WILDLIFE HEALTH AUSTRALIA

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 protection when interacting with bats.

A companion information document How to report a suspect case of white-nose syndrome[1] is available as a resource for bat carers, ecologists, cavers and members of the public

BACKGROUND

The WHA White-Nose Syndrome Fact Sheet[2] is available on the WHA website and provides detailed information on WNS.

a. Species likely to be affected by WNS in Australia

Australia is home to a number of genera of microbats 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 per 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 megabats (flying foxes/fruit bats, tube-nosed bats and 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 behaviours (such as flying during the day or increased arousal/activity during a period of torpor).

[1] www.wildlifehealthaustralia.com.au/ProgramsProjects/BatHealthFocusGroup.aspx#WNS
[2] www.wildlifehealthaustralia.com.au/FactSheets.aspx

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

KAREN VANDERWOLF/NB MUSEUM
http://blog.cwf-fcf.org/?author=5

KAREN VANDERWOLF/NB MUSEUM
http://blog.cwf-fcf.org/?tag=geomyces-destructans

KAREN VANDERWOLF
http://blog.cwf-fcf.org/?tag=pseudogymnoascus-destructans

More images of WNS in microbats are available on the USGS National Wildlife Health Center[3] website

The overgrowth of saprophytic fungi on dead microbats 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.

Based on the above information, if you suspect WNS, please call your local State / Territory Wildlife Health Australia (WHA) Coordinator[4], if possible prior to collecting or submitting any samples.

[3] www.nwhc.usgs.gov/disease_information/white-nose_syndrome/

[4] www.wildlifehealthaustralia.com.au/AboutUs/ContactDetails.aspx

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, people handling bats should use safe work practices and personal protective equipment (PPE). For further information see the US Fish and Wildlife Service website[5]

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. If you find an injured or sick bat, contact a wildlife care organisation or your local veterinarian.

People trained in the care of bats should be vaccinated for protection against ABLV and always use appropriate protection 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 5 minutes and a virucidal antiseptic applied[6]. 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.

The Queensland Government Workplace Health and Safety website provides further information on rabies vaccination, PPE and other ABLV risk management considerations (Australian bat lyssavirus and handling bats[7]). The website also provides a safe bat handling[8] video, with further information on PPE[9].

The Australian Veterinary Association (AVA) Guidelines for veterinary personal biosecurity[10] provides general advice regarding personal biosecurity.

[5] www.whitenosesyndrome.org/national-plan/general-practices

[6] Department of Health. Rabies Virus and Other Lyssavirus (Including Australian Bat Lyssavirus) Exposures and Infections. CDNA National Guidelines for Public Health Units. Canberra. 2014. Available from www.health.gov.au/internet/main/publishing.nsf/Content/cdna-song-ablv-rabies.htm

[7] www.worksafe.qld.gov.au/agriculture/workplace-hazards/diseases-from-animals

[8] www.worksafe.qld.gov.au/forms-and-resources/films/safe-bat-handling

[9] www.worksafe.qld.gov.au/forms-and-resources/guides-and-fact-sheets

[10] www.ava.com.au/biosecurity-guidelines

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, and this provides 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, 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 considered 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 inherent risks 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 setting. For example, the most universally available option for treatment of clothing, footwear and equipment that can be immersed in water is submersion for a minimum of 20 minutes at a temperature of at least 55°C. The National White-Nose Syndrome Decontamination Protocol (US)[11] details specific, effective disinfectants for porous and non-porous surfaces and PPE.

d. Sample Collection

A list of information to record is provided in Table 1.
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, please call your local State / Territory Wildlife Health Australia (WHA) Coordinator[12], or contact the corresponding laboratory in your jurisdiction prior to collecting or submitting any samples.

e. Sample Submission and Testing

Samples must be submitted to respective State/Territory government laboratories. Molecular testing available at Australian Animal Health Laboratory (AAHL) include a *Pseudogymnoascus destructans* specific-PCR and a pan-fungi PCR.

[11] www.whitenosesyndrome.org/sites/default/files/resource/national_wns_revise_final_6.25.12.pdf
[12] www.wildlifehealthaustralia.com.au/AboutUs/ContactDetails.aspx

To ensure the collected samples are stored appropriately during transport, please call your local State / Territory Wildlife Health Australia (WHA) Coordinator[13], or contact the corresponding laboratory in your jurisdiction prior to collecting or submitting any samples.

State/Territory government laboratories may subsequently refer your samples to the Australian Animal Health Laboratory or other laboratories for further testing including specific molecular assays and fungal culture.

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

[13] www.wildlifehealthaustralia.com.au/AboutUs/ContactDetails.aspx

TABLE 2 – Sample collection and testing

***Please note**:*

- *To ensure samples are submitted and stored appropriately during transport, please call your local State / Territory Wildlife Health Australia (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 AAHL.*

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**
Whole carcass	4°C	• Molecular assays • Fungal culture • Histopathology	
Photographs of lesions		n/a	• To accompany any sample submissions
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**
Biopsy: wing membrane or skin	4°C	• Molecular assays • Fungal culture • Histopathology	• Usually performed on areas of wing membrane with visible fungus or characteristic fluorescence on wing membranes. • When collecting wing membrane biopsies, avoid bones and blood vessels. • Skin biopsies can be collected from other affected areas with consideration of the impact to the live bat. • Methodology: Appendix D of the USGS NWHC WNS Submission Protocol[14]
	Formalin	• Histopathology • Molecular assays (fresh tissue preferred)	
Swab of affected area	4°C Dry	• Fungal culture may be possible • Molecular assays may be possible	• Methodology: Appendix E of the USGS NWHC WNS Submission Protocol[13]
ANCILLARY TESTING	These sampling techniques should ALWAYS accompany submission of the whole carcass, a biopsy or swab. Confirmation or exclusion of WNS cannot be achieved if these sampling techniques are performed in isolation.		
Sticky tape of affected area (muzzle preferred)	4°C	• Microscopic examination of organism	• This technique will NOT confirm WNS on bats and should not be used as the sole sampling methodology. • No specific expertise at AAHL for identification by microscopic examination. • Methodology: Appendix C of the USGS NWHC WNS Submission Protocol[13]
UVA screening of wing membranes		• Detection of pale yellow-orange fluorescence spots on wings	• To be used in conjunction with other targeted sampling techniques • Methodology: Appendix F of the USGS NWHC WNS Submission Protocol[13]

[14] Further information can be found in the USGS NWHC WNS Submission Protocol (PDF) accessed via www.nwhc.usgs.gov/disease_information/white-nose_syndrome/