications or products in Table 1.

Table 1. Applications and products with demonstrated efficacy against Pd^{3,4,5,6,&7}. Remember to consult equipment labels, registered product labels, and the appropriate SDS for regulations on safe and acceptable use.

	Tested Applications & Products ^{3,4,5,6,&7}	Federal Reg No.:	Laboratory Results
Preferred Applications	Equipment Dedication	N/A	Clean according to manufacturer standards and dedicated to a site
	Submersion in Hot Water ^{4,6,&7}	N/A	Laboratory effectiveness demonstrated upon submersion in water with sustained temperature $\geq 55^{\circ}\text{C}$ (131°F) for 20 minutes.
Other Products	Ethanol (60% or greater) ^{4,6,&7}	CAS - 64-17-5	Laboratory effectiveness demonstrated upon exposure in solution for at least 1 minute.
	Isopropanol (60% or greater) ^{4,6,&7}	CAS - 67-63-0	
	Isopropyl Alcohol Wipes (70%) ^{4,6,&7}	CAS - 67-63-0	Laboratory effectiveness demonstrated immediately following contact and associated drying time.
	Hydrogen Peroxide Wipes (3%) ^{4,6,&7}	CAS - 7722-84-1	
	Accel ^{®4,5,6,&7}	EPA - 74559-4	Laboratory effectiveness demonstrated when used in accordance with product label.
	Clorox [®] Bleach ^{3,4,5,6,&7}	EPA - 5813-100	
	Clorox [®] Wipes ^{4,5,6,&7}	EPA - 5813-79	
	Clorox [®] Clean-Up Cleaner + Bleach ^{4,5,6,&7}	EPA - 5813-21	
	Hibiclens ^{®4,5,6,&7}	NDA - 017768	
Lysol [®] IC Quaternary Disinfectant Cleaner ^{3,4,5,6,&7}	EPA - 47371-129		

Other effective treatments with similar water based applications or chemical formulas (e.g., a minimum of 0.3% quaternary ammonium compound) may exist but remain untested at this time. Find more information on the EPA or FDA registered product labels by accessing the individual hyperlink or searching EPA or FDA Registration Numbers at: <http://iaspub.epa.gov/apex/pesticides/f?p=PPLS:1> or <http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm>.

Products with USEPA registration numbers mitigate persistence of living organisms on surfaces and are regulated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, 7 USC 136, et seq.). FIFRA provides for federal regulation of pesticide distribution, sale, and use. Within FIFRA, pesticides are defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. FIFRA further defines pests as any insect, rodent, nematode, fungus, weed, or any other form of terrestrial or aquatic plant or animal life or virus, bacteria, or other micro-organism (except viruses, bacteria, or other micro-organisms on or in living man or other living animals) which the Administrator declares to be a pest under section 25(c)(1). Find more information on FIFRA at: <http://www.epa.gov/oecaogct/lfra.html>.

VI. EQUIPMENT AND ACTIVITY SPECIFIC RECOMMENDATIONS:

It is the responsibility of the users of this protocol to read and follow the product label and SDS. The product label is the law!

A. Clothing & Footwear:

IMPORTANT: All clothing (i.e., inner and outer layers) and footwear should be decontaminated after every site visit using the most appropriate Application/Product in Table 1 or otherwise cleaned and dedicated for use at individual sites or areas as determined appropriate in Section IV.

Use of a disposable suit (e.g., Tyvek® or ProShield®) or site-dedicated, reusable suit (i.e., coveralls) is an appropriate strategy to minimize sediment/soil accumulation on clothing during a cave/mine or bat research activity. As stated earlier, all clothing layers should still be decontaminated or otherwise cleaned and dedicated after every use.

Disposable items, regardless of condition, should not be reused. Contain all used equipment in plastic bags upon final exit from a site, separating disposable materials from reusable equipment. Seal and store plastic bags in plastic containers until trash can be properly discarded, and/or exposed reusable equipment can be properly decontaminated off site.

B. Cave/Mine and other Subterranean Equipment:

Dedicate, as necessary, or decontaminate all cave/mine equipment (e.g., backpacks, helmets, harness, lights, ropes, etc.) using the most appropriate guidance in Section V. Most types of equipment, including but not limited to, technical and safety equipment, have not undergone testing for safety and integrity after decontamination. Therefore carefully review and adhere to the manufacturer's care and use standards to maintain equipment functionality and safety protective features. If the application/product options in Table 1 are not approved by the manufacturer's care and use standards for the respective type of equipment, clean and inspect equipment according to manufacturer's specification and dedicate to similarly classified caves/mines/bat roosts and only reuse in progressively more contaminated caves/mines/bat roosts.

C. Scientific Equipment:

Always consider the use of disposable scientific equipment and materials between individual bats. All disposable scientific equipment (e.g., work surfaces, bags/containers/envelopes, exam gloves, etc.) should only be used on one bat, then discarded after use. Re-useable equipment (e.g., cotton bags, plastic containers, etc.) must be decontaminated between individual bats using the most appropriate application or product in Table 1. In all cases, use breathable bags (e.g., paper, cotton, mesh, etc.).

