WHITE-NOSE SYNDROME
RESPONSE TEAM

WNS Case Definitions – revised January 2020

Note: Diagnostic categories described in this document apply to individual specimens submitted for diagnostic evaluation and may differ from site-level designations used for official reporting purposes based on pending guidance for interpreting the weight of evidence.

Diagnostic Criteria for Reporting Cases of Bat White-Nose Syndrome (WNS) at the individual specimen level

1. **Positive for WNS** – Characteristic histologic lesions of WNS[1] are present on an individual bat **AND** the bat is positive for *Pseudogymnoascus destructans* (*Pd*) either by qPCR[2] or by fungal culture.

2. **Suspect for WNS** – (one of the following criteria must be met)
   a) Characteristic histologic lesions of WNS[1] are present on an individual bat but *Pd* is not detected, the test result for *Pd* is inconclusive (either by qPCR[2] or by fungal culture), or testing for *Pd* is not performed.
   b) One or more field signs* are observed in a bat colony **AND** *Pd* is detected (either by qPCR[2], fungal culture, or a tapelift performed directly on visible fungal growth on bat skin) on at least one individual of the same species AND histopathology is negative or is not performed.
   c) **MULTIPLE** field signs* are observed among species known to be susceptible to WNS **AND** are within the currently recognized range of WNS but no samples are collected for diagnostic evaluation.
   d) Individual bats that are part of a confirmed WNS morbidity/mortality event are submitted to, but not tested by, a diagnostician. This criterion is for instances in which multiple samples from the same site are submitted, but only a subset of those samples is tested. The untested samples may be classified as suspect for WNS if the subset of tested samples is **Positive for WNS** and consists of the same species as the untested samples. Representatives of all species involved in the disease event should be tested.

3. **Negative for WNS** – Characteristic histologic lesions of WNS[1] are not present **AND** bat is negative for *Pd* (either by qPCR[2] or fungal culture).

Diagnostic Criteria for Reporting the Detection of *Pseudogymnoascus destructans (Pd)* at the individual specimen level in the absence of field signs of WNS

1. ***Pd* Positive** – *Pd* detected by qPCR[2] or by fungal culture in accordance with criteria **(Appendix A)** accepted by the WNS Diagnostic Laboratory Network in an environmental sample or on an individual bat with no other field signs of WNS* observed within the surveyed population. Bat carcasses submitted for diagnostic testing are placed in this category if *Pd* is detected on the carcass but there were no field signs of WNS observed on the individual AND histopathology is negative or is not performed. Repeat testing and/or independent secondary confirmation of ***Pd* Positive** results is warranted before this designation is applied to bat species of unknown susceptibility to WNS or areas outside the known geographic distribution of *Pd*.

2. **Inconclusive for *Pd*** – Non-negative results by qPCR[2] that are outside the range of accepted, standardized laboratory-defined criteria for ***Pd* Positive (Appendix A)**. This designation is made independent of other epidemiological evidence. The real-time PCR assay for *Pd* provides a cycle threshold (Ct) value that is inversely related to the amount of target DNA in that sample (i.e. lower Ct values indicate higher amounts of DNA). Interlaboratory testing indicates that across a range of techniques and platforms, results of Ct ≤ 37 exhibit reliable reproducibility. Results with a Ct > 37 but ≤ 40 are categorized as inconclusive. While

these results may indicate the presence of very small amounts of target DNA, they are less likely to be reproducible, which makes them more difficult to differentiate from laboratory contamination. Thus, no determination of *Pd* status can be made about the specimen based on an inconclusive result. Caution is urged in using inconclusive results for making inference about the presence of *Pd* in a broader population, hibernaculum, or geographic area. Additional sampling is warranted before broader inferences can be made about the true presence or absence of *Pd* at the survey site for official reporting purposes.

3. ***Pd* Negative** – *Pd* is not detected (either by qPCR[2] **[Appendix A]** or fungal culture) in an environmental sample or on an individual bat. [Note: Although a negative qPCR or fungal culture result indicates that *Pd* was not detected in the tested sample, this does not guarantee the roost site or bat colony from which the sample was collected is free of *Pd*. A lack of observed field signs* in the resident bat population is also not sufficient for assuming that a roost is *Pd*-free. Consistently negative results from a statistically robust sample size can, however, increase confidence that *Pd* is absent from the sampled population or environment.]

***Field Signs Associated with WNS in Bats**

Winter/Spring – excessive or unexplained mortality at or near a hibernaculum; visible fungus on flight membranes, muzzle, or ears of live or fresh dead bats; abnormal behaviors including daytime activity, premature egression from the hibernaculum, or unexpected population shift to entrance of the hibernaculum; moderate to severe wing damage[3] in nontorpid bats or thin body condition (each considered a nonspecific field sign when observed by itself); yellow-orange fluorescent pattern of non-haired skin under UVA light.[4]

Summer/Fall – There are not consistent field signs associated with WNS during summer/fall.

Additional Comments

When screening for the presence of *Pd*, qPCR is preferred over fungal culture or tapelift due to the greater sensitivity of the qPCR assay.

Results obtained using testing methodologies other than those referenced here will be considered preliminary and further testing with accepted standard methodologies is necessary for official reporting purposes.

For management purposes, hibernacula should be considered contaminated with *Pd* if they contain at least one sample (bat or environmental) that is determined to be **Positive for WNS**, **Suspect for WNS**, or ***Pd* Positive** as described above. A contaminated hibernaculum retains this designation indefinitely. The ability of *Pd* to persist long-term outside of hibernacula is not currently well understood however, appropriate biosecurity measures are advised to minimize the risk of human-mediated spread.

Citations

1. Meteyer, C.U., E.L. Buckles, D.S. Blehert, A.C. Hicks, D.E. Green, V. Shearn-Bochsler, N.J. Thomas, A. Gargas, and M.J. Behr. 2009. Histopathologic crtieria to confirm white-nose syndrome in bats. *Journal of Veterinary Diagnostic Investigation* 21: 411-414.
2. Muller, L.K., J.M. Lorch, D.L. Lindner, M. O'Connor, A. Gargas, and D.S. Blehert. 2013. Bat white-nose syndrome: a real-time TaqMan polymerase chain reaction test targeting the intergenic spacer region of *Geomyces destructans*. *Mycologia* 105: 253-259.
3. Reichard, J.D. and T.H. Kunz. 2009. White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*). *Acta Chriopterologica* 11: 457-464.
4. Turner, G.G., C.U. Meteyer, H. Barton, J.F. Gumbs, D.M. Reeder, B. Overton, H. Bandouchova, T. Bartonička, N. Martínková, J. Pikula, J. Zukal, and D.S. Blehert. 2014. Non-lethal screening of bat-wing skin with the use of UV fluorescence to detect lesions indicative of white-nose syndrome. *Journal of Wildlife Diseases* 50: 566-573.

APPENDIX A

For Pd qPCR diagnostic interpretation:

Ct ≤ 37 = Pd positive
Ct > 37 but ≤ 40 = Pd inconclusive
Ct > 40, or no amplification = Pd negative

When testing replicates*, readily interpretable categories include:

Ct ≤ 37 for all replicates = Pd positive
Ct > 37 but ≤ 40 in all replicates = Pd inconclusive
Ct > 40 or no amplification in all replicates = Pd negative

Running multiple replicates increases the probability of detecting small amounts of target DNA in the extracted material. However, replicate results that fall into multiple diagnostic categories present a challenge for interpretation. Divergence between results can generally be categorized as:

1. **Extreme**: Differences for which there is no biological or statistical explanation. Example: A mixture of positive (Ct ≤ 37) and negative (above 40 or no amplification) replicates; or a mixture of inconclusive replicates near or equal to 40 together with a strong (i.e., low Ct) positive.
2. **Minimal to moderate**: Differences for which there is a biological or statistical explanation. Example: A mix of positive and inconclusive replicates with Ct values near the cut-off threshold of 37, or a mix of inconclusive and negative replicates.

Whenever discordant results arise, all possible explanations, including user error or contamination, should be explored. Discordant replicates determined by the laboratory to be **extreme**, must be reconciled prior to reporting. If extremely discordant results cannot be reconciled, the result should be reported as invalid.

Discordant replicates determined by the laboratory to be **minimal or moderate**, should be reported as follows:

Any mixture of:	Result
Pd positive and Inconclusive	Pd Positive
Pd negative and Inconclusive	Pd Inconclusive

Laboratories or submitters may elect to have samples with an original Ct value ≤ 37 retested in a different laboratory if the result represents a novel documentation of *Pd* (by species or geographic area). Final sample categorization should be agreed upon by both laboratories. It is not likely productive to send samples with original Ct values greater than 37 to other laboratories for confirmatory testing because the low amount of target DNA in these samples makes replication less probable. If independent verification is desired, re-sampling should occur, with duplicate samples taken and submitted directly to the separate laboratories for parallel testing.

* Replicates are defined as any number of qPCR reactions amplified from the same sample extract