WHITE-NOSE SYNDROME RESPONSE TEAM

WNS Case Definitions – revised May 2019

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 are present (Meteyer et al.) on an individual bat **AND** the bat is positive for *Pseudogymnoascus destructans* (*Pd*) either by Muller et al. qPCR or by fungal culture.

2. **Suspect for WNS** – (one of the following criteria must be met)
   a) Characteristic histologic lesions of WNS are present on an individual bat but *Pd* is not detected, the test result for *Pd* is inconclusive (either by Muller et al. qPCR or by fungal culture), or further testing for *Pd* is not performed.
   b) One or more field signs* are observed in a bat colony **AND** *Pd* is detected (either by Muller et al. qPCR, 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 are not present **AND** bat is negative for *Pd* (either by Muller et al. qPCR 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 Muller et al. qPCR or by fungal culture in accordance with laboratory-defined criteria 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 Muller et al. qPCR that are outside the range of accepted, standardized laboratory-defined criteria for ***Pd* Positive**. Results in this category may reflect the presence of minimal target DNA in the sample, representing a low-level *Pd* detection indicative of early infection. However, other possible explanations must be considered, such as contamination, non-specific amplification, or artifact from degradation of qPCR reaction components in the late stages of

thermocycling. A designation of **Inconclusive for *Pd*** is made independent of other epidemiological evidence. Additional sampling and/or testing is warranted before broader inferences can be made about the presence of *Pd* at the survey site for official reporting purposes.

3. ***Pd* Negative** – *Pd* is not detected (either by Muller et al. qPCR 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 hibernaculum 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 hibernaculum 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 in nontorpid bats [Reichard et al.] 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 [Turner et al.]

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 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 tests ***Pd* Positive** by the Muller et al. qPCR or fungal culture regardless of whether field signs of the disease were observed within the hibernaculum. A contaminated hibernaculum retains this designation indefinitely. The ability of *Pd* to persist long-term outside of hibernacula is not currently well understood.

Citations

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.

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.

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.

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.