At the completion of daily activities and when allowable by equipment and product labels, equipment may be autoclaved before reuse; otherwise use the guidance in Section V to determine the relevant procedure for decontamination of all work surface area(s) and equipment (e.g., light boxes, banding pliers, holding bags, rulers, calipers, scale, scissors, wing biopsy punches, weighing containers, etc.).

D. Mist-Nets:

Contamination of trapping equipment is possible year-round when used at *Pd* contaminated hibernacula (NWHC, unpublished data). Dedicate, as necessary, or decontaminate all netting equipment (e.g., netting, tie ropes, poles, stakes, etc.) using the most appropriate guidance in Section V for the particular equipment. All nets that are contacted by one or more bats must be decontaminated after each night of use according to the submersion in hot water application (Table 1). All nets should be completely dry prior to the next use.

E. Harp Traps:

Contamination of trapping equipment is possible year-round when used at *Pd* contaminated hibernacula (NWHC, unpublished data). Dedicate, as necessary, or decontaminate all trapping equipment (e.g., lines, National White-Nose Syndrome Decontamination Protocol v 04.12.2016

frame, feet, bags, etc.) using the most appropriate guidance in Section V for the particular equipment. All trapping equipment that comes in contact with one or more bats OR enters a cave/mine/bat roost must be decontaminated after each night of use according to the most appropriate application or product (Table 1). Explore the use of disposable trap bags or liners to reduce transmission risks throughout each trapping effort. Disposable trap bags should be discarded at the end of each night.

F. Acoustic Monitor, Camera, and Related Electronic Equipment:

Dedicate, as necessary, or decontaminate all acoustic monitoring, camera, and related electronic equipment (e.g., detector, camera, tablets, cell phones, laptops, carrying case, lenses, microphone(s), mounting devices, cables, etc.) using the most appropriate guidance in Section V for the particular equipment. The material composition of this equipment requires careful review and adherence to the manufacturer’s care and use standards to maintain their functionality and protective features. If application/product options in Table 1 are not approved by the manufacturer’s care and use standards for the respective type of equipment, clean equipment accordingly and dedicate to similarly classified caves/mines/bat roosts or only reuse in progressively more contaminated caves/mines/bat roost. Electronic devices used as terrestrial equipment, independent of bat handling work, pose a limited risk of transmission (i.e., driving transects or fixed point detector surveys not associated with a cave/mine/bat roost entrance).

Equipment used in a cave/mine/bat roost may be placed in a sealed plastic casing, plastic bag, or plastic wrap to reduce the potential for contact/exposure with contaminated environments. Prior to opening or removing any plastic protective wrap, first clean, then remove, and discard all protective wrap. This technique has not been tested and could result in damage to, or the improper operation of, equipment.

These recommendations are the product of the multi-agency WNS Decontamination Team, a sub-group of the Disease Management Working Group established by the National WNS Plan (A National Plan for Assisting States, Federal Agencies, and Tribes in Managing White-Nose Syndrome in Bats, finalized May 2011). On 15 March 2012 a national decontamination protocol was approved and adopted by the WNS Executive Committee, a body consisting of representatives from Federal, State, and Tribal agencies which oversees the implementation of the National WNS Plan. The protocol will be updated as necessary to include the most current information and guidance available.

- 1 To find published addenda and/or supplemental information, visit <http://www.whitenosesyndrome.org/topics/decontamination>.
- 2 Visit <http://www.whitenosesyndrome.org/resources/map> for the most updated information on the status of county and state. County and state level determination is made after a laboratory examination and subsequent classification of bats according to the current WNS case definitions. Definitions for the classification can be found at http://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/Case%20Definitions%20for%20WNS.pdf. Contaminated determination includes both confirmed and suspect WNS classifications.
- 3 Information from : V. Shelley, S. Kaiser, E. Shelley, T. Williams, M. Kramer, K. Haman, K. Keel, and H.A. Barton – Evaluation of strategies for the decontamination of equipment for *Geomyces destructans*, the causative agent of White-Nose Syndrome (WNS) Journal of Cave and Karst Studies, v. 75, no. 1, p. 1–10. DOI: 10.4311/2011LSC0249
- 4 Efficacy of these agents and treatments are subject to ongoing investigation by the Northern Research Station, USDA Forest Service Cooperative Agreement 13-IA-11242310-036 (U.S. National Park Service and U.S. Forest Service) & 16IA11242316017 (U.S. Fish and Wildlife Service and U.S. Forest Service). Information contained in this protocol from work associated with either agreement will continue to be revised, as necessary, pending results of these investigations.
- 5 The use of trade, firm, or corporation names in this protocol is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by state and/or federal agencies of any product or service to the exclusion of others identified in the protocol that may also be suitable for the specified use.
- 6 Product guidelines should be consulted for compatibility of use with one another before using any decontamination product. Also, detergents and quaternary ammonium compounds (i.e., Lysol® IC Quaternary Disinfectant Cleaner) should not be mixed directly with bleach as this will inactivate the bleach and in some cases produce a toxic chlorine gas. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.
- 7 Final determination of suitability for any decontaminant is the sole responsibility of the user. All users should read and follow all labeled instructions for the products/applications and/or understand associated risks prior to their use. Treatments and the corresponding procedures may cause irreversible harm, injury, or death to humans, bats, equipment or the environment when used improperly. Always use personal protective equipment in well-ventilated spaces to reduce exposure to these products or applications.

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