National guidelines for sample submission
White-nose syndrome exclusion testing

This document provides a framework to assist veterinarians with the appropriate collection and submission of samples to facilitate the exclusion of white-nose syndrome (WNS) in Australia.

Veterinarians should be vaccinated for protection against ABLV and always use appropriate personal protective equipment when interacting with bats.

A companion information document How to report a suspect case of white-nose syndrome is available as a resource for bat carers, ecologists, cavers and members of the public.

Background

The WHA White-Nose Syndrome Fact Sheet provides detailed information on WNS in the Australian context. Further information is available at www.whitenosesyndrome.org.

a. Species likely to be affected by WNS in Australia

Australia is home to a number of genera of insectivorous bats that are affected by WNS in North America. The detection of clinical signs in any Australian species of microbat, similar to those described for WNS in North America, should prompt WNS exclusion testing as outlined in this document. WNS should also be considered as a differential diagnosis for mass mortalities of Australian microbats, with or without clinical signs consistent with WNS.

Australia's fruit and nectar-eating bats (flying foxes, tube-nosed bats, blossom bats) do not currently warrant WNS exclusion testing.

b. Clinical signs of WNS in microbats

The causative fungus *Pseudogymnoascus destructans* has **not** been detected in Australia. WNS exclusion testing should be considered when Australian microbats display any of the following clinical signs:

- Presence of white or grey powdery fungus (see Figure 1)
- Wing membrane damage (membrane thinning, depigmented areas, flaky appearance or non-traumatic holes)
- Mass mortality
- Aberrant behaviour (such as flying during the day, increased arousal/activity during a period of torpor).

Figure 1 – Clinical signs of white-nose syndrome in microbats

Photos: Karen Vanderwolf/NB Museum http://blog.cwf-fcf.org. More images of WNS in microbats are available on the USGS National Wildlife Health Center website.

The overgrowth of saprophytic fungi on dead bats may present similarly to WNS. Detailed and accurate records of the circumstances and state of decomposition at the time the bat was discovered will assist in determining, in discussion with your WHA Coordinator, if exclusion testing is indicated.

WNS is a nationally notifiable disease in Australia. If you suspect WNS, please call your local State/Territory WHA Coordinator (if possible prior to collecting or submitting any samples) or call the Emergency Animal Disease Hotline (1800 675 888).

Sample submission guidelines

a. Human health precautions

No human health risk from WNS has been identified; there is no information indicating that people or other animals have been affected after exposure to the fungus. However, there is a risk of exposure through handling bats to other diseases such as Australian bat lyssavirus (ABLV).

Members of the public should not handle bats. For an injured or sick bat, contact a wildlife care organisation or local veterinarian.

People trained in the care of bats should be vaccinated for protection against ABLV and always use appropriate personal protective equipment when interacting with bats.

ABLV is transmitted by the saliva of an infected animal introduced via a bite or scratch, or by contamination of mucous membranes or broken skin. In the event of a bat bite, scratch or other significant contact, **seek medical attention URGENTLY.** Bite or scratch wounds should immediately be washed thoroughly with soap and copious water for approximately 15 minutes and a virucidal antiseptic applied. Bat saliva in the eyes or mouth should be rinsed out immediately and thoroughly with water. For more information contact your local Public Health agency for advice.

For further information on ABLV risk management:

- WHA: Personal Protective Equipment (PPE) Information for Bat Handlers
- Qld WHS: Australian bat lyssavirus and handling bats and safe bat handling video
- Links to state/territory ABLV resources
- AVA: Guidelines for veterinary personal biosecurity

b. Disease transmission and biosecurity

Transmission of WNS occurs via direct contact between bats. The fungus has also been found to persist in the environment for long periods without the presence of bats, providing the opportunity for bats to become infected from environments contaminated with the pathogen. Humans may also facilitate the spread of the disease by transferring spores on clothing, footwear, equipment or other fomites.

Any bat where WNS is suspected should be kept separately and isolated from all other bats and animals to reduce the risk of disease transmission.

The use of PPE and decontamination as outlined above and below should be adopted to minimise the potential transfer of *P. destructans* between individual bats and between bats and the environment, and to limit the spread within the environment. Consideration of how clothing and equipment will be decontaminated (see below) should be made before any contact with suspect cases.

c. Decontamination

Guidelines for the disinfection of materials and equipment exposed to *P. destructans* have been published in the USA. These decontamination procedures largely aim to address the risk of humans transferring the fungus between affected and non-affected sites / caves, however many of the recommendations for disinfection can be equally applied in the veterinary clinical setting. The preferred treatment for clothing, footwear and equipment is cleaning to remove dirt and debris, followed by submersion in hot water maintaining a temperature of at least 55°C for a minimum of 5 continuous minutes, followed by rinsing in clean water and complete drying. Equipment that cannot be immersed in water can be treated by

disinfection. The US National White-nose Syndrome Decontamination Protocol (October 2020)[1] details specific, effective disinfectants (see Table 1 in the Protocol).

d. Sample Collection

A list of information to record is provided in Table 1 below. **Photographs should be taken PRIOR to any sampling effort or packaging of carcasses.** The sampling process may disrupt the delicate attachment of the fungus to the animal. Visible spores are also extremely fragile and prone to being dislodged during sampling or packaging for shipment.

Details of samples to be collected and available tests are provided in Table 2. To ensure the most appropriate samples are submitted and stored appropriately during transport, contact your local State/Territory WHA Coordinator or the corresponding laboratory in your jurisdiction prior to collecting or submitting any samples, if possible.

e. Sample Submission and Testing

Samples must be submitted to the relevant State/Territory government laboratory. Molecular testing available at the Australian Centre for Disease Preparedness (ACDP) includes a *Pseudogymnoascus destructans* specific-PCR.

To ensure the collected samples are stored appropriately during transport, contact your local State/Territory WHA Coordinator, or the corresponding laboratory in your jurisdiction prior to collecting or submitting any samples, if possible. State/Territory government laboratories may subsequently refer your samples to the ACDP or other laboratories for further testing, including real-time PCR.

TABLE 1 - Information to record

- Date found
- Location
- Bat species
- Number of bats affected
- Presenting signs
- Any comments/observations on the status of the colony
- Body condition
- Weight
- Types of samples submitted
- Photographs of lesions
- Colour of lesions
- Size of lesions
- Number of lesions
- Location of lesions

[1] For the latest version of the US Decontamination Protocol and further decontamination information: https://www.whitenosesyndrome.org/static-page/decontamination-information

TABLE 2 – Sample collection and testing

***Please note**:*

- *To ensure samples are submitted and stored appropriately during transport, please call your local State/Territory WHA Coordinator, or contact the corresponding laboratory in your jurisdiction prior to collecting or submitting any samples.*
- *Samples must be sent to respective State/Territory government laboratories in the first instance and must not to be sent directly to ACDP.*

PREFERRED SAMPLES	The whole carcass should be submitted where possible, to allow histopathology to be conducted and to maximise the opportunity for testing.		
Sample	**Storage**	**Available tests**	**Notes**
Photographs of lesions	N/A	N/A	• To accompany any sample submissions
Whole carcass	4°C	• Real-time PCR • (+/-) Histopathology	
OTHER SAMPLE OPTIONS (LIVE BATS)	Non-lethal sampling techniques may not allow confirmation of WNS and may have a reduced reliability of detection as compared to whole carcass evaluation.		
Sample	**Storage**	**Available tests**	**Notes**
Swab of affected area	4°C Nuclease-free water[2]	• Real-time PCR	• Methodology: Appendix D of the NWHC Bat White-Nose Syndrome/Pd Surveillance Submission Guidelines (Winter 2022 - 2023) • Collect prior to biopsy if collecting both sample options from the same bat.
Punch biopsy of wing membrane	4°C	• Real-time PCR • (+/-) Histopathology	• Ideally performed on areas of wing membrane with visible fungus or characteristic fluorescence on wing membranes (see UVA screening of wing membrane, below). • If this is the only sample type submitted, it is recommended 2 biopsies are collected (from different wings) of each bat. When collecting wing membrane biopsies, avoid bones and blood vessels. • Methodology: Appendix E of the NWHC Bat White-Nose Syndrome/Pd Surveillance Submission Guidelines (Winter 2022 - 2023) • Furred skin or non-flight membrane biopsy from live bats is not recommended.
	Formalin	• Histopathology • (+/-) Real-time PCR (fresh tissue preferred)	
ANCILLARY TESTING	Submission of the whole carcass, a biopsy or swab should ALWAYS accompany these sampling techniques. Confirmation or exclusion of WNS cannot be achieved if these sampling techniques are performed in isolation.		
UVA screening of wing membranes	N/A	• Detection of pale yellow-orange fluorescence spots on wings	• To be used in conjunction with other targeted sampling techniques. • Can be used to help optimise biopsy placement. • Methodology: Appendix F of the NWHC Bat White-Nose Syndrome/Pd Surveillance Submission Guidelines (Winter 2022 - 2023)

[2] Nuclease-free water (buffer) is ideal for PCR testing. However if not available, sterile PBS, VTM or other equivalent buffer is acceptable for PCR